Tufts Medical Center

Protocol Title:	Effect of Intra-Articular Steroids on Structural Progression of Knee OA: A Randomized Controlled Trial
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1. Summary

Triamcinolone 40 mg will be compared to placebo in a randomized placebo-controlled clinical trial testing the effect of triamcinolone versus placebo given intra-articularly (into the index knee joint with osteosoarthritis (OA)) once every three months over two years for a total of eight knee injections. Tolerability and safety will be assessed by adverse effects (AE) reporting, physical examination, vital signs, and clinical laboratory tests. The primary clinical outcome will be assessed by changes in the WOMAC questionnaire. The primary pathological process measure will be change in knee cartilage volume as measured by magnetic resonance imaging (MRI).

2. Background and Significance

2.1 Osteoarthritis Background

Symptomatic knee OA has an estimated overall incidence of 240/100,000 person years[38], and is the most frequent cause of dependency in lower limb tasks, especially in the elderly[39]. It causes 68 million work loss days per year and more than 5% of the annual retirement rate[11, 12]. It has considerable economic and societal costs, in terms of work loss[40] and hospital admission[41]. Furthermore, OA is the most frequent reason for joint replacement at a cost to the community of billions of dollars per year[12]. There are currently no therapies for OA confirmed to have an effect on disease progression. Treatments commonly used for OA include intra-articular corticosteroids (IACS), a widely employed invasive intervention that is of particular note because its structural effects on cartilage and bone are uncertain.

2.2 Knee Osteoarthritis and Knee Synovitis

OA is characterized by focal and progressive damage to articular hyaline cartilage accompanied by a hypertrophic 'reaction' in peri-articular bone[42]. The prevailing view of knee OA has been one of a non-inflammatory disorder but chronic inflammatory changes in the synovium are seen in all stages of knee OA[43-47], appear to be of clinical significance[17, 19, 48, 49], are associated with regions of cartilage damage[19, 50] and predict structural progression[19]. Inflamed synovium is also the most plausible target for IACS in knee OA[19].

It is likely that synovial inflammation in osteoarthritic knees is triggered and sustained by particles and crystals abraded from damaged cartilage – a "detritic synovitis"[18]. These generate in synovial macrophages and fibroblasts a broad range of inflammatory responses that resemble those found in inflammatory joint disorders such as rheumatoid arthritis. According to this model, synovial inflammation should likely occur as a late consequence of damage to cartilage and subchondral bone. However, it is also possible that it occurs earlier, as a paracrine response to disordered homeostasis in the articular environment[18].

Synovial biopsy studies of osteoarthritic knees have found a high prevalence of inflammation. One study of 63 patients with moderate to severe knee OA found thickening of the lining layer, increased vascularity, and inflammatory cell infiltration in synovial membranes in all knees, with the most prominent features in those with advanced disease[44]. However, synovitis appears common even in mild knee OA - Myers studied the mild end of the spectrum and found synovitis in 55% of biopsied knees[43]. Another biopsy study of nine selected knees with mild OA and short symptom duration also showed a mild chronic synovitis in all cases[47].

Synovial changes in the knee also appear to be associated with pain and structural damage. An MRI study of over 400 individuals from both Veterans Affairs and community sources, found more effusions and synovial thickening among those with knee pain[49]. Among the subset with symptomatic radiographic knee OA, synovial thickening was associated with the severity of knee pain. In a subsequent longitudinal

analysis of 270 participants in that study who had MRI evaluations at 15 and 30 months, change in synovitis (especially in the infrapatellar fat pad) correlated with the change in pain ($r = 0.21$, $p = 0.0003$)[17]. Loeille et al, in scrutinizing the relationships between synovitis and cartilage damage among 39 patients with knee OA using arthroscopy, biopsy and MRI, found a correlation of synovitis with medial tibiofemoral cartilage damage ($r = 0.5$, $p=0.001$)[50].

There is also evidence that synovitis participates in the structural progression of knee OA[19, 51]. Ayral studied a cohort of 422 individuals with medial compartment tibiofemoral OA who participated in a (null) clinical trial that included a systematic assessment of synovitis and chondropathy using arthroscopy[19]. Twenty-one percent of participants had inflammatory changes in the synovium at baseline and these individuals were three times more likely to exhibit progression of cartilage damage (25.8% vs. 11.9%, adjusted odds ratio = 3.11, 95% confidence limits 1.07 - 5.69). Pelletier et al, in a 2-month observational study of 29 knee OA patients with MRI-confirmed synovitis found that the baseline synovitis score correlated significantly with the percentage loss in cartilage volume over this relatively short period ($r\sim0.6$)[51].

2.3 Mechanisms of Joint Damage from Synovitis in Knee Osteoarthritis

The synovium has important roles in articular health, so it is logical to infer that pathological changes and functional compromise may contribute to progression of OA. Two important synovial roles in articular homeostasis include provision of vascular support to distant chondrocytes and facilitation of low-friction movement between cartilaginous surfaces[52]. The synovium produces two critical components of synovial lubrication; (i) the macromolecule hyaluronan, which confers to synovial fluid its viscosity and (ii) the boundary layer lubricant lubricin. In situations of joint injury, the synovium increases its secretion of hyaluronan, which may dampen inflammation in the synovial space. Typical OA effusions are transudative and have high viscosity[52]. However, this viscosity is lost in the presence of inflammation[53]. Experimental data also show reduction in synovial secretion of lubricin in the presence of inflammation and proinflammatory cytokines such as IL-1 and TNF-α[54, 55].

The synovium may also participate in the pathophysiology of OA through the secretion of proteolytic enzymes and cytokines. Indeed, cytokines are fairly abundant in OA synovium, at least in late-stage disease (Table 1)[18]. Immunohistological studies of synovium obtained from OA and rheumatoid (RA) joints show quantitative rather than qualitative differences between the two disorders[44]. Production of IL-1α, IL-1β, and TNFα is present in synovial membranes from all patients with OA, irrespective of the degree of articular cartilage damage[44]. Thus, chronic inflammatory changes with production of proinflammatory cytokines are a feature of synovial membranes from patients with early OA, with the most severe changes resembling those of RA.

IL-1 and TNF-α are the dominant cytokines in inflamed OA joints, of which IL-1 appears to have the greatest potential to mediate cartilage damage[56, 57]. IL-1 is produced in considerable quantities in OA synovium[58, 59] and appears to be the driving force in the production of destructive enzymes (matrix metalloproteinases) and IL-6[60]. However, there are numerous other cytokines that may have specific and important roles in OA. IL-6, for example, may upregulate matrix metalloproteinase expression in cartilage, stimulate hepatic production of acute phase proteins and mediate bone destruction[18]. The non-peptide mediator, Nitric oxide (NO), is also produced by synovial cells and chondrocytes in OA joints[61] and may contribute cartilage destruction.

There is evidence that IL-1 and TNF-α may be partly responsible for increased amounts of proteases found in OA synovium[18]. Coordinate synthesis of IL-1 and stromelysin has been found in canine OA[62], as has a correlation in human OA synovium between the number of inflammatory cells and levels of neutral proteases[63]. Expression of collagenase and stromelysin is evident in OA synovium, albeit at lower levels than in rheumatoid disease[64, 65]. Tissue inhibitor of metalloproteinase (TIMP) is elevated in OA synovium, with a protease:inhibitor ratio that suggests a role for proteases in tissue destruction. Indeed, many cartilage proteinases have now been identified, among which collagenase 3 (MMP-13) and

aggrecanase 1 (ADAMTS4) appear to play major roles in degradation of human cartilage matrix[52]. Activation of collagenase 3 (MMP-13) appears to be at least partly cytokine dependent[66, 67], but there is a lack of agreement on the effect of cytokines on ADAMTS4[52].

Of course, the factors that are pivotal to maintaining this balance or the cartilage response are largely unknown. It follows, then, that perturbation of the system using pharmaceuticals may have effects on the balance between catabolism and anabolism that are not entirely predictable. It is suggested, for example, that intra-articular corticosteroids (IACS) might benefit OA knees by suppressing IL-1 mediated stimulation of metalloproteinase synthesis[68]. While this seems plausible, the net effects of IACS on measures of cartilage health need to be tested in a clinical environment.

2.4 IACS: Current Clinical Usage in the Treatment of Knee Osteoarthritis

IACS are widely employed in the management of knee OA. It is estimated that 95% of rheumatologists in the U.S. perform IACS for OA, 53% frequently[69]. The use of IACS for OA is endorsed by the American College of Rheumatology (ACR)[15], the European League Against Rheumatism (EULAR)[16], the Osteoarthritis Research Society International (OARSI)[13, 14], and the National Collaborating Centre for Chronic Conditions[70]. The latter was a thoughtful document and commented "...intra-articular corticosteroid injections provide short-term reduction in osteoarthritis pain... The risks ... are generally small. The question of steroid-arthropathy, ... remains controversial and is currently based on animal model and retrospective human studies".

2.5 Effectiveness of IACS for Knee Osteoarthritis Pain

There have been at least 12 randomized controlled trials (RCTs) testing IACS for OA *pain*[1, 71-82], and two recent meta-analyses[26, 83]. The most recent and comprehensive meta-analysis[26] was based on 28 short-duration trials (except one[1]). In the pooled analyses, IACS was more effective than placebo for pain reduction one-week post injection (relative risk 1.4; 95% confidence limits 1.1 - 1.8), 2 weeks (1.8; 1.1 - 3.0) and 3 weeks (3.1, 1.6 - 6.1). They recommended that future trials have standardized outcomes, run longer, investigate different patient subgroups, and clinical predictors of response, specifically those associated with inflammation and structural damage. The proposed study will accommodate these recommendations by testing a long-term (2-year) IACS intervention among the subset of KOA with inflammatory features, using standardized clinical and structural outcomes.

2.6 Previous Studies Attempting to Evaluate the Effect of IACS in Clinical Samples

Three studies have attempted to examine the structural effects of IACS on OA progression[1, 84, 85]. The first was an observational report of 61 patients with knee OA who had documentation of IACS treatment over a 10-year period[85]. In men only, the knees that had received IACS had more severe osteoarthritis at follow-up, as measured by the tibiofemoral angle of alignment. However, this study has serious methodologic problems including bidirectional censorship and confounding by indication.

The second was an observational study of 67 patients with temporomandibular joint OA that had not responded to non-surgical treatment[84]. Twenty-three proceeded immediately to high condylectomy, while the remaining 44 received two intra-articular injections of triamcinolone acetonide, 20 mgs, with a 2-month interval. It is not clear how this treatment assignment was made. Eighteen of the 44 patients who received IACS later underwent high condylectomy due to 'treatment failure'. Histological examination of the surgical specimens from this subset exhibited more extensive joint damage than those in the non-steroid group. However, the observational non-randomized nature of this study introduces complexities that prevent causal inference.

Raynauld evaluated the safety of triamcinolone acetonide 40mgs, or saline control, administered every 3 months for 2 years in a randomized controlled trial for knee OA[1]. Structural progression was assessed using fluoroscopically-positioned knee x-rays[86, 87]. They enrolled 68 participants, of whom 66 completed the trial. Most had mild OA (~65% had Kellgren & Lawrence grade 2 changes). There was no difference between groups in extent of change in radiographic joint space width. The clinical outcomes were also

identical, with the exception of area-under-the-curve (AUC) analyses for night pain and stiffness that favored treated knees (AUC for night pain; treated =-0.7; untreated =-0.3; p = 0.005; stiffness; treated =-0.7; untreated =-0.3; p = 0.05). This study is somewhat reassuring in that it provides evidence for symptomatic benefit without evidence of increased structural deterioration. On the other hand, there are methodologic factors that limit its interpretation. The participants had mild disease and exhibited little structural progression. In fact, there was no attempt to enroll a phenotype at higher risk for progression nor was there evaluation or stratification for indicators of inflammatory OA that might predicate responsiveness to IACS. Thus, the findings are not especially informative in respect of the potential for structural modification in the pertinent phenotypes. The radiograph is also fundamentally flawed due to its inability to image soft-tissue articular structures such as cartilage, and high precision error relative to expected change, especially in a small sample such as this[88].

2.7 Are Intra-Articular Corticosteroids Safe?

Joint sepsis is a primary concern in relation to intra-articular injection. However, while its incidence is difficult to measure with certainty, it is clearly infrequent. A 1999 estimate from a retrospective survey in France suggests an incidence rate of 13 per million injections, lower with pre-packaged syringes[89]. Concordant with this low estimate, a survey of all cases of septic arthritis incident between 1982 to 1991 in Nottingham, UK (which had a base population of 600,000) identified only 3 cases attributable to IACS[90].

More frequent, but of less medical concern, are local aseptic inflammatory reactions to IACS injections[91]. These have been reported with rates up to 24% in some series[73-75, 92]. The biologic basis for these reactions is uncertain but may relate to the crystalline nature of the injected corticosteroid formulations.

There is systemic absorption of steroid injected into a joint. Peak plasma levels after an intra-articular injection of 80 mgs methyl prednisolone are in the order of 169 ng/ml, achieved 8 hours post injection[93]. A measurable transient effect on the pituitary axis is apparent with injections of 40 mg of methyl prednisolone[94] or >40 mg triamcinolone diacetate[95]. However, it seems unlikely that this has any longterm effects at the frequency generally recommended in rheumatology practice (< 3 months). Problems with the pituitary axis were also apparently not encountered in an RCT in which IACS were administered every 3 months over a 24 month period[1]. While links have been made between IACS and various systemic features (immunosuppression[96], impaired diabetes control[97, 98], flushing, menstrual irregularities[99]) substantial data quantifying their frequency is generally lacking[83, 100]. One study provided data showing elevation in blood pressure and blood glucose levels following epidural corticosteroid injections, but the doses used were greater than those proposed in this study[97]. Anaphylaxis after IACS has been rarely reported[101, 102], as has steroid psychosis[103].

The most systematic evaluation of adverse effects from IACS was performed by Bellamy, in a Cochrane review of their efficacy for OA[26]. He found that side effects were uncommon and noted no reports of *serious* adverse events among the trials reviewed. Reported adverse events included post-injection flare, crystal-induced synovitis, tissue atrophy, fat necrosis, calcification, joint sepsis, steroid arthropathy, avascular necrosis, hematoma, fluid retention, hyperglycemia and hypertension.

Raynauld's RCT of IACS is pertinent to this proposal in that it employed a similar intervention dosage and time frame[1]. In that study, "No infections (local or systemic) or acute flares were associated with the injections during the study. Findings of laboratory tests performed during the study were within normal limits, and there were no differences between the treatment groups."

2.8 Effects of Intra-Articular Corticosteroids on Cartilage – Helpful or Adverse?

A number of case studies emerged in the 1950s and 1960s that suggested a link between IACS injections and joint destruction (cited by Salter[104], Mankin[105]). Chandler and Wright, for example, observed that repeated intra-articular injection of hydrocortisone in patients with rheumatoid arthritis seemed to improve the extent of synovitis but increase the rate of radiological deterioration[106]. Chandler

et al subsequently drew an analogy with a Charcot joint in a description of apparent acceleration of progression of hip OA in a doctor's wife who had received frequent IACS injections[107]. Similar inferences based on clinical observations recur even in recent literature[85]. While such inferences are innately confounded by indication, their validity seemed to be substantiated by early studies in animals that showed anti-anabolic effects of IACS on cartilage[105, 108, 109].

2.9 Effects of Corticosteroids on Healthy Cartilage In-Vitro

There is evidence that corticosteroids have anti-anabolic influences on cartilage metabolism in-vitro[110-112]. Farquhar tested for interactions between corticosteroid exposure and joint loading on cartilage metabolism and observed that high dose steroids depressed protein and proteoglycan synthesis[110]. Heavy loading further exacerbated the loss of matrix solids. Murphy et al, in pursuit of the optimal therapeutic concentration of IACS, found that proteoglycan synthesis in normal cultured equine articular cartilage was severely depressed by the presence of methylprednisolone 10 mg/mL, and failed to recover after 13 days of culture without methylprednisolone[112]. Cartilage treated with concentrations in the range of 5 mg/mL also exhibited adverse metabolic responses, but these tended to recover after methylprednisolone removal. Lower concentrations did not have significant effects. More recently, Fubini scrutinized corticosteroid-induced changes in collagen expression at transcriptional and translational levels in articular cartilage from young adult horses[111]. Steady-state levels of type II procollagen mRNA decreased as methylprednisolone concentrations increased in a dose-dependent fashion, dropping below 10% of control values by a concentration of 1×10^5 pg/ml. Cytotoxicity occurred as methylprednisolone levels were increased beyond 1×10^8 pg/ml. Based on a comparison of their data with information on the pharmacokinetics of intra-articular methylprednisolone in clinical practice, they estimated that synovial fluid methylprednisolone may remain above a cytotoxic concentration for more than 36 hours following a typical clinical IACS injection. Pelletier et al found that hydrocortisone reduced proteoglycan catabolism in cartilage explants (which they attributed to suppression of metalloprotease synthesis) but also proteoglycan synthesis. They observed extensive vesicular dilatation of chondrocyte endoplasmic reticulum in explants treated with hydrocortisone[113].

2.10 Effects of Corticosteroids on Healthy Cartilage in Animal Models

In-vivo studies of the effects of corticosteroids on healthy cartilage tend to show adverse effects, although their findings are not always consistent. Moskowitz in 1970, and Behrens in 1975, separately observed deleterious effects of intra-articular triamcinolone acetonide on rabbit cartilage[27, 28]. These included nuclear degeneration, cyst formation, cartilage fissures, and reduced synthesis of proteoglycans and matrix proteins. Lewandowski induced 'OA-like damage' in rats using repeated intramuscular injections of dexamethasone[114]. Catabolic or anti-anabolic effects of IACS have also been seen in studies of horses[108] and rabbits[115]. One study testing interactions of intra-articular hydrocortisone and running on cartilage in rats found that the combination was more detrimental than either intervention alone[116]. Studies testing cartilage effects of intra-articular methylprednisolone acetate on the healing of osteochondral defects in horse joints had somewhat discordant results. Shoemaker found degenerative effects[117] but Foland did not observe significant changes[118]. Of course, the extent to which effects in animal models can be generalized to human situation is uncertain.

2.11 Effects of Corticosteroids on Degenerative Cartilage in Animal Models

In contrast to the prior scenarios, these studies provide evidence that IACS may reduce cartilage degradation in certain pathophysiologic situations. Williams & Brandt tested the effect of intra-articular triamcinolone hexacetonide on the development of degenerative cartilage changes that follows a single intra-articular injection of sodium iodoacetate (a metabolic poison)[21]. These features include cartilage fibrillation, loss of staining with Safranin O, depletion of chondrocytes, and prominent osteophytes, without evidence of synovitis. However, when triamcinolone hexacetonide was injected into the ipsilateral knee 24 hours after the intra-articular injection of iodoacetate, fibrillation was noted in only 1 of 6 samples, osteophytes were less prominent, pericellular staining with Safranin O persisted, and cell loss

was less extensive. Thus, triamcinolone hexacetonide produced a marked, dose-dependent protective effect in this model of chemically-induced articular cartilage damage.

Corticosteroids also apparently reduce progression of degenerative lesions in the cruciate ligament transaction dog model of OA[22, 23]. Pelletier & Martel-Pelletier treated 6 animals with oral prednisone and 6 with intra-articular triamcinolone hexacetonide at surgery and 4 weeks later[22]. Twelve other operated dogs received no treatment. Four of 15 normal control dogs received intra-articular triamcinolone. All dogs were sacrificed 8 weeks post-surgery. Operated untreated dogs developed significant cartilage lesions on the femoral condyles and tibial plateaus with prominent osteophytes. Operated dogs treated with oral or intra-articular steroids developed significantly less osteophytosis. Cartilage erosions on femoral condyles appeared in 25% of the untreated dogs, 8% of the dogs receiving oral prednisone, and none of the dogs receiving intra-articular triamcinolone. In both groups of treated dogs, the size of the tibial plateau lesions was significantly smaller than in the operated untreated dogs. Histologically, corticosteroids also significantly reduced the severity of cartilage structural degeneration. Electron microscopy revealed no increase in cell degeneration or death associated with steroids.

The same research group later performed a similar experiment testing the effect of intra-articular methylprednisolone acetate on the development of osteoarthritic lesions in the dog model of OA[23]. Treatment with methylprednisolone reduced the incidence and size of osteophytes and the histologic severity of cartilage damage. Immunohistochemistry of the OA cartilage revealed a marked increase in staining for stromelysin in chondrocytes and throughout the matrix. Treatment with methylprednisolone reduced this toward normal in both chondrocytes and matrix.

In a subsequent mechanistic study using the same animal model, Pelletier et al examined the effects of triamcinolone hexacetonide on cartilage metalloproteases and expression of cytokines/oncogenes (IL-1 beta, c-Fos, and c-Myc)[119]. This 30-dog study had 4 groups: (1) no treatment; (2) triamcinolone at time of surgery and at 4 and 8 weeks later; (3) triamcinolone at 4 and 8 weeks after surgery; (4) triamcinolone at 8 weeks after surgery. As before, intra-articular injections of triamcinolone reduced the development of osteophytes and the histologic severity of cartilage lesions. Immunohistochemistry revealed that triamcinolone reduced the percentage of chondrocytes immunoreactive for stromelysin in a dose-response fashion. They inferred that the effect of triamcinolone may be mediated through a reduction in the expression of proteolytic enzymes, such as stromelysin.

2.12 Effects of Corticosteroids on Inflammatory Cartilage Damage in Animal Models

The potential for corticosteroids to attenuate cartilage damage resulting from inflammatory milieus appears more clear-cut. Corticosteroids have diverse anti-inflammatory effects that could lead to a reduction in activity of downstream catabolic pathways[120, 121]. In inflammatory arthritis models, corticosteroids reduce proteoglycan breakdown, suggesting an inhibitory effect on enzymatic degradation of cartilage[24, 122, 123]. Sedgwick et al found evidence that suppression of synovial expression of inflammatory mediators is a critical mediator of this effect[24]. Using an in-vivo inflammatory model of cartilage destruction, they observed that (soluble) hydrocortisone sodium succinate reduced while (insoluble) hydrocortisone acetate enhanced proteoglycan loss[24]. Injection of the same dose of hydrocortisone acetate into the inflamed lining tissue reversed this effect. However, these results are also compatible with the possibility that corticosteroids formulated to provide persistent intra-articular concentrations have catabolic effects on cartilage.

Joosten et al compared rimexolone and triamcinolone hexacetonide injected into joints of mice with monoarticular antigen-induced arthritis[25]. In control joints, both drugs suppressed proteoglycan synthesis. However, in the inflamed joints, they counteracted the usual severe suppression of proteoglycan synthesis, and reduced the extent of osteophyte formation (a characteristic of this type of experimental arthritis). Thus, in the presence of overt inflammation, the overall effect of corticosteroids on cartilage appears to be favorable.

2.13 Intra-Articular Corticosteroids and Peri-Articular Bone

Peri-articular bone has an important role in protecting cartilage from injury. It is estimated that 30-50% of a load across a knee is absorbed by peri-articular bone compared to only 1-3% by articular cartilage[124-127]. In OA there are widespread pathologic changes in peri-articular bone that include thickening and remodeling of the subchondral plate[128], increased bone turnover[129], trabecular microstructural abnormalities[130,131,132] and macroscopic damage[133]. All of these impair the biomechanical properties of peri-articular bone and could contribute to structural progression. Clinical observations bear this out: peri-articular scintigraphic abnormalities and bone marrow lesions (both common in OA) predict radiographic progression[134] and correlate with subsequent joint space loss ($r \sim 0.2 - 0.3$, $p < 0.05$)[135,136,137] while systemic bone density and tibial peri-articular BMD are highly predictive of structural progression of knee OA[138, 138, 139,140]. Bruyere found a correlation between subchondral BMD and 1-year change in radiographic joint space width of 0.43, highlighting the large effect of bone health on cartilage loss[140]. Zhang et al, examined the relationship of femoral neck BMD with progression of radiographic knee OA among 473 women over 8 years[138]. Compared to those in the lowest quartile of BMD, the adjusted odds ratios for progression were 0.3, 0.2, and 0.1 among women in the 2nd, 3rd, and highest BMD quartiles (p for trend = 0.04).

Glucocorticoids have effects on bone metabolism that include suppression of osteoblast proliferation and reduced protein synthesis[141]. The reduction in protein synthesis by osteoblasts is probably mediated by direct glucocorticoid receptor regulation of important osteoblast genes. This results in loss of bone mass at trabecular sites and a propensity for fracture. Surprisingly, we have not found any previous studies addressing how these biological effects might interact with processes in osteoarthritic peri-articular bone. It may be reassuring that studies of IACS for knee OA, including a 2-year intervention[1], make no note of any adverse effect on bone health[26]. Nevertheless, the biologic effects of corticosteroids on bone, together with epidemiologic observations on the relationships of bone density with progression of OA, indicate that effects of IACS on bone health need to be evaluated. This proposed study will incorporate measures of peri-articular and distant bone mineral density using DXA, and trabecular morphometry using MRI.

2.14 Ultrasonographic Screening for Synovitis in Osteoarthritic Knees

Clinical and radiological characteristics are poor predictors of inflammation in knee OA[142]; ultrasonography and MRI both have the capability to demonstrate synovial changes[34, 143, 144]. Of these two modalities, ultrasonography has greater clinical utility as a screening tool because of portability, lower cost and increasing deployment in rheumatology practice. These factors make it a more desirable technology than MRI to evaluate knee OA synovitis in a clinical setting, especially since the probable outcome of the proposed trial is a therapeutic strategy (IACS) that is contingent on screening for the presence of knee joint synovitis.

In general, musculoskeletal ultrasonography has high sensitivity and specificity in the detection of joint inflammation[145, 146], and is sensitive to change induced by therapeutic interventions[147-149]. Ultrasound may be slightly less predictive than MRI in the detection of synovial thickening[150]. One study compared B-mode and power Doppler ultrasound to *contrast-enhanced* MRI -effusion or synovial thickening was detected by B-mode ultrasound in 58%, power Doppler in 63% and contrast-enhanced MRI in 82%[151]. Using MRI as the reference standard there was a sensitivity of 72% for effusion in the superior recess and 81% for effusion in the lateral recess. There have been a couple of pathological validation studies of ultrasonographically-detected synovitis. The first showed that power Doppler sonography can reliably measure the extent of vascularity in the synovium of knee joints, with correlations between power Doppler and pathological grading of vascularity of ~0.8[152]. Another compared ultrasound assessments of synovitis (classified according to the intensity of the power Doppler signal) against histopathology in 3 OA and 7 rheumatoid knees[153]. The power Doppler ultrasound synovitis score correlated with synovial tissue cell infiltrate (r~0.6), synovial lining layer thickness (r~0.6), and vascularity (r~0.6).

The primary role of the ultrasonography assessment is to enrich the sample with participants who have synovitis using a technology that can be easily deployed in clinical practice; a role for which its performance characteristics are sufficient.

2.15 MRI Assessment of Synovitis in Osteoarthritic Knees

There is currently no clear consensus on the optimal MRI approach for assessment of synovitis in clinical studies[154-156]. While non-enhanced MRI has been used in epidemiologic and clinical studies to classify synovitis in osteoarthritic knees[17, 47, 49, 50, 150, 157-159], this was generally confined to measurement of signal intensity in Hoffa's fat pad. Histological validation of this approach appears to be limited to only two cases in a series of nine patients[47, 50]. Although the performance of this approach may be adequate for large-scale studies, it is uncertain that it is suitable for following synovitis in a clinical trial.

Other studies indicate that *gadolinium-enhanced* MRI offers greater specificity in the detection of synovitis[50, 160], and there seems to be a greater enthusiasm for use of this technique[154-156]. However, a number of factors militate against its use in the proposed study. Firstly, intravenous gadolinium confers a risk of severe complications[161] that may be unacceptable in the context of a OA research study. Secondly, contrast enhancement appears to diminish sensitivity to change of cartilage volume measures[162], the primary structural outcome in this study. Thirdly, the procedure carries substantial participant and staff burden and adds to the cost of the endeavor. While the opportunity will exist to score our MRIs for synovitis, especially if clarity emerges about how best to use non-enhanced images for this purpose, we will not view this as a primary measure of this covariate.

2.16 Appeal and Validity of MRI in the Assessment of Knee OA Structural Progression

Radiographs have been widely used to measure the severity of knee OA but their ability to provide precise, reproducible and valid measurement of the structures of interest (cartilage, synovium, bone) is limited. MR, on the other hand can image soft-tissue structures, provide insight into the tissue characteristics, and indicate the state of subchondral trabecular bone[163]. Recent MRI studies of knee OA have revealed wide-ranging damage to soft-tissue structures[164, 165], and have revealed pathologies such as bone marrow lesions and synovitis[17]. A particular appeal of MR is the inherent digital acquisition permits quantification of signal intensities from discrete structures, generation of 3D representations of individual anatomic structures, and computation of their volumes.

An OMERACT-OARSI workshop on imaging technologies published a comprehensive review of the MRI data on (1) pulse sequences for morphological analysis of articular cartilage (2) techniques for segmenting cartilage (3) semi-quantitative scoring of cartilage status and (4) technical validity, precision and sensitivity to change of quantitative measures of cartilage morphology[30]. Among their conclusions was that quantitative assessment of cartilage morphology using fat-suppressed gradient echo sequences and appropriate image analysis techniques, displays high accuracy and adequate precision (e.g., root-mean-square standard deviation medial tibia=61 μm^3) for longitudinal studies in OA patients. Longitudinal studies show changes in cartilage volume of the order of -4% to -6% occur per annum in OA in most knee compartments (e.g., -90 μm^3 in medial tibia). Annual changes in cartilage volume exceed the precision errors and appear to be associated with clinical symptoms as well as with time to knee arthroplasty.

Cartilage volume and thickness measurements have been validated in at least nine studies using cadaveric knees or patient specimens from arthroplasty[166-173]. Most of these document a close linear relationship between the two measures with differences in the range of 5 – 10%. MRI cartilage volume and thickness measurements obtained prior to arthroplasty have been validated against actual cartilage volume from the resected specimen[174]. Cartilage volume assessments were lower using MRI (range 7-27%) but there was a high correlation between the two measures (r=0.98, standard error 7%). This study provides estimates of absolute cartilage volume loss, e.g. mean medial tibia cartilage loss in patients with medial compartment OA was 1290 mm^3 [174].

The reproducibility of cartilage volume measurements appears to be excellent[30]. Raynauld et al demonstrated inter-reader ICCs of 0.986-0.995 for total cartilage volume, and intra-reader ICCs of 0.99[175]. We have also examined the reproducibility of *longitudinal* measurement of cartilage volume loss over a 2-year period and demonstrated intra-reader ICCs of ~0.93.

MRI assessments of OA severity appear clinically relevant and have discriminative validity. Comparison of cartilage volume measured by MRI with severity of radiographic joint space narrowing showed a mean reduction in tibial cartilage volume of 1.00 ml (s.d. 0.32) in the medial compartment and 0.53 ml (s.d. 0.25) in the lateral compartment each increment in joint space narrowing score[176]. Estrogen-users were found to have more cartilage than non-users (adjusted difference = 0.30 ml, 95% confidence interval 0.08 - 0.52)[177]. MRI can detect in-vivo deformation of patellar cartilage shortly after joint loading[178], diurnal variation in cartilage thickness[179], and is more sensitive in assessment of OA progression than radiography and arthroscopy[136]. Cartilage volume loss also predicts symptoms[180] and arthroplasty[181].

Currently there are at least thirteen publications describing the measurement characteristics of this approach among clinical cohorts with knee OA followed for periods up to three years[182-191]. With one exception[187], these show progressive cartilage volume loss with a mean value in the tibia, or medial tibia, of ~5%, s.d. of ~5%, range between 3.8 – 7.4%. One study detected cartilage volume loss in the absence of change in joint space width, confirming that MRI has greater sensitivity to change than radiography[186].

A number of semi-quantitative scoring methods for whole-organ OA and cartilage status have been developed[192-196]. These have shown adequate reliability, specificity and sensitivity, and ability to detect lesion progression over 1-2 years[30]. Intra-class correlation coefficients for components of these scales are in the range 0.59 – 0.92, with the most reliable assessments produced for osteophytosis (0.91)[192, 194].

2.17 Peri-articular Trabecular Morphometry in Knee OA using MRI

The importance of trabecular micro-architecture as a determinant of bone strength prompted a number of investigators to develop and validate an approach to measurement of trabecular parameters using MRI[163, 197-199]. MRI trabecular morphometry has been validated against trabecular bone using vertebral, femoral, and calcaneal specimens as a gold standard[200-202], and against bone mechanical properties[202]. MR trabecular parameters are reproducible[163], correlate closely with those derived from optical images of the bone specimens[201], and can discriminate between postmenopausal osteoporotic and non-osteoporotic women[203]. MRI trabecular morphometry has been evaluated in clinical studies of knee OA[163], and we are currently deploying it in the Osteoarthritis Initiative (OAI)[204] (see Section C.6). As an indication of its *discriminative validity*, the apparent trabecular bone volume fraction and apparent trabecular spacing differs between OA and non-OA knees, varies within knees reflecting the localization of OA[163], and relates to articular cartilage degeneration[198], and correlates with subchondral BMD.

3. Study Objectives

This study is a two-year stratified and block-randomized double-blind, placebo controlled clinical trial of the effect of intra-articular triamcinolone 40 mg injectable suspension, administered every 3 months over two years (eight doses). The randomization will be stratified by the baseline radiographic severity of knee OA (Kellgren and Lawrence grade of 2 or 3). An interim analysis will be conducted after the first half of participants has completed the trial. This interim analysis will allow the trial to be stopped early for either success or futility, or allow the trial to continue if neither success nor futility has been established.

The primary structural outcome objective in this study is cartilage volume loss; secondary structural outcomes include peri-articular bone marrow lesions (BML), attrition, tibial peri-articular bone density ratio (tsBMD), and apparent bone volume fraction (aBVF). The primary clinical outcome objective is the pain domain of the WOMAC; secondary clinical outcomes include WOMAC stiffness and function scores,

physical function tests and healthcare utilization (medication use, physician visits for knee OA, and arthroplasty).

4. Investigation Sites

This study will be a single site study performed at Tufts Medical Center.

5. Duration of Study

The entire study will run over five years (a study timeline can be found in Appendix A). For each study participant participation is twenty six months. **In this study a month is defined as 28 days**.

6. Study Sample

6.1 Number of Study Participants

The number of study participants to be randomized for this study is 140. Randomized participants who withdraw study participation or who are prematurely terminated will not be replaced.

6.2 Primary Diagnosis

Study participants will have a diagnosis of knee OA as defined a) chronic knee discomfort, b) tibiofemoral or patellofemoral OA by radiograph, c) clinical exam confirming pain/discomfort referable to the knee joint.

6.3 Inclusion Criteria

- Female or Male, Age ≥ 45 years
- Chronic knee discomfort based on affirmative response to the question "During the past 12 months, have you had any pain, aching, or stiffness in or around your knee(s) on most days for at least one month?"
- Baseline (Month 0) Pain score ≥2 on at least one of the WOMAC weight-bearing pain questions; and total weight-bearing pain score ≤8
- Tibiofemoral or patellofemoral OA on posterior-anterior weight-bearing semi-flexed or lateral knee radiographs with severity equivalent to Kellgren and Lawrence grade 2 or 3
- Evidence of synovitis on ultrasound at screening
- Clinical examination confirming knee pain or discomfort referable to the knee joint
- Prepared to discontinue NSAID(s)/analgesic(s) for 2 days prior to each assessment

6.4 Exclusion Criteria

- Prior septic (study) knee joint
- Prior reconstructive surgery in the study knee
- Prior osteonecrosis (avascular necrosis of bone)
- Chronic use of oral corticosteroids; knee intra-articular corticosteroid injection within 3 months of Month 0 (baseline) visit
- Ongoing use of doxycycline, indomethacin, glucosamine and/or chondroitin; or use of these within 2 months of Screening visit
- Evidence of other inflammatory joint disease (e.g., gout, CPPD)
- Serious medical conditions or impairments that, in the view of the investigator, would obstruct their participation in the trial such as uncontrolled diabetes, uncontrolled hypertension, opiate dependency
- Plan to permanently relocate from the region, or take an extended vacation for greater than 3 months during the trial period
- Planned arthroscopy and/or arthroplasty in the study knee.

- Any contra-indication to having an MRI

6.5 Selection of the Study Knee In The Event That Both Are Eligible

- If both knees are eligible, then the more symptomatic knee will be selected (greater WOMAC pain subscale score)
- If both knees have equivalent WOMAC pain subscale scores, the knee with a greater K/L grade will be chosen
- Finally, if both the WOMAC pain subscales scores and K/L grades are equivalent for both knees, the study knee will be randomly assigned

7. Study Intervention

7.1 Investigational Product

Triamcinolone Acetonide (i.e., Kenalog-40mg/mL for injection)
Active Ingredient: Triamcinolone Acetonide - 40 mg/mL
Excipients: Benzyl Alcohol - 0.9%; Carboxymethylcellulose Sodium - 0.75%; Polysorbate 80 - 0.04%; may contain Sodium Hydroxide to adjust ph; may contain Hydrochloric Acid to adjust ph; Sodium Chloride
pH: 5.0-7.5
Form: Injection suspension

Manufacturer: Bristol-Myers Squibb
P.O. Box 4500
Princeton, NJ 08543-4500
Business hours: www.bms.com (web site)
Business hours: phone (800) 321-1335 (drug information)
Business hours: fax (609) 897 6663
Product ID: 2150509
AAPCC Code: 132000-Corticosteroids

7.2 Placebo Product

0.9% Sodium Chloride Injection (sterile, nonpyrogenic, isotonic solution of sodium chloride and water for injection)
Active Ingredient: Sodium chloride 9mg/mL
Excipients: The solution may contain hydrochloric acid or sodium hydroxide for pH adjustment. Sodium Chloride, USP is chemically designated NaCl, a white crystalline compound freely soluble in water.
How supplied: 0.9% Sodium Chloride Inj. Single-dose Fliptop Vial 10 mL that contains no bacteriostat or antimicrobial agent or added buffer. Sodium Chloride, USP is chemically designated NaCl, a white crystalline compound freely soluble in water.
mOsmol (calc): 0.308/mL
pH: 5.3 (4.5 to 7.0)
Storage requirement: 20 to 25°C (68 to 77°F). [See USP Controlled Room Temperature.
Supplier: Hosperia, Inc., Lake Forest, IL 60045 USA
List Number: 4888

7.3 Investigational Product and Placebo Storage

Both the investigational product (IP) and placebo will be stored in the Research Pharmacy at Tufts Medical Center according to manufacturer storage instructions. Intra-articular injections of IP and placebo will be prepared by the research pharmacy and will be blinded to the research team members.

7.4 Investigational Product and Placebo Preparation

Syringes will be pre-filled by the research pharmacist with triamcinolone acetonide or saline according to the randomization code kept in the research pharmacy. The syringe contents will be masked by the research pharmacist prior to transportation using opaque labels and an opaque three-way stopcock (3). A 0.2 ml airlock will ensure that no contents are visible in the syringe tip. The needle will be affixed with the syringe in a vertical orientation so that the airlock migrates upwards to the plunger and ensures full delivery of the medication.

7.5 Dosage and Administration

Participants will receive either triamcinolone acetonide 40 mg/mL or matching 0.9% normal placebo 1mL intra-articularly (IA) to the index knee every 12 weeks for 8 doses.

IA injections will be performed by a rheumatologist with ultrasound guidance for needle placement. After introduction of the needle into the joint cavity the ultrasound machine will be switched off so that the medication flowing into the joint is not identifiable on the screen, thus maintaining the study allocation blind.

7.6 Duration of Treatment

Each randomized participant will receive a total of eight doses of intra-articular triamcinoline actinide 40 mg/mL or placebo every 12 weeks over a 2-year period (eight scheduled IA injections). In this study a month is defined as 28 days.

7.7 Warnings and Precautions

7.7.1 Triamcinolone

Joint sepsis is a primary concern in relation to intra-articular injection. However, while its incidence is difficult to measure with certainty, it is clearly very infrequent. A 1999 estimate from a retrospective survey in France suggests an incidence rate of 13 per million injections, lower with pre-packaged syringes[89]. Concordant with this low estimate, a survey of all cases of septic arthritis incident between 1982 to 1991 in Nottingham, UK (which had a base population of 600,000) identified only 3 cases attributable to IACS[90].

More frequent, but of less medical concern, are local aseptic inflammatory reactions to IACS injections[91]. These self-limiting events have been reported with rates up to 24% in some series[73-75, 92]. The biologic basis for these reactions is uncertain but may relate to the crystalline nature of the injected corticosteroid formulations.

There is systemic absorption of steroid injected into a joint. Peak plasma levels after an intra-articular injection of 80 mgs methyl prednisolone are in the order of 169 ng/ml, achieved 8 hours post injection[93]. A measurable transient effect on the pituitary axis is apparent with injections of 40 mg of methyl prednisolone[94] or >40 mg triamcinolone diacetate[95]. However, it seems unlikely that this has any longterm effects at the frequency planned in this study (3 months). Problems with the pituitary axis were also apparently not encountered in an RCT in which IACS were administered every 3 months over a 24 month period[1]. While links have been made between IACS and various systemic features (immunosuppression[96], impaired diabetes control[97, 98], flushing, menstrual irregularities[99]) substantial data quantifying their frequency is generally lacking[83, 100]. One study provided data showing elevation in blood pressure and blood glucose levels following epidural corticosteroid injections, but the doses used were greater than those proposed in this study[97]. Anaphylaxis after IACS has been rarely reported[101, 102], as has steroid psychosis[103].

The most systematic evaluation of adverse effects from IACS was performed by Bellamy, in a Cochrane review of their efficacy for OA26. He found that side effects were uncommon and noted no reports of serious adverse events among the trials reviewed. Reported adverse events included post-injection flare,

crystal-induced synovitis, tissue atrophy, fat necrosis, calcification, joint sepsis, steroid arthropathy, avascular necrosis, hematoma, fluid retention, hyperglycemia and hypertension.

Pregnancy Category: Rating C (FDA) Studies in animals have revealed adverse effects on the fetus (teratogenic or embryocidal or other) and there are no well-controlled studies in women, or studies in women and animals are not available. Drugs should be given only if the potential benefit justifies the potential risk to the fetus.

Participants will be given a handout post injection of post procedure care and signs and symptoms that require medical attention.

7.7.2 0.9% Sodium Chloride (placebo)

Though reactions to a physiological fluid are not expected, adverse events could occur because of the technique of administration or contamination of the fluid. These could result in a febrile response, local tenderness, abscess, tissue necrosis, thrombosis or infection at the site of injection. Based on the data described above, the likely incidence of such events is extremely low.

Participants will be given a handout post injection of post procedure care and signs and symptoms that require medical attention.

8. Procedures and Monitoring

8.1 Randomization

Randomization will be stratified by baseline radiographic severity of knee OA at baseline (Kellgren and Lawrence grade of 2 or 3). Specially designed software will be used to generate the random numbers. The statistician will review lists prior to release and will keep a copy of the randomization list in a locked filing cabinet. The randomization list will be released to the research pharmacist at Tufts Medical Center who will be unblinded to participants' treatment allocation / randomization.

8.2 Good Clinical Practice (GCP) and Monitoring

This study will be performed under U.S Code of Federal Regulations (CFR 21), International Committee on Harmonization (ICH) and Good Clinical Practice (GCP) guidelines. The protocol, informed consent form (ICF), and recruitment materials will be submitted to the Institutional Review Board (IRB) of the Tufts Health Sciences Campus and Tufts Medical Center for review and approval. Annual continuing review of the project will be submitted to the IRB and any other governmental agencies as required.

The Tufts Medical Center IRB will be informed of any serious adverse events (SAEs) within 5 working days of knowledge of the event.

The investigator agrees to any inspections of study-related records by the National Institutes of Health (NIH), FDA, CDER, and / or Tufts Medial Center IRB and other regulatory bodies as required.

8.3 Electronic Data Capture

We will use electronic data capture (EDC) for Case Report Form (CRF) completion and some source documentation. We will use for this our secure website, which is hosted on a TMC intranet domain.

8.3.1 Security of the Electronic Data

Our web server is located within the Tufts Medical Center domain and so is inaccessible from the general internet. The TMC intranet is highly protected by its architecture, firewalls and virus software, and user

authentication protocols. Within this structure, our website will have an additional layer of user authentication using high level time-expiring passwords. We will also operate user-type permission levels to constrain access to the different functions and data (role-based security). We will encrypt in the database highly sensitive information such as passwords. As part of the institutional network protections and maintenance, our database will regular back-ups (at least daily), which will ensure that if any data can be recovered. Finally, each software component will be updated regularly by our IT group. Patches and other updates on servers are done daily to ensure that Microsoft's vulnerabilities can be identified and solved quickly.

8.3.2 HIPAA

In order to be compliant with Health Insurance Portability and Accountability Act (HIPAA) requirements, we will operate the following additional procedures:

1. Database normalization. Using a practice known as standard normalization of data, participant names will not be stored in the same database table as their contact information. It is through this framework that two database tables will need to be queried in order to link a participant's name with their protected health information (PHI)
2. use of encryption for sensitive information such as passwords
3. Database audit trails. Deletions and major changes made to a participants stored information will be tracked automatically
4. Any paper records that we store (e.g. consent forms) will be kept in our secure office environment protected by two levels of lockage (e.g. in a locked filing cabinet in a locked office area)

8.4 Consents

8.4.1 Informed Consent

Prior to study participation, each potential participant will be provided with an informed consent form (ICF) to read. At the first screening visit each potential participant will have a discussion about the study requirements and expectations with the principal investigator (PI) or a sub-investigator (Sub-I). If the potential participant agrees to the study requirements and expectations then the ICF will be signed and dated by both the participant and the PI or Sub-I. A copy of the signed ICF will be given to the participant and the original kept in the research files in the Division of Rheumatology.

Any amendments that warrant changes to the ICF will be submitted to the IRB for review and approval. Any new version of the ICF will be presented to the active participants at their next study visit and discussed. The participants will then decide if they want to continue their study participation. If they choose to continue to participate then the participants will sign the new ICF. A copy of the signed ICF will be given to the participant.

8.4.2 Research Authorization Form

To comply with the Health Insurance Portability and Accountability Act (HIPAA) of 1996 each participant will provide written permission to allow their personal/protected health information (PHI) to be disclosed within the context of this research project.

A valid Research Authorization Form (RAF) is an individual's signed permission that allows a covered entity, in this study Tufts Medical Center, to use or disclose the individual's PHI for the purposes and to the recipients stated in the RAF. When a research authorization is obtained, the Privacy Rule requires that it pertain only to a specific research study, not to future, unspecified study projects.

Once the RAF is signed, the participant will be given a copy of the signed RAF. Participants that choose not to sign a RAF will be excluded from the study.

A copy of the RAF will be submitted to and filed in the IRB.

8.4.3 E-Mail

Research participants desiring to communicate with research team members during the study shall provide written consent as per Tufts Medical Center's guidelines for electronic mail communication of protected health information.

8.4.4 Photography/Video-Recording

Research participants who are willing to participate in the gait analysis portion of the study will be presented with a photography/video consent form.

Participants who do not wish to sign the photography/video-recording consent form will not be excluded from participation into the study.

8.5 Observations and Measurements

8.5.1 Study Visits

All efforts will be made to keep the time of participants' study visits consistent (i.e., all a.m. or all p.m.) throughout the study to minimize intra-patient variability. Additionally, keeping a participant's study visits at approximately the same time will be important to laboratory specimen collection and MRI scanning. Laboratory collections and MRI scans done at approximately the same time for each participant will decrease biorhythm variability in the laboratory samples and MRI scans. The time of blood draw and urine collection will be noted at each visit, as well as the time of participant's last meal. Participants who will be having an MRI scan will be asked to refrain from strenuous physical activity the day before their scheduled MRI scan.

Beginning at Month 0, the study visit window will be +/- 1 week however, MRI will have up to -2 weeks prior to Month 0, 12, and 24. The visit schedule and procedures that will occur at each visit are summarized in Appendix B.

Month -1; Screening

This is the initial screening visit. Prior to any research procedures being done informed consent will be obtained. The remaining procedures may be done at this visit:

- Obtaining informed consent and research authorization (and other applicable consents, e.g., e-mail communication, photography/video-recording) from the potential participant
- Participant WOMAC pain subscale – Right knee
- Participant WOMAC pain subscale – Left knee
- Collection of demographic data
- Collect rheumatic history, include type, duration, and treatment history of OA as well as notation of any known complications of disease or therapies
- Medical & surgical history to include past medical history, secondary diagnoses, concomitant medications, all medications and treatments taken within the last two months
- Bilateral knee examinations
- MRI coil fit check
- Knee x-rays – bilateral, weight bearing, semi-flexed, posteroanterior (PA)
- Bilateral knee ultrasound scans
- Laboratory studies (which will be analyzed in real-time to determine eligibility)
  - Hemoglobin A1c
  - 25(OH)D
  - Rheumatoid factor, anti-CCP and hsCRP
- Provide and teach use of participant diary/calendar/journal

Month 0 Randomization (+/- 1 week window)

This is the first visit of the double-blind treatment period. Only those participants that fulfill inclusion/exclusion criteria will be randomized. The following procedures will be done at this visit:

- Review of informed consent – study requirements and expectations
- Height/Weight
- Participant WOMAC, SF-36, HAQ, global assessment
- Randomization
- Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences
  - Re-educate about diary/calendar/journal
  - Present and discuss calendar of target/ideal visit dates and times for Months 3-24 visits
- Knee examination
- Participant physical function tests (chair stand, 20 meter walk)
- Laboratory studies / collections
  - Hemoglobin A1c
  - Serum CrossLaps
  - Serum for archive
  - Plasma for archive
  - Urine CrossLaps
  - Urine for storage
  - White Blood Cell (Buffy Coat) archive
  - Synovial fluid (if obtained) for cell count, crystal exam
  - Synovial fluid (if obtained) for archive
- MRI of study knee (this exam will be done up to two weeks prior to Month 0 to review for any exclusionary criteria)
- DXA – bilateral hip, knee and lumbar spine
- Knee ultrasound exam
- Knee injection of triamcinolone or placebo by ultrasound guidance

Month 3 (+/- 1 week window)

The following procedures will be done at this visit:

- Participant WOMAC, HAQ, global assessment
  - Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences
  - Analgesia/NSAID accounting
- Laboratory studies
  - Hemoglobin A1c
  - Synovial fluid (if obtained) for cell count, crystal exam
  - Synovial fluid (if obtained) for archive
- Knee injection of triamcinolone or placebo by ultrasound guidance

Month 6 (+/- 1 week window)

The following procedures will be done at this visit:

- Participant WOMAC, HAQ, global assessment
- Participant physical function tests (chair stand, 20 meter walk)

- Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences
- Laboratory studies / collections
  - Hemoglobin A1c
  - Synovial fluid (if obtained) for cell count, crystal exam, and/or culture analysis
  - Synovial fluid (if obtained) for archive
- Knee ultrasound exam
- Knee injection of triamcinolone or placebo by ultrasound guidance

Month 9 (+/- 1 week window)

The following procedures will be done at this visit:

- Participant WOMAC, HAQ, global assessment
- Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences
- Laboratory studies / collections
  - Hemoglobin A1c
  - Synovial fluid (if obtained) for cell count, crystal exam
  - Synovial fluid (if obtained) for archive
- Knee injection of triamcinolone or placebo by ultrasound guidance

Month 12 (+/- 1 week window)

The following procedures will be done at this visit:

- Height/Weight
- Participant WOMAC, SF-36, HAQ, global assessment
- Participant physical function tests (chair stand, 20 meter walk)
- Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences
- Knee examination
- Laboratory studies / collections
  - Hemoglobin A1c
  - Serum CrossLaps
  - Serum for archive
  - Plasma for archive
  - Urine CrossLaps
  - Urine for storage
  - White Blood Cell (Buffy Coat) archive
  - Synovial fluid (if obtained) for cell count, crystal exam
  - Synovial fluid (if obtained) for archive
- MRI of study knee (this exam will be done up to two weeks prior to Month 12)
- DXA – bilateral hip, knee and lumbar spine
- Knee ultrasound exam
- Knee injection of triamcinolone or placebo by ultrasound guidance

Month 15 (+/- 1 week window)

The following procedures will be done at this visit:

- Participant WOMAC, HAQ, global assessment
- Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences
- Laboratory studies / collections
  - Hemoglobin A1c
  - Synovial fluid (if obtained) for cell count, crystal exam
  - Synovial fluid (if obtained) for archive
- Knee injection of triamcinolone or placebo by ultrasound guidance

Month 18 (+/- 1 week window)

The following procedures will be done at this visit:

- Participant WOMAC, HAQ, global assessment
- Participant physical function tests (chair stand, 20 meter walk)
- Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences
  - Analgesia/NSAID accounting
- Laboratory studies / collections
  - Hemoglobin A1c
  - Synovial fluid (if obtained) for cell count, crystal exam
  - Synovial fluid (if obtained) for archive
- Knee ultrasound exam
- Knee injection of triamcinolone or placebo by ultrasound guidance

Month 21 (+/- 1 week window)

The following procedures will be done at this visit:

- Participant WOMAC, HAQ, global assessment
- Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences
- Laboratory studies / collections
  - Hemoglobin A1c
  - Synovial fluid (if obtained) for cell count, crystal exam
  - Synovial fluid (if obtained) for archive
- Knee injection of triamcinolone or placebo by ultrasound guidance

Month 24 (+/- 1 week window)

Month 24 is the final visit of the double-blind treatment period. The following procedures will be done at this visit:

- Height/Weight
- Participant WOMAC, SF-36, HAQ, global assessment
- Participant physical function tests (chair stand, 20 meter walk)
- Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences

- Knee examination
- Laboratory studies / collections
  - Hemoglobin A1c
  - Serum CrossLaps
  - Serum for archive
  - Plasma for archive
  - Urine CrossLaps
  - Urine for storage
  - White Blood Cell (Buffy Coat) archive
  - Synovial fluid (if obtained) for cell count, crystal exam
  - Synovial fluid (if obtained) for archive
- MRI of study knee (this exam will be done up to two weeks prior to Month 24)
- DXA – bilateral hip, knee and lumbar spine
- Knee ultrasound exam

Premature Termination / Early Termination

A participant may choose with withdraw his or her study participation, or be withdrawn from the study by the investigator. The following procedures, as applicable, should be done:

- Height/Weight
- Participant WOMAC, SF-36, HAQ, global assessment
- Participant physical function tests (chair stand, 20 meter walk)
- Collect and review diary
  - Concomitant medications
  - Adverse experiences
- Knee examination
- Laboratory studies /collections
  - Hemoglobin A1c
  - Serum CrossLaps
  - Serum for archive
  - Plasma for archive
  - Urine CrossLaps
  - Urine for storage
  - White Blood Cell (Buffy Coat) archive
  - Synovial fluid (if obtained) for cell count, crystal exam
  - Synovial fluid (if obtained) for archive
- MRI of study knee
- DXA – bilateral hip, knee and lumbar spine
- Knee ultrasound exam

Safety Follow-Up

One month (28 days) following final study visit, certain participants will be contacted to follow-up on any adverse experiences that were noted at Month 24 (or premature termination) visit.

8.5.2 Laboratory

Tufts Medical Center will analyze blood samples for Hemoglobin A1c, Vitamin D, i.e., (25(OH)D, Rheumatoid factor, anti-CCP and hsCRP.

8.5.3 Radiology

Bilateral knee radiographs will be collected on all potential participants at screening. Participants will be screened for evidence of tibiofemoral OA with a Kellgren and Lawrence (K/L) score of 2 or 3, as evidenced on bilateral, PA semi-flexed, weight bearing knee x-rays. X-rays are obtained using a Synaflexor Positioning Frame (Synarc, Inc. San Francisco, CA). X-rays will be evaluated for K/L score and joint space width (JSW) measurements.

8.5.4 Knee MRI

MRIs for this protocol will be done in the Department of Radiology (MRI suite) at Tufts Medical Center under the direction of Dr. R. Ward, utilizing a Phillips Achieva X-Series 3.0 Tesla scanner. This scanner includes the SmartKnee technology, an automated algorithm that ensures replication of the original parameters and positioning (and hence reproducibility) of repeat scans210. Participants will have three MRIs of their study knee; at Month 0, 12, and (or PT/ET if indicated).

8.5.5 Knee Ultrasonography

Ultrasound examination of the knee will be performed in the Rheumatology Outpatient Clinic at Tufts Medical Center under the direction of Dr. J. Yinh. Participants will have five ultrasound knee exams of their study knee; at Month –1 (screening), 6, 12, 18, and 24 study visits. All IA injections will be done under ultrasound guidance to verify needle placement in the joint space.

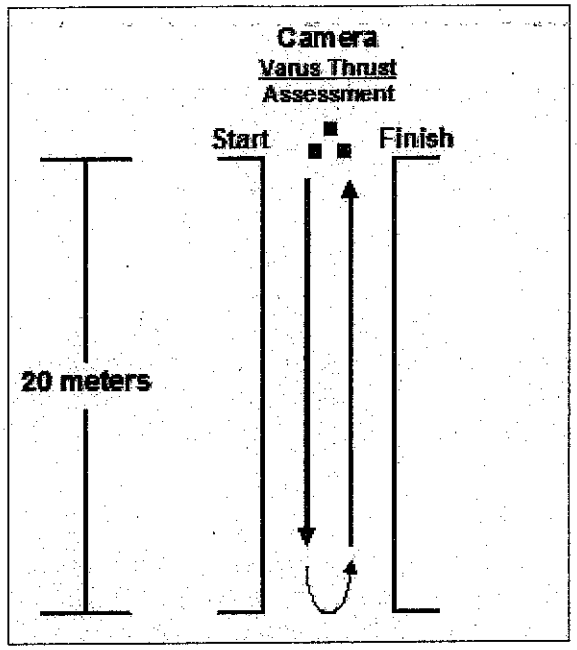
8.5.6 DXA (Bone Density Testing)

Bilateral hip, study knee and lumbar spine DXA scans will be done in the Rheumatology Outpatient Clinic at Tufts Medical Center. These measurements will be obtained using the GE Lunar Prodigy Advance machine dedicated for this study. Randomized participants will undergo three DXA studies; Month 0, 12, and 24. The weight and height measurements obtained at the time of each DXA exam.

8.5.7 Observed Gait Analysis

In order to obtain dynamic gait assessments, including varus thrust, we will perform observational gait analysis (OGA). We will video record the participants walking barefooted 20 meters down a corridor and turning around to walk another 20 meters back, at the participant's chosen speed.

OGA will be obtained on consenting participants at Month 0, 12, and 24 study visits. Subjects who do not want to be videotaped may opt out of this portion of the protocol. Similarly, participants who require the use of a walking aid will be excluded from this portion of the protocol while still being able to participate in the remainder of this study.



8.6 Participant Compliance

8.6.1 Investigational Product

Compliance of the IP will be ensured as the participant is either receiving the IACS or the IA placebo of Normal Saline at each study visit.

8.6.2 Study Visits

It is expected that randomized participants will complete all study visits. Participants missing visits will be contacted to reschedule missed appointments.

8.7 Concomitant Medications

The use of concomitant medications is allowed, **except** for the following:

- Other investigational drugs
- MMP-like medications (i.e., tetracyclines or related compounds e.g., Doxycycline)
- Chronic glucocorticoid use
- Glucocorticoid intra-articular injections
- Hyaluronic acid formulation intra-articular injections

At the screening visit all concomitant medications (prescription, over-the-counter, and complementary alternative medicines (CAMs)) taken within the previous 90 days will be recorded. The name, dose, frequency, and start/stop dates will be noted. Throughout the study all concomitant medications will be recorded in similar fashion.

8.7.1 Washout Period

Participants will be asked to discontinue use of any analgesics (NSAIDs, opiods) two full days prior to each pain assessment (including the baseline eligibility evaluation) and take only acetaminophen (up to 3 grams/day) if needed. If acetaminophen is consumed in the two days prior to the study visit, they will be asked to record this in their study calendar/journal/diary.

8.8 Adverse Experiences/Events (AE)

8.8.1 AE Definitions

Adverse Event: An adverse event is any condition that appears or worsens after the initiation of investigational agent (IACS or placebo) and described as an undesirable and unintended event, although not necessarily unexpected.

Adverse events will be classified using the National Cancer Institute's (NCI) Common Toxicity Criteria as follows:

Unrelated:	The AE is clearly not related to the investigational agent
Unlikely:	The AE is doubtfully related to the investigational agent
Possible:	The AE may be related to the investigational agent
Probable:	The AE is likely related to the investigational agent
Definite:	The AE is clearly related to the investigational agent

Adverse event severity likewise will be scored according to NCI Common toxicity Criteria as follows:

Grade 1 is mild
Grade 2 is moderate
Grade 3 is severe
Grade 4 is life threatening
Grade 5 is death related to the adverse event

8.8.2 AE Reporting

Grade 1 and 2, non-serious, adverse events will be summarized and reported to the Data Safety Monitoring Board (DSMB) and to the Tufts Medical Center IRB annually. These events may be reported more frequently if requested by the DSMB or Tufts Medical Center IRB.

8.8.3 Serious Adverse Event (SAE) Reporting

Investigators are required to report serious or unexpected adverse events, which occur during the course of a human research study to the Tufts Medical Center IRB within five (5) working days of learning of the event occurrence.

A serious adverse event is defined as follows:

1. All deaths (if known), whether or not they are attributable to research participation.
2. Events of a serious nature, even if not related to the study, for example, chest pain requiring hospitalization and/or treatment, MI, stroke, major surgery, etc.
3. Events that are identified in the consent form, but that are more severe and longer lasting than expected.
4. Events that are not expected to occur during the course of the study and that are not identified in the consent form.

8.8.4 AE / SAE Monitoring

An independent Data Safety Monitoring Board (DSMB) will be assembled under the direction of KAI Research, Inc. (under contract with NIAMS). KAI will assemble the DSMB and the protocols for AE/SAE reporting and monitoring.

Study participants will be monitored for occurrence of adverse events. This will be done pro-actively by (i) posing structured and open-ended questions at each visit to obtain information on undesirable

experiences and (ii) by exerting surveillance of all laboratory tests. Also, a telephone number will be provided to participants to enable them to report any such experiences at any time to study personnel. In the event that an adverse experience occurs outside of office hours, participants will be provided with hospital page operator to contact the rheumatology fellow on call.

8.9 Early Withdrawal/Termination of Participants

Every effort will be made to keep enrolled participants in the study since it has an 'intent-to-treat' design and withdrawals / early terminations / 'drop-outs' will not be replaced.

Participants may be prematurely terminated from the study for the following reasons:

- An adverse experience with such severity that continuation in the study is unsafe
- Failure to keep study appointments
- Relocation such that the participant is not able to travel to Tufts Medical Center
- Participant personal reasons
- Lost to follow-up after all reasonable attempts to locate the participant for early termination visit
- Joint replacement surgery, osteotomy, arthroscopy, arthrocentesis or other invasive procedure on the study knee

9. Computational Analyses

Hypotheses 1a,b: Knees with OA and synovitis treated with IACS for 2 years will exhibit (a) slower rates of cartilage volume loss (our primary *structural* outcome) (b) less progression in bone marrow lesions and subchondral bone attrition.

Hypotheses 2a,b: The treated knees will exhibit less change in (a) tibial subchondral bone mineral density and (b) apparent bone volume fraction.

Hypotheses 3a,b,c: The group assigned to IACS will have less (a) overall pain (our primary *clinical* outcome), (b) decline in physical function and (c) healthcare utilization

We will perform descriptive analyses of baseline characteristics of the treated and placebo groups to permit assessment of generalizability and success of randomization. We will use either Student t-tests or rank-based nonparametric tests whenever appropriate. Our primary analytic approach will be to use intention- to-treat (ITT) analyses with mixed effects regression models for the longitudinal repeated measures data. We will also evaluate the dropout and adherence patterns of trial subjects to see if these issues may change the assessment of treatment effect.

Our analyses will evaluate the cartilage volumes and WOMAC pain scores over time using mixed effects regression models (incorporating both fixed and random effects) for longitudinal repeated measures to see if there is a difference in the trajectory of the structural and clinical outcome measures over time between the treatment groups[249]. The two co-primary endpoints are (i) cartilage loss (structural)

Table 4. Conceptualization of the study measures

	Structural outcome	***Clinical outcome***	***Covariate***
Primary	cartilage volume	WOMAC pain	knee alignment
Secondary	BML Score subchondral attrition tibial medial:lateral BMD ratio tibial subchondral aBVF tibial BMD femoral neck BMD	WOMAC function Physical function healthcare utilization	femoral neck BMD serum 25(OH)D (synovitis; US and/or MRI)

and WOMAC pain (clinical). The models will include a random intercept for each subject, 3 dummy variables corresponding to the 4 randomization strata (gender and radiographic severity of KOA at entry to the trial (Kellgren and Lawrence grades 2 vs. 3)), use of analgesia (acetaminophen) at the times of outcome measurement, a time effect, an effect of treatment, and the interaction between time and treatment that will provide the basis for testing hypothesis 1 (i.e. testing if the trajectories are different between treatment groups). The effect of time will be assumed to be linear in this model unless preliminary data analysis suggests that the cartilage volumes or WOMAC score have a distinctly non-linear trend over time. The compound symmetry variance structure will be assumed for the correlation between repeated measures over time within a subject. If the likelihood cannot be maximized for this model, then the covariance structure will be simplified by removing the random intercept. In secondary analyses we will evaluate whether the clinical conclusions drawn from this trial are sensitive to the choice of different modeling methods.

For secondary analyses, we will employ the Student-t test and the rank-based Wilcoxon test to evaluate the difference between treatment groups in the change in WOMAC scores or cartilage volume, as that is how these outcomes have often been analyzed. The LOCF approach will be used to address dropout from the trial in these analyses of change over the duration of the trial. Additional secondary analyses will use alternative regression modeling approaches, including ordinal logistic regression[250], and regression tree models[251].

The influence of exposures that might exert intermediary or confounding effects on the outcomes (e.g. knee OA severity, serum 25(OH)D, radiographic knee alignment, use of analgesia, and BMI) will be tested by performing stratified analyses and by entering them as covariates in the linear regression models (Table 4). The ways in which all these variables might interact are complex so we will interpret our results in respect of differentiating confounding from an intermediary role on the basis of biologically based a priori hypotheses.

10. Adaptive Interim Monitoring

For interim monitoring, we will use the adaptive approach due to Bauer and Köhne[6]. This approach to interim monitoring is based on the use of Fisher's method of combing p-values from separate experiments[252] rather than repeated analyses of accruing data, such as the Peto method. Fisher's method rejects a common null hypothesis based on two independent experiments with significance of 0.05 if $p_1 * p_2 \leq 0.0087$, where this cutoff is based on the chi-square distribution with 4 degrees of freedom. In this case, the p_1 would be the p-value for the null hypothesis of no treatment effect in the first half of the subjects, and p_2 would be the same for the second half of the trial subjects. Using this relationship, Bauer and Köhne develop cutoffs for p_1 would that would allow early stopping while maintaining a significance level of 0.05 for the product of the p-values. Using the values from their Table 1 we will stop the trial for success if $p_1 \leq 0.0233$. The trial would be stopped for futility if $p_1 > 0.5$. If p_1 satisfies neither of these conditions, then the trial would continue, and the second half of the subjects would be analyzed separately at the end of the trial for p_2; then the product of p_1 and p_2 would be compared to 0.0087. This adaptive method for interim monitoring allows us to use the mixed effects analysis as outlined in section E.15 for the primary outcome of cartilage volume; this will be performed on the first half of the trial subjects at the interim analysis, and, if the trial continues, separately on the second half of the subjects at the trial's end.

Bauer and Köhne note that the power loss due to the adaptive interim analysis is quite small when compared to the best single test on the complete data when the sub-samples are the same size, and when stopping for futility is only done when p_1 is at least as large as 0.5. In this setting they found 77.8% power for trials where a single complete analysis would have 80% power. In our simulations presented in section E.17 of the grant proposal, we found a slightly larger, but manageable, loss of power due to the adaptive interim analysis. The gain from this interim analysis is from the possibility of early stopping

of the trial, which would significantly reduce the number of MRIs that need to be aequired and read in the second half of the subjects, and the truncation of their treatment and follow-up. It would also allow early closing of the study and earlier dissemination of study results. In 60-70% of the simulations presented in section D.17, the studies would have been stopped early.

The interim analysis of cartilage volume (pain will not be evaluated in the interim analysis) will be conducted by the study statistician and presented to the DSMB for the trial. The DSMB will evaluate these results in light of the stopping rules above and advise whether the trial should continue or stop.

11. Investigator's Regulatory Obligations

11.1 Institutional Review Board

The study protocol, participant informed consent form, and any recruitment materials will be reviewed and approved by the Tufts Medical Center IRB prior to the start of participant recruitment.

11.2 Tufts Medical Center Rheumatology Outpatient Clinic

The study will occur at the Tufts Medical Center's Adult Rheumatology Outpatient Clinic.

11.3 Data and Safety Monitoring Board (DSMB)

NIAMS has developed guidelines for data safety monitoring board activities for NIAMS funded studies and has appointed KAI to assemble and administer a DSMB for this trial. Tufts Medical Center Division of Rheumatology will work with NIAMS and KAI Research, Inc in the formation of and procedures for data and safety monitoring.

DSMB meeting minutes will be copied and forwarded to the Tufts Medical Center IRB.

11.4 Records/Data Retention

Research records and data will be kept for a minimum of 2 years after study completion.

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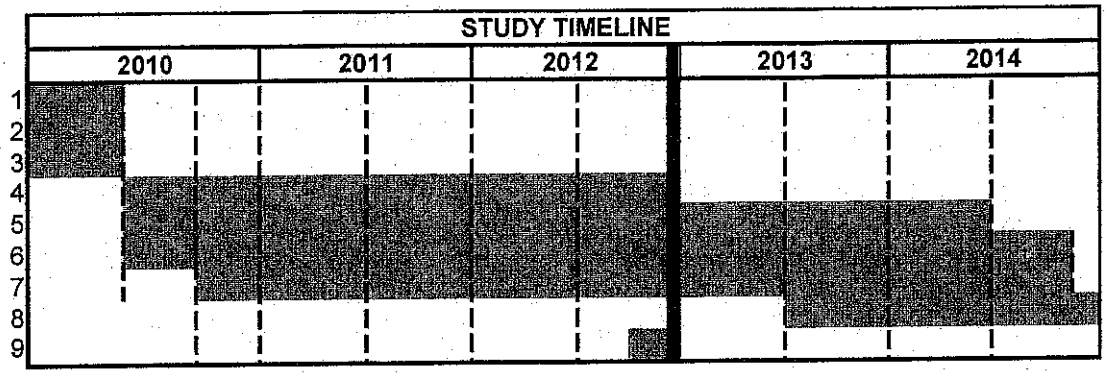
Appendix A:

Study Timeline



1. Development and testing of the website and electronic data capture systems
2. Construction of the manual of operations, assembly of DSMB, start-up DSMB meeting
3. Acquisition and deployment of study materials (e.g. injection kit, ultrasound scanner, medication)
4. Advertising and recruitment
5. Conduct of the clinical trial
6. Quality control of study DXA scans and MRIs
7. Interpretation, scoring of paired MRIs
8. Data processing, cleaning & analysis; presentation of findings; manuscript preparation
9. **Adaptive interim analysis**. Timeline for subsequent study activities will be truncated if the study is stopped early.

Appendix B

Schedule of Events and Examinations

When→ What ↓	Month -1 Screen	Month 0	Month 3	Month 6	Month 9	Month 12	Month 15	Month 18	Month 21	Month 24	Early Term (ET)*
Consents	X										
Medical-Surgical history	X										
Height & weight	X	X				X				X	X
Knee exam	X	X				X				X	X
Knee x-rays	X										
Knee ultrasound	X	X		X		X		X		X	X
MRI of study knee		X				X				X	X
Bone density test		X				X				X	X
Knee study injection		X	X	X	X	X	X	X	X		
Blood tests	X	X	X	X	X	X	X	X	X	X	X
Knee synovial fluid for storage		X	X	X	X	X	X	X	X	X	X
Blood collection (serum, plasma, and white cell) for storage		X				X				X	X
Urine collection for storage		X				X				X	X
Questionnaires	X	X	X	X	X	X	X	X	X	X	X
Physical function tests		X		X		X		X		X	X
Observed Gait Analysis (optional)		X				X				X	
Review all medicines	X	X	X	X	X	X	X	X	X	X	X
Review of adverse events / side effects		X	X	X	X	X	X	X	X	X	X
Review your study calendar/diary/journal		X	X	X	X	X	X	X	X	X	X

*Early Termination (ET) visit is performed only if the participant withdraws from the study prior to completion.

Appendix C

ACR Criteria for Classification of Idiopathic Knee Osteoarthritis

AMERICAN COLLEGE OF RHEUMATOLOGY
EDUCATION • TREATMENT • RESEARCH

[1986] Criteria for Classification of Idiopathic Osteoarthritis (OA) of the Knee*.

Clinical and laboratory	**Clinical and radiographic**	**Clinical†**
Knee pain	Knee pain	Knee pain
+ at least 5 of 9:	+ at least 1 of 3:	+ at least 3 of 6:
- Age > 50 years	- Age > 50 years	- Age > 50 years
- Stiffness < 30 minutes	- Stiffness < 30 minutes	- Stiffness < 30 minutes
- Crepitus	- Crepitus	- Crepitus
- Bony Tenderness	+ Osteophytes	- Bony Tenderness
- Bony enlargement		- Bony enlargement
- No palpable warmth		- No palpable warmth
- ESR <40 mm/hour		
- RF <1:40		
- SF OA		
92% sensitive	91% sensitive	95% sensitive
75% specific	86% specific	69% specific

* ESR = erythrocyte sedimentation rate (Westergren); RF = rheumatoid factor; SF OA = synovial fluid signs of OA (clear, viscous, or white blood cell count <2,000/mm^3).

† Alternative for the clinical category would be 4 of 6, which is 84% sensitive and 89% specific.

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Appendix D

Kellgren and Lawrence Radiographic Grading System for Osteoarthritis

Grade	Classification	Description
0	Normal	No features of OA
1	Doubtful	Minute osteophyte, doubtful significance
2	Minimal	Definite osteophyte, unimpaired joint space
3	Moderate	Moderate diminution of joint space
4	Severe	Joint space greatly impaired with sclerosis of subchondral bone

Appendix E

WOMAC Questionnaire

The Western Ontario and McMaster Universities OA Index (WOMAC), is a disease –specific instrument used to measure symptoms and physical functioning of patients with osteoarthritis of the hip and knee. It contains twenty-four items (5 pain, 2 stiffness, and 17 physical function) and takes approximately ten minutes for the participant to complete. The validity, reliability, and responsiveness of the WOMAC has been demonstrated in the OA population, and has become a standard instrument utilized in clinical studies.

SF-36v2™

The SF-36 is a multi-purpose, short-form health survey consisting of 36 questions. It yields an 8-scale profile of functional health and well-being scores as well as psychometrically-based physical and mental health summary measures and a preference-based health utility index. It is a generic measure, as opposed to one that targets a specific age, disease, or treatment group. Accordingly, the SF-36 has proven useful in surveys of general and specific populations, comparing the relative burden of diseases, and in differentiating the health benefits produced by a wide range of different treatments.

Stanford Health Assessment Questionnaire

The Standford Health Assessment Questionnaire (HAQ) is a well-validated and widely used instrument that originated from work on rheumatic disease populations and collects data on domains of disability, pain, adverse treatment effects and healthcare costs[37]. The elements that we will utilize for this study include the *HAQ Disability Index,* which assesses a patient's level of functional ability, as well as questions about drug toxicity, and medical cost data both direct (e.g. physician visits, medications) and indirect (e.g. loss of productivity).

Global Knee Pain Severity Scale

Knee pain severity (not activity-specific) during the past 30 days assessed using a 0-10 numerical rating scale. The validity of numerical rating scales has been well documented, they are easy to administer and score, and can be used with a greater variety of participants than can a visual analog scale. Numeric rating scales can also be administered over the telephone to participants who become unable to visit the clinic.

Appendix E

MRI Screening Form

TUFTS MEDICALCENTER

DEPARTMENT OF RADIOLOGY-OUTPATIENT MRI

1) Patient Name:_____ Hospital #:_____

2) Has placement of an intravenous line been difficult in the past?

Yes:_____ No:_____ If yes, describe: _____

3) Do you have an intravenous access device?

Yes:____ No:_____ If yes, which? Broviac____ Hickman____ Infusaport_____

4) Do you have any allergies to food, medications or latex?

Yes:_____ No:_____ If yes, list.

5) Please list medications, including over the counter medications, with the doses that you currently take.

6) Please list surgical operations you have had.

7) Do you have any of the following medical problems?

Asthma or Shortness of breath:	Yes:_____ No:_____
Hypertension:	Yes:_____ No:_____
Diabetes:	Yes:_____ No:_____
Kidney failure:	Yes:_____ No:_____
Sickle cell anemia:	Yes:_____ No:_____
Multiple myeloma:	Yes:_____ No:_____
Pheochromocytoma:	Yes:_____ No:_____

Heart disease: Yes:_____ No:_____

8) Have you eaten in the last four hours? Yes:_____ No:_____

9) Are you pregnant? Yes:___ No:____ Do not know: ____ Date of last period?_____

10) Patient Weight_____ lb.

11) Have you had a prior MRI exam(s)? Yes_____ No_____ If Yes, where and date_____

MRI: MR Safety - Please check the appropriate box if you have any of the following:

	YES	NO		YES	NO
Cardiac pacemaker, pacer wires			Renal transplant clips		
EKG leads			Insulin pump		
Transdermal medication patches			Transcutaneous nerve stimulator		
Implantable cardiac defibrillator (ICD)			Biostimulator		
Intracranial aneurysm clips			Cardiac arrhythmias		
Vascular clips			Kidney problems		
History of metal in eyes or metal work, Shrapnel			Back or neck surgery		
Heart valve prosthesis			Back or neck pain		
Joint or limb prosthesis			Numbness or tingling		
Joint rods, screws, pins, etc.			Hair Wig		
Middle ear prosthesis			Claustrophobia		
Eye / Orbital prosthesis			Dentures, retainers or braces		
Hearing aid			Metal IUD		
Body Piercing	Date/location		Tattoo	Date/location	

Have you ever had an IV injection of Gadolinium (MRI IV Contrast)? Yes:_____ No:_____

If yes, did you experience any difficulty with it? (please explain)_____
_____

Technologist signature completing medication reconciliation_____Date_____

Tufts Medical Center

Protocol Title:	Effect of Intra-Articular Steroids on Structural Progression of Knee OA: A Randomized Controlled Trial
Protocol Version Date:	April 15, 2014 (Protocol changes) May 2, 2012 (Protocol changes) February 29, 2012 (Protocol changes) August 3, 2011 (Protocol changes) April 11, 2011 (Protocol changes) February 16, 2011 (DSMB requested changes) October 8, 2010 (DSMB requested changes) June 29, 2010 (IRB requested changes) June 18, 2010 (IRB requested changes) April 12, 2010 (Initial)
Principal Investigator:	Timothy E. McAlindon, MD, MPH Chief, Division of Rheumatology Tufts Medical Center 800 Washington Street, Tufts Box 406 Boston, MA 02111
NIAMS Grant:	R01 AR057802-01

STUDY PROTOCOL

TABLE of CONTENTS

Abbreviations

aBVF	apparent bone volume fraction
ACR	American College of Rheumatology
AE	adverse effects/events
AHA	American Heart Association
AUC	area under the curve
BMD	bone mineral density
BMI	body mass index
BML	bone marrow lesion
CAM	complementary alternative medicine
CBI	Center for Biomedical Imaging at Boston University Medical Campus
CDER	Center for Drug Evaluation and Research
CPPD	calcium pyrophosphate deposition disease
CFR	Code of Federal Regulations
CRF	case report form
DAR	Drug Accountability Record
DSMB	Data and Safety Monitoring Board
DXA/DEXA	dual X-ray absorptiometry
eCRF	electronic case report form
EDC	electronic data capture
ET	early termination
EULAR	European League Against Rheumatism
FDA	Food and Drug Administration
GCP	good clinical practice
HAQ	PROMIS Health Assessment Questionnaire
HIPAA	Health Insurance Portability and Accountability Act
IA	intra-articular(ly)
IACS	intra-articular corticosteroid(s)
ICC	intraclass correlation coefficient
ICF	informed consent form
ICH	International Committee on Harmonization
IP	investigational product
IRB	Institutional Review Board
ITT	intent-to-treat
JSW	joint space width
JNC 7	The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure
K/L	Kellgren and Lawrence
KAI	KAI Research, Inc.
KOA	knee osteoarthritis
LOCF	last observation carried forward
MI	myocardial infarction
MR	magnetic resonance
MRI	magnetic resonance imaging
NCI	National Cancer Institute
NIAMS	National Institute of Arthritis and Musculoskeletal and Skin Diseases
NIH	National Institutes of Health
NO	nitric oxide
NS	normal saline

NSAID	non-steroidal anti-inflammatory drug
OA	osteoarthritis
OAI	Osteoarthritis Initiative
OARSI	Osteoarthritis Research Society International
OGA	observational gait analysis
PA	posteroanterior
paBMD	tibial periarticular bone mineral density
PHI	protected health information
PI	principal investigator
PROMIS	Patient Reported Outcome Measurement Information System
RA	rheumatoid arthritis
RAF	research authorization form
RCT	randomized controlled trial
SAE	serious adverse effects/events
SF-36	Short Form 36
Sub-I	sub-investigator
TIMP	tissue inhibitor metalloproteinase
TMC	Tufts Medical Center
TUHS	Tufts University Health Sciences
WOMAC© LK3.1	Western Ontario and McMaster Osteoarthritis Index

1. Summary

Triamcinolone 40 mg will be compared to placebo in a randomized placebo-controlled clinical trial testing the effect of triamcinolone versus placebo given intra-articularly (into the index knee joint with osteoarthritis (OA)) once every three months over two years for a total of eight knee injections. Tolerability and safety will be assessed by adverse effects (AE) reporting, physical examination, vital signs, and clinical laboratory tests. The primary clinical outcome will be assessed by changes in the WOMAC© LK3.1 questionnaire. The primary pathological process measure will be change in knee cartilage volume as measured by magnetic resonance imaging (MRI).

2. Background and Significance

2.1 Osteoarthritis Background

Symptomatic knee OA has an estimated overall incidence of 240/100,000 person years[1], and is the most frequent cause of dependency in lower limb tasks, especially in the elderly[2]. It causes 68 million work loss days per year and more than 5% of the annual retirement rate[3, 4]. It has considerable economic and societal costs, in terms of work loss[5] and hospital admission[6]. Furthermore, OA is the most frequent reason for joint replacement at a cost to the community of billions of dollars per year[4]. There are currently no therapies for OA confirmed to have an effect on disease progression. Treatments commonly used for OA include intra-articular corticosteroids (IACS), a widely employed invasive intervention that is of particular note because its structural effects on cartilage and bone are uncertain.

2.2 Knee Osteoarthritis and Knee Synovitis

OA is characterized by focal and progressive damage to articular hyaline cartilage accompanied by a hypertrophic 'reaction' in peri-articular bone[7]. The prevailing view of knee OA has been one of a non-inflammatory disorder but chronic inflammatory changes in the synovium are seen in all stages of knee OA[8-12], appear to be of clinical significance[13-16], are associated with regions of cartilage damage[13, 17] and predict structural progression[13]. Inflamed synovium is also the most plausible target for IACS in knee OA[13].

It is likely that synovial inflammation in osteoarthritic knees is triggered and sustained by particles and crystals abraded from damaged cartilage – a "detritic synovitis"[18]. These generate in synovial macrophages and fibroblasts a broad range of inflammatory responses that resemble those found in inflammatory joint disorders such as rheumatoid arthritis. According to this model, synovial inflammation should likely occur as a late consequence of damage to cartilage and subchondral bone. However, it is also possible that it occurs earlier, as a paracrine response to disordered homeostasis in the articular environment[18].

Synovial biopsy studies of osteoarthritic knees have found a high prevalence of inflammation. One study of 63 patients with moderate to severe knee OA found thickening of the lining layer, increased vascularity, and inflammatory cell infiltration in synovial membranes in all knees, with the most prominent features in those with advanced disease[9]. However, synovitis appears common even in mild knee OA - Myers studied the mild end of the spectrum and found synovitis in 55% of biopsied knees[8]. Another biopsy study of nine selected knees with mild OA and short symptom duration also showed a mild chronic synovitis in all cases[12].

Synovial changes in the knee also appear to be associated with pain and structural damage. An MRI study of over 400 individuals from both Veterans Affairs and community sources, found more effusions and synovial thickening among those with knee pain[16]. Among the subset with symptomatic radiographic knee OA, synovial thickening was associated with the severity of knee pain. In a subsequent longitudinal analysis of 270 participants in that study who had MRI evaluations at 15 and 30 months, change in synovitis (especially in the infrapatellar fat pad) correlated with the change in pain ($r = 0.21$, $p = 0.0003$)[15]. Loeille et al, in scrutinizing the relationships between synovitis and cartilage

damage among 39 patients with knee OA using arthroscopy, biopsy and MRI, found a correlation of synovitis with medial tibiofemoral cartilage damage (r = 0.5, p=0.001)[17].

There is also evidence that synovitis participates in the structural progression of knee OA[13, 19]. Ayral studied a cohort of 422 individuals with medial compartment tibiofemoral OA who participated in a (null) clinical trial that included a systematic assessment of synovitis and chondropathy using arthroscopy[13]. Twenty-one percent of participants had inflammatory changes in the synovium at baseline and these individuals were three times more likely to exhibit progression of cartilage damage (25.8% vs. 11.9%, adjusted odds ratio = 3.11, 95% confidence limits 1.07 - 5.69). Pelletier et al, in a 2-month observational study of 29 knee OA patients with MRI-confirmed synovitis found that the baseline synovitis score correlated significantly with the percentage loss in cartilage volume over this relatively short period (r~0.6)[19].

2.3 MECHANISMS OF JOINT DAMAGE FROM SYNOVITIS IN KNEE OSTEOARTHRITIS

The synovium has important roles in articular health, so it is logical to infer that pathological changes and functional compromise may contribute to progression of OA. Two important synovial roles in articular homeostasis include provision of vascular support to distant chondrocytes and facilitation of low-friction movement between cartilaginous surfaces[20]. The synovium produces two critical components of synovial lubrication; (i) the macromolecule hyaluronan, which confers to synovial fluid its viscosity and (ii) the boundary layer lubricant lubricin. In situations of joint injury, the synovium increases its secretion of hyaluronan, which may dampen inflammation in the synovial space. Typical OA effusions are transudative and have high viscosity[20]. However, this viscosity is lost in the presence of inflammation[21]. Experimental data also show reduction in synovial secretion of lubricin in the presence of inflammation and proinflammatory cytokines such as IL-1 and TNF-α[22, 23].

The synovium may also participate in the pathophysiology of OA through the secretion of proteolytic enzymes and cytokines. Indeed, cytokines are fairly abundant in OA synovium, at least in late-stage disease (Table 1)[18]. Immunohistological studies of synovium obtained from OA and rheumatoid (RA) joints show quantitative rather than qualitative differences between the two disorders[9]. Production of IL-1α, IL-1β, and TNFα is present in synovial membranes from all patients with OA, irrespective of the degree of articular cartilage damage[9]. Thus, chronic inflammatory changes with production of proinflammatory cytokines are a feature of synovial membranes from patients with early OA, with the most severe changes resembling those of RA.

IL-1 and TNF-α are the dominant cytokines in inflamed OA joints, of which IL-1 appears to have the greatest potential to mediate cartilage damage[24, 25]. IL-1 is produced in considerable quantities in OA synovium[26, 27] and appears to be the driving force in the production of destructive enzymes (matrix metalloproteinases) and IL-6[28]. However, there are numerous other cytokines that may have specific and important roles in OA. IL-6, for example, may upregulate matrix metalloproteinase expression in cartilage, stimulate hepatic production of acute phase proteins and mediate bone destruction[18]. The non-peptide mediator, nitric oxide (NO), is also produced by synovial cells and chondrocytes in OA joints[29] and may contribute to cartilage destruction.

There is evidence that IL-1 and TNF-α may be partly responsible for increased amounts of proteases found in OA synovium[18]. Coordinate synthesis of IL-1 and stromelysin has been found in canine OA[30], as has a correlation in human OA synovium between the number of inflammatory cells and levels of neutral proteases[31]. Expression of collagenase and stromelysin is evident in OA synovium, albeit at lower levels than in rheumatoid disease[32, 33]. Tissue inhibitor of metalloproteinase (TIMP) is elevated in OA synovium, with a protease:inhibitor ratio that suggests a role for proteases in tissue destruction. Indeed, many cartilage proteinases have now been identified, among which collagenase 3 (MMP-13) and aggrecanase-1 (ADAMTS4) appear to play major roles in degradation of human cartilage matrix[20]. Activation of collagenase 3 (MMP-13) appears to be at least partly cytokine dependent[34, 35], but there is a lack of agreement on the effect of cytokines on ADAMTS4[20].

Of course, the factors that are pivotal to maintaining this balance or the cartilage response are largely unknown. It follows, then, that perturbation of the system using pharmaceuticals may have effects on the balance between catabolism and anabolism that are not entirely predictable. It is suggested, for example, that intra-articular corticosteroids (IACS) might benefit OA knees by suppressing IL-1 mediated stimulation of metalloproteinase synthesis[36]. While this seems plausible, the net effects of IACS on measures of cartilage health need to be tested in a clinical environment.

2.4 IACS: CURRENT CLINICAL USAGE IN THE TREATMENT OF KNEE OSTEOARTHRITIS

IACS are widely employed in the management of knee OA. It is estimated that 95% of rheumatologists in the U.S. perform IACS for OA, 53% frequently[37]. The use of IACS for OA is endorsed by the American College of Rheumatology (ACR)[38], the European League Against Rheumatism (EULAR)[39], the Osteoarthritis Research Society International (OARSI)[40, 41], and the National Collaborating Centre for Chronic Conditions[42]. The latter was a thoughtful document and commented "…intra-articular corticosteroid injections provide short-term reduction in osteoarthritis pain… The risks … are generally small. The question of steroid-arthropathy, … remains controversial and is currently based on animal model and retrospective human studies."

2.5 EFFECTIVENESS OF IACS FOR KNEE OSTEOARTHRITIS PAIN

There have been at least 12 randomized controlled trials (RCTs) testing IACS for OA *pain*[43-55], and two recent meta-analyses[56, 57]. The most recent and comprehensive meta-analysis[57] was based on 28 short-duration trials (except one[51]). In the pooled analyses, IACS was more effective than placebo for pain reduction one-week post injection (relative risk 1.4; 95% confidence limits 1.1 - 1.8), 2 weeks (1.8; 1.1 - 3.0) and 3 weeks (3.1, 1.6 - 6.1). They recommended that future trials have standardized outcomes, run longer, investigate different patient subgroups, and clinical predictors of response, specifically those associated with inflammation and structural damage. The proposed study will accommodate these recommendations by testing a long-term (2-year) IACS intervention among the subset of knee osteoarthritis (KOA) with inflammatory features, using standardized clinical and structural outcomes.

2.6 PREVIOUS STUDIES EVALUATING THE EFFECT OF IACS IN CLINICAL SAMPLES

Three studies have attempted to examine the structural effects of IACS on OA progression[51, 58, 59]. The first was an observational report of 61 patients with knee OA who had documentation of IACS treatment over a 10-year period[59]. In men only, the knees that had received IACS had more severe osteoarthritis at follow-up, as measured by the tibiofemoral angle of alignment. However, this study has serious methodologic problems including bidirectional censorship and confounding by indication.

The second was an observational study of 67 patients with temporomandibular joint OA that had not responded to non-surgical treatment[58]. Twenty-three proceeded immediately to high condylectomy, while the remaining 44 received two intra-articular injections of triamcinolone acetonide, 20 mg, with a 2-month interval. It is not clear how this treatment assignment was made. Eighteen of the 44 patients who received IACS later underwent high condylectomy due to 'treatment failure'. Histological examination of the surgical specimens from this subset exhibited more extensive joint damage than those in the non-steroid group. However, the observational non-randomized nature of this study introduces complexities that prevent causal inference.

Raynauld evaluated the safety of triamcinolone acetonide 40mg, or saline control, administered every 3 months for 2 years in a randomized controlled trial for knee OA[51]. Structural progression was assessed using fluoroscopically-positioned knee x-rays[60, 61].They enrolled 68 participants, of whom 66 completed the trial. Most had mild OA (~65% had Kellgren & Lawrence grade 2 changes). There was no difference between groups in extent of change in radiographic joint space width. The clinical outcomes were also identical, with the exception of area-under-the-curve (AUC) analyses for night pain and stiffness that favored treated knees (AUC for night pain; treated =-0.7; untreated =-0.3; p = 0.005; stiffness; treated =-0.7; untreated =-0.3; p = 0.05). This study is somewhat reassuring in that it provides

evidence for symptomatic benefit without evidence of increased structural deterioration. On the other hand, there are methodologic factors that limit its interpretation. The participants had mild disease and exhibited little structural progression. In fact, there was no attempt to enroll a phenotype at higher risk for progression nor was there evaluation or stratification for indicators of inflammatory OA that might predicate responsiveness to IACS. Thus, the findings are not especially informative in respect of the potential for structural modification in the pertinent phenotypes. The radiograph is also fundamentally flawed due to its inability to image soft-tissue articular structures such as cartilage, and high precision error relative to expected change, especially in a small sample such as this[62].

2.7 ARE INTRA-ARTICULAR CORTICOSTEROIDS SAFE?

Joint sepsis is a primary concern in relation to intra-articular injection. However, while its rate of occurrence is difficult to measure with certainty, it is clearly low. A 1999 estimate from a retrospective survey in France suggests a rate of 13 infections per million injections, lower with pre-packaged syringes[63]. Concordant with this low estimate, a survey of all cases of septic arthritis incident between 1982 to 1991 in Nottingham, UK (which had a base population of 600,000) identified only 3 cases attributable to IACS[64].

More frequent, but of less medical concern, are local aseptic inflammatory reactions to IACS injections[65]. These have been reported with rates up to 24% in some series[45-47, 66]. The biologic basis for these reactions is uncertain but may relate to the crystalline nature of the injected corticosteroid formulations.

There is systemic absorption of steroid injected into a joint. Peak plasma levels after an intra-articular injection of 80 mg methyl prednisolone are in the order of 169 ng/ml, achieved 8 hours post injection[67]. A measurable transient effect on the pituitary axis is apparent with injections of 40 mg of methyl prednisolone[68] or >40 mg triamcinolone diacetate[69]. However, it seems unlikely that this has any long term effects at the frequency generally recommended in rheumatology practice (< 3 months). Problems with the pituitary axis were also apparently not encountered in an RCT in which IACS were administered every 3 months over a 24 month period[51]. While links have been made between IACS and various systemic features (immunosuppression[70], impaired diabetes control[71, 72], flushing, menstrual irregularities[73]) substantial data quantifying their frequency is generally lacking[56, 74]. One study provided data showing elevation in blood pressure and blood glucose levels following epidural corticosteroid injections, but the doses used were greater than those proposed in this study[71]. Anaphylaxis after IACS has been rarely reported[75, 76], as has steroid psychosis[77].

The most systematic evaluation of adverse effects from IACS was performed by Bellamy, in a Cochrane review of their efficacy for OA[57]. He found that side effects were uncommon and noted no reports of *serious* adverse events among the trials reviewed. Reported adverse events included post-injection flare, crystal-induced synovitis, tissue atrophy, fat necrosis, calcification, joint sepsis, steroid arthropathy, avascular necrosis, hematoma, fluid retention, hyperglycemia and hypertension.

Raynauld's RCT of IACS is pertinent to this proposal in that it employed a similar intervention dosage and time frame[1]. In that study, "No infections (local or systemic) or acute flares were associated with the injections during the study. Findings of laboratory tests performed during the study were within normal limits, and there were no differences between the treatment groups."

2.8 EFFECTS OF INTRA-ARTICULAR CORTICOSTEROIDS ON CARTILAGE

A number of case studies emerged in the 1950s and 1960s that suggested a link between IACS injections and joint destruction (cited by Salter[78], Mankin[79]). Chandler and Wright, for example, observed that repeated intra-articular injection of hydrocortisone in patients with rheumatoid arthritis seemed to improve the extent of synovitis but increase the rate of radiological deterioration[80]. Chandler et al subsequently drew an analogy with a Charcot joint in a description of apparent acceleration of progression of hip OA in a doctor's wife who had received frequent IACS injections[81]. Similar

inferences based on clinical observations recur even in recent literature[59]. While such inferences are innately confounded by indication, their validity seemed to be substantiated by early studies in animals that showed anti-anabolic effects of IACS on cartilage[79, 82, 83].

2.9 Effects of Corticosteroids on Healthy Cartilage In-Vitro

There is evidence that corticosteroids have anti-anabolic influences on cartilage metabolism in-vitro[84-86]. Farquhar tested for interactions between corticosteroid exposure and joint loading on cartilage metabolism and observed that high dose steroids depressed protein and proteoglycan synthesis[84]. Heavy loading further exacerbated the loss of matrix solids. Murphy et al, in pursuit of the optimal therapeutic concentration of IACS, found that proteoglycan synthesis in normal cultured equine articular cartilage was severely depressed by the presence of methylprednisolone 10 mg/mL, and failed to recover after 13 days of culture without methylprednisolone[86]. Cartilage treated with concentrations in the range of 5 mg/mL also exhibited adverse metabolic responses, but these tended to recover after methylprednisolone removal. Lower concentrations did not have significant effects. More recently, Fubini scrutinized corticosteroid-induced changes in collagen expression at transcriptional and translational levels in articular cartilage from young adult horses[85]. Steady-state levels of type II procollagen mRNA decreased as methylprednisolone concentrations increased in a dose-dependent fashion, dropping below 10% of control values by a concentration of 1×10^5 pg/ml. Cytotoxicity occurred as methylprednisolone levels were increased beyond 1×10^8 pg/ml. Based on a comparison of their data with information on the pharmacokinetics of intra-articular methylprednisolone in clinical practice, they estimated that synovial fluid methylprednisolone may remain above a cytotoxic concentration for more than 36 hours following a typical clinical IACS injection. Pelletier et al found that hydrocortisone reduced proteoglycan catabolism in cartilage explants (which they attributed to suppression of metalloprotease synthesis) but also proteoglycan synthesis. They observed extensive vesicular dilatation of chondrocyte endoplasmic reticulum in explants treated with hydrocortisone[87].

2.10 Effects of Corticosteroids on Healthy Cartilage in Animal Models

In-vivo studies of the effects of corticosteroids on healthy cartilage tend to show adverse effects, although their findings are not always consistent. Moskowitz in 1970, and Behrens in 1975, separately observed deleterious effects of intra-articular triamcinolone acetonide on rabbit cartilage[88, 89]. These included nuclear degeneration, cyst formation, cartilage fissures, and reduced synthesis of proteoglycans and matrix proteins. Lewandowski induced ‘OA-like damage’ in rats using repeated intramuscular injections of dexamethasone[90]. Catabolic or anti-anabolic effects of IACS have also been seen in studies of horses[82] and rabbits[91]. One study testing interactions of intra-articular hydrocortisone and running on cartilage in rats found that the combination was more detrimental than either intervention alone[92]. Studies testing cartilage effects of intra-articular methylprednisolone acetate on the healing of osteochondral defects in horse joints had somewhat discordant results. Shoemaker found degenerative effects[93] but Foland did not observe significant changes[94]. Of course, the extent to which effects in animal models can be generalized to human situation is uncertain.

2.11 Effects of Corticosteroids on Degenerative Cartilage in Animal Models

In contrast to the prior scenarios, these studies provide evidence that IACS may reduce cartilage degradation in certain pathophysiologic situations. Williams & Brandt tested the effect of intra-articular triamcinolone hexacetonide on the development of degenerative cartilage changes that follows a single intra-articular injection of sodium iodoacetate (a metabolic poison)[95]. These features include cartilage fibrillation, loss of staining with Safranin O, depletion of chondrocytes, and prominent osteophytes, without evidence of synovitis. However, when triamcinolone hexacetonide was injected into the ipsilateral knee 24 hours after the intra-articular injection of iodoacetate, fibrillation was noted in only 1 of 6 samples, osteophytes were less prominent, pericellular staining with Safranin O persisted, and cell loss was less extensive. Thus, triamcinolone hexacetonide produced a marked, dose-dependent protective effect in this model of chemically-induced articular cartilage damage.

Corticosteroids also apparently reduce progression of degenerative lesions in the cruciate ligament transaction dog model of OA[96, 97]. Pelletier & Martel-Pelletier treated 6 animals with oral prednisone and 6 with intra-articular triamcinolone hexacetonide at surgery and 4 weeks later[96]. Twelve other operated dogs received no treatment. Four of 15 normal control dogs received intra-articular triamcinolone. All dogs were sacrificed 8 weeks post-surgery. Operated untreated dogs developed significant cartilage lesions on the femoral condyles and tibial plateaus with prominent osteophytes. Operated dogs treated with oral or intra-articular steroids developed significantly less osteophytosis. Cartilage erosions on femoral condyles appeared in 25% of the untreated dogs, 8% of the dogs receiving oral prednisone, and none of the dogs receiving intra-articular triamcinolone. In both groups of treated dogs, the size of the tibial plateau lesions was significantly smaller than in the operated untreated dogs. Histologically, corticosteroids also significantly reduced the severity of cartilage structural degeneration. Electron microscopy revealed no increase in cell degeneration or death associated with steroids.

The same research group later performed a similar experiment testing the effect of intra-articular methyl-prednisolone acetate on the development of osteoarthritic lesions in the dog model of OA[97]. Treatment with methylprednisolone reduced the incidence and size of osteophytes and the histologic severity of cartilage damage. Immunohistochemistry of the OA cartilage revealed a marked increase in staining for stromelysin in chondrocytes and throughout the matrix. Treatment with methylprednisolone reduced this toward normal in both chondrocytes and matrix.

In a subsequent mechanistic study using the same animal model, Pelletier et al examined the effects of triamcinolone hexacetonide on cartilage metalloproteases and expression of cytokines/oncogenes (IL-1 beta, c-Fos, and c-Myc)[98]. This 30-dog study had 4 groups: (1) no treatment; (2) triamcinolone at time of surgery and at 4 and 8 weeks later; (3) triamcinolone at 4 and 8 weeks after surgery; (4) triamcinolone at 8 weeks after surgery. As before, intra-articular injections of triamcinolone reduced the development of osteophytes and the histologic severity of cartilage lesions. Immunohistochemistry revealed that triamcinolone reduced the percentage of chondrocytes immunoreactive for stromelysin in a dose-response fashion. They inferred that the effect of triamcinolone may be mediated through a reduction in the expression of proteolytic enzymes, such as stromelysin.

2.12 Effects of Corticosteroids on Cartilage Damage in Animal Models

The potential for corticosteroids to attenuate cartilage damage resulting from inflammatory milieus appears more clear-cut. Corticosteroids have diverse anti-inflammatory effects that could lead to a reduction in activity of downstream catabolic pathways[99, 100]. In inflammatory arthritis models, corticosteroids reduce proteoglycan breakdown, suggesting an inhibitory effect on enzymatic degradation of cartilage[101-103]. Sedgwick et al found evidence that suppression of synovial expression of inflammatory mediators is a critical mediator of this effect[102]. Using an in-vivo inflammatory model of cartilage destruction, they observed that (soluble) hydrocortisone sodium succinate reduced while (insoluble) hydrocortisone acetate enhanced proteoglycan loss[102]. Injection of the same dose of hydrocortisone acetate into the inflamed lining tissue reversed this effect. However, these results are also compatible with the possibility that corticosteroids formulated to provide persistent intra-articular concentrations have catabolic effects on cartilage.

Joosten et al compared rimexolone and triamcinolone hexacetonide injected into joints of mice with monoarticular antigen-induced arthritis[104]. In control joints, both drugs suppressed proteoglycan synthesis. However, in the inflamed joints, they counteracted the usual severe suppression of proteoglycan synthesis, and reduced the extent of osteophyte formation (a characteristic of this type of experimental arthritis). Thus, in the presence of overt inflammation, the overall effect of corticosteroids on cartilage appears to be favorable.

2.13 Intra-Articular Corticosteroids and Peri-Articular Bone

Peri-articular bone has an important role in protecting cartilage from injury. It is estimated that 30-50% of a load across a knee is absorbed by peri-articular bone compared to only 1-3% by articular cartilage[105-

108]. In OA there are widespread pathologic changes in peri-articular bone that include thickening and remodeling of the subchondral plate[109], increased bone turnover[110], trabecular microstructural abnormalities[111],[112],[113] and macroscopic damage[114]. All of these impair the biomechanical properties of peri-articular bone and could contribute to structural progression. Clinical observations bear this out: peri-articular scintigraphic abnormalities and bone marrow lesions (both common in OA) predict radiographic progression[115] and correlate with subsequent joint space loss ($r \sim 0.2 - 0.3$, $p < 0.05$)[116],[117, 118] while systemic bone density and tibial peri-articular bone mineral density (BMD) are highly predictive of structural progression of knee OA[119], [119, 120],[121]. Bruyere found a correlation between subchondral BMD and 1-year change in radiographic joint space width of 0.43, highlighting the large effect of bone health on cartilage loss[121]. Zhang et al, examined the relationship of femoral neck BMD with progression of radiographic knee OA among 473 women over 8 years[119]. Compared to those in the lowest quarter of BMD, the adjusted odds ratios for progression were 0.3, 0.2, and 0.1 among women in the 2nd, 3rd, and highest BMD quarters (p for trend = 0.04).

Glucocorticoids have effects on bone metabolism that include suppression of osteoblast proliferation and reduced protein synthesis[122]. The reduction in protein synthesis by osteoblasts is probably mediated by direct glucocorticoid receptor regulation of important osteoblast genes. This results in loss of bone mass at trabecular sites and a propensity for fracture. Surprisingly, we have not found any previous studies addressing how these biological effects might interact with processes in osteoarthritic peri-articular bone. It may be reassuring that studies of IACS for knee OA, including a 2-year intervention[51], make no note of any adverse effect on bone health[57]. Nevertheless, the biologic effects of corticosteroids on bone, together with epidemiologic observations on the relationships of bone density with progression of OA, indicate that effects of IACS on bone health need to be evaluated. This proposed study will incorporate measures of peri-articular and distant bone mineral density using dual x-ray absorptiometry (DXA), and trabecular morphometry using MRI.

2.14 Ultrasonographic Screening for Synovitis in Osteoarthritic Knees

Clinical and radiological characteristics are poor predictors of inflammation in knee OA[123]: Ultrasonography and MRI both have the capability to demonstrate synovial changes[124-126]. Of these two modalities, ultrasonography has greater clinical utility as a screening tool because of portability, lower cost and increasing deployment in rheumatology practice. These factors make it a more desirable technology than MRI to evaluate knee OA synovitis in a clinical setting, especially since the probable outcome of the proposed trial is a therapeutic strategy (IACS) that is contingent on screening for the presence of knee joint synovitis.

In general, musculoskeletal ultrasonography has high sensitivity and specificity in the detection of joint inflammation[127, 128], and is sensitive to change induced by therapeutic interventions[129-131]. Ultrasound may be slightly less predictive than MRI in the detection of synovial thickening[132]. One study compared B-mode and power Doppler ultrasound to *contrast-enhanced* MRI -effusion or synovial thickening was detected by B-mode ultrasound in 58%, power Doppler in 63% and contrast-enhanced MRI in 82%[133]. Using MRI as the reference standard there was a sensitivity of 72% for effusion in the superior recess and 81% for effusion in the lateral recess. There have been a couple of pathological validation studies of ultrasonographically-detected synovitis. The first showed that power Doppler sonography can reliably measure the extent of vascularity in the synovium of knee joints, with correlations between power Doppler and pathological grading of vascularity of ~0.8[134]. Another compared ultrasound assessments of synovitis (classified according to the intensity of the power Doppler signal) against histopathology in 3 OA and 7 rheumatoid knees[135]. The power Doppler ultrasound synovitis score correlated with synovial tissue cell infiltrate (r~0.6), synovial lining layer thickness (r~0.6), and vascularity (r~0.6).

The primary role of the ultrasonography assessment is to enrich the sample with participants who have synovitis using a technology that can be easily deployed in clinical practice; a role for which its performance characteristics are sufficient.

2.15 MRI Assessment of Synovitis in Osteoarthritic Knees

There is currently no clear consensus on the optimal MRI approach for assessment of synovitis in clinical studies[136-138]. While non-enhanced MRI has been used in epidemiologic and clinical studies to classify synovitis in osteoarthritic knees[12, 15-17, 132, 139-141], this was generally confined to measurement of signal intensity in Hoffa's fat pad. Histological validation of this approach appears to be limited to only two cases in a series of nine patients[12, 17]. Although the performance of this approach may be adequate for large-scale studies, it is uncertain that it is suitable for following synovitis in a clinical trial.

Other studies indicate that *gadolinium-enhanced* MRI offers greater specificity in the detection of synovitis[17, 142], and there seems to be a greater enthusiasm for use of this technique[136-138]. However, a number of factors militate against its use in the proposed study. Firstly, intravenous gadolinium confers a risk of severe complications[143] that may be unacceptable in the context of a OA research study. Secondly, contrast enhancement appears to diminish sensitivity to change of cartilage volume measures[144], the primary structural outcome in this study. Thirdly, the procedure carries substantial participant and staff burden and adds to the cost of the endeavor. While the opportunity will exist to score our MRIs for synovitis, especially if clarity emerges about how best to use non-enhanced images for this purpose, we will not view this as a primary measure of this covariate.

2.16 MRI in the Assessment of Knee OA Structural Progression

Radiographs have been widely used to measure the severity of knee OA but their ability to provide precise, reproducible and valid measurement of the structures of interest (cartilage, synovium, bone) is limited. MR, on the other hand can image soft-tissue structures, provide insight into the tissue characteristics, and indicate the state of subchondral trabecular bone[145]. Recent MRI studies of knee OA have revealed wide-ranging damage to soft-tissue structures[146, 147], and have revealed pathologies such as bone marrow lesions and synovitis[15]. A particular appeal of MR is the inherent digital acquisition permits quantification of signal intensities from discrete structures, generation of 3D representations of individual anatomic structures, and computation of their volumes.

An OMERACT-OARSI workshop on imaging technologies published a comprehensive review of the MRI data on (1) pulse sequences for morphological analysis of articular cartilage (2) techniques for segmenting cartilage (3) semi-quantitative scoring of cartilage status and (4) technical validity, precision and sensitivity to change of quantitative measures of cartilage morphology[148]. Among their conclusions was that quantitative assessment of cartilage morphology using fat-suppressed gradient echo sequences and appropriate image analysis techniques, displays high accuracy and adequate precision (e.g., root-mean-square standard deviation medial tibia=61 μm^3) for longitudinal studies in OA patients. Longitudinal studies show changes in cartilage volume of the order of -4% to -6% occur per annum in OA in most knee compartments (e.g., -90 μm^3 in medial tibia). Annual changes in cartilage volume exceed the precision errors and appear to be associated with clinical symptoms as well as with time to knee arthroplasty.

Cartilage volume and thickness measurements have been validated in at least nine studies using cadaveric knees or patient specimens from arthroplasty[149-156]. Most of these document a close linear relationship between the two measures with differences in the interval of 5 – 10%. MRI cartilage volume and thickness measurements obtained prior to arthroplasty have been validated against actual cartilage volume from the resected specimen[157]. Cartilage volume assessments were lower using MRI (interval 7-27%) but there was a high correlation between the two measures (r=0.98, standard error 7%). This study provides estimates of absolute cartilage volume loss, e.g. mean medial tibia cartilage loss in patients with medial compartment OA was 1290 mm^3 [157].

The reproducibility of cartilage volume measurements appears to be excellent[148]. Raynauld et al demonstrated inter-reader ICCs of 0.986-0.995 for total cartilage volume, and intra-reader intraclass correlation coefficient (ICC) of 0.99[158]. We have also examined the reproducibility of ***longitudinal*** measurement of cartilage volume loss over a 2-year period and demonstrated intra-reader ICCs of ~0.93.

MRI assessments of OA severity appear clinically relevant and have discriminative validity. Comparison of cartilage volume measured by MRI with severity of radiographic joint space narrowing showed a mean reduction in tibial cartilage volume of 1.00 ml (s.d. 0.32) in the medial compartment and 0.53 ml (s.d. 0.25) in the lateral compartment each increment in joint space narrowing score[159]. Estrogen-users were found to have more cartilage than non-users (adjusted difference = 0.30 ml, 95% confidence interval 0.08 - 0.52)[160]. MRI can detect in-vivo deformation of patellar cartilage shortly after joint loading[161], diurnal variation in cartilage thickness[162], and is more sensitive in assessment of OA progression than radiography and arthroscopy[117]. Cartilage volume loss also predicts symptoms[163] and arthroplasty[164].

Currently there are at least thirteen publications describing the measurement characteristics of this approach among clinical cohorts with knee OA followed for periods up to three years[165-174]. With one exception[170], these show progressive cartilage volume loss with a mean value in the tibia, or medial tibia, of ~5%, s.d. of ~5%, interval between 3.8 – 7.4%. One study detected cartilage volume loss in the absence of change in joint space width, confirming that MRI has greater sensitivity to change than radiography[169].

A number of semi-quantitative scoring methods for whole-organ OA and cartilage status have been developed[175-179]. These have shown adequate reliability, specificity and sensitivity, and ability to detect lesion progression over 1-2 years[148]. Intra-class correlation coefficients for components of these scales are in the interval 0.59 – 0.92, with the most reliable assessments produced for osteophytosis (0.91)[175, 177].

2.17 PERI-ARTICULAR TRABECULAR MORPHOMETRY IN KNEE OA USING MRI

The importance of trabecular micro-architecture as a determinant of bone strength prompted a number of investigators to develop and validate an approach to measurement of trabecular parameters using MRI[145, 180-182]. MRI trabecular morphometry has been validated against trabecular bone using vertebral, femoral, and calcaneal specimens as a gold standard[183-185], and against bone mechanical properties[185]. MR trabecular parameters are reproducible[145], correlate closely with those derived from optical images of the bone specimens[184], and can discriminate between postmenopausal osteoporotic and non-osteoporotic women[186]. MRI trabecular morphometry has been evaluated in clinical studies of knee OA[145], and we are currently deploying it in the Osteoarthritis Initiative (OAI)[187] (see Section C.6). As an indication of its *discriminative validity*, the apparent trabecular bone volume fraction and apparent trabecular spacing differs between OA and non-OA knees, varies within knees reflecting the localization of OA[145], relates to articular cartilage degeneration[181], and correlates with subchondral BMD.

3. Study Objectives

This study is a two-year stratified and block-randomized double-blind, placebo controlled clinical trial of the effect of intra-articular triamcinolone 40 mg injectable suspension, administered every 3 months over two years (for a total of 8 doses). The randomization will be stratified by the baseline radiographic severity of knee OA (Kellgren and Lawrence grade of 2 or 3) and gender. An interim analysis will be conducted after the first half of participants has completed the trial. This interim analysis will allow the trial to be stopped early for either success or futility, or allow the trial to continue if neither success nor futility has been established.

The primary structural outcome objective in this study is cartilage volume loss; secondary structural outcomes include peri-articular bone marrow lesions (BML), attrition, tibial peri-articular bone density ratio (paBMD), and apparent bone volume fraction (aBVF). The primary clinical outcome objective is the pain domain of the WOMAC© LK3.1; secondary clinical outcomes include WOMAC© LK3.1 stiffness and function scores, physical function tests and healthcare utilization (medication use, physician visits for knee OA, and arthroplasty).

4. Investigation Sites

This study will be a single site study performed at Tufts Medical Center.

5. Duration of Study

The entire study will run over five years (a study timeline can be found in Appendix A). For each study participant, participation is 26 months. **In this study a month is defined as 28 days**.

6. Study Sample

6.1 NUMBER OF STUDY PARTICIPANTS

The number of study participants to be randomized for this study is 140. Randomized participants who withdraw study participation or who are prematurely terminated will not be replaced.

6.2 PRIMARY DIAGNOSIS

Study participants will have a diagnosis of knee OA, defined as a) chronic knee discomfort, b) tibiofemoral or patellofemoral OA by radiograph, and c) clinical exam confirming pain/discomfort referable to the knee joint. If all criteria are satisfied, the participant will automatically meet American College of Rheumatology criteria for osteoarthritis of the knee.

6.3 INCLUSION CRITERIA

- Age ≥ 45 years
- Chronic knee discomfort based on affirmative response to the question “During the past 12 months, have you had any pain, aching, or stiffness in or around your knee(s) on most days for at least one month?”
- Screening WOMAC© LK3.1 pain subscale score ≥2 on at least one of the WOMAC© LK3.1 weight-bearing pain questions and total weight-bearing pain score ≤ 8
- Tibiofemoral or patellofemoral OA on posteroanterior weight-bearing semi-flexed or lateral knee radiographs with severity equivalent to Kellgren and Lawrence (K/L) grade 2 or 3
- Evidence of synovitis on ultrasound at screening
- Clinical examination confirming knee pain or discomfort referable to the knee joint
- Prepared to discontinue NSAID(s)/analgesic(s) for 2 days (i.e, 48 hours) prior to each assessment (visit)

6.4 EXCLUSION CRITERIA

- Prior septic (study) knee joint
- Prior reconstructive surgery in the study knee
- Prior or present osteonecrosis (avascular necrosis of the index knee)
- Chronic use of oral corticosteroids; knee intra-articular corticosteroid injection within 3 months of Baseline (Month 0) visit
- Knee injection of hyaluronic acid within 6 months of Baseline (Month 0) visit
- Ongoing use of doxycycline, indomethacin, glucosamine and/or chondroitin; or use of these within 2 months of Screening visit
- Evidence (e.g., on ultrasound or by history) of other inflammatory joint disease (e.g., gout, CPPD)

- Serious medical conditions or impairments that, in the view of the investigator, would obstruct participation in the trial such as uncontrolled HIV infection, uncontrolled diabetes, uncontrolled hypertension, opiate dependency
- Plan to permanently relocate from the region, or take an extended vacation for greater than 3 months during the trial period
- Planned arthroscopy and/or arthroplasty in the study knee
- Any contra-indication to having an MRI (including weight >350 lbs)
- Inability to speak or comprehend English

6.5 SELECTION OF THE STUDY KNEE IN THE EVENT THAT BOTH ARE ELIGIBLE

- If both knees are eligible, then the more symptomatic knee will be selected (greater WOMAC© LK3.1 pain subscale score).
- If both knees have equivalent WOMAC© LK3.1 pain subscale scores, the knee with a greater K/L grade will be chosen.
- Finally, if both the WOMAC© LK3.1 pain subscales scores and K/L grades are equivalent for both knees, the study knee will be randomly assigned.

7. Study Intervention

7.1 INVESTIGATIONAL PRODUCT

Triamcinolone Acetonide (i.e., Kenalog-40mg/mL for injection)
Active Ingredient: Triamcinolone Acetonide - 40 mg/mL
Excipients: Benzyl Alcohol - 0.9%; Carboxymethylcellulose Sodium - 0.75%; Polysorbate 80 - 0.04%; may contain Sodium Hydroxide to adjust ph; may contain Hydrochloric Acid to adjust ph; Sodium Chloride
pH: 5.0-7.5
Form: Injection suspension

Manufacturer: Bristol-Myers Squibb
P.O. Box 4500
Princeton, NJ 08543-4500
Business hours: www.bms.com (web site)
Business hours: phone (800) 321-1335 (drug information)
Business hours: fax (609) 897 6663
Product ID: 2150509
AAPCC Code: 132000-Corticosteroids

7.2 PLACEBO PRODUCT

0.9% Sodium Chloride Injection (sterile, nonpyrogenic, isotonic solution of sodium chloride and water for injection)
Active Ingredient: Sodium chloride 9mg/mL
Excipients: The solution may contain hydrochloric acid or sodium hydroxide for pH adjustment.
Sodium Chloride, USP is chemically designated NaCl, a white crystalline compound freely soluble in water.
How supplied: 0.9% Sodium Chloride Inj. Single-dose Fliptop Vial 10 mL that contains no bacteriostat or antimicrobial agent or added buffer. Sodium Chloride, USP is chemically designated NaCl, a white crystalline compound freely soluble in water.
mOsmol (calc): 0.308/mL
pH: 5.3 (4.5 to 7.0)
Storage requirement: 20 to 25°C (68 to 77°F). [See USP Controlled Room Temperature].

Supplier: Hosperia, Inc., Lake Forest, IL 60045 USA
List Number: 4888

7.3 INVESTIGATIONAL PRODUCT AND PLACEBO STORAGE

Both the investigational product (IP) and placebo will be stored in the Research Pharmacy at Tufts Medical Center according to manufacturer storage instructions. Intra-articular injections of IP and placebo will be prepared by the research pharmacy and will be blinded to the research team members. No one involved with screening procedures will be able to seek or anticipate treatment allocation.

7.4 INVESTIGATIONAL PRODUCT AND PLACEBO PREPARATION

Syringes will be pre-filled by the research pharmacist with triamcinolone acetonide or saline according to the randomization code kept in the research pharmacy. The syringe contents will be masked by the research pharmacist prior to transportation using opaque labels and an opacified three-way stopcock. A 0.2 ml airlock will ensure that no contents are visible in the syringe tip. The needle will be affixed with the stopcock in a vertical orientation so that the airlock migrates upwards to the plunger and ensures full delivery of the medication.

7.5 INVESTIGATIONAL PRODUCT DOSAGE AND ADMINISTRATION

Participants will receive either triamcinolone acetonide 40 mg/mL or matching 0.9% normal placebo 1mL intra-articularly (IA) to the index knee every 12 weeks for a total of 8 doses.

IA injections will be performed by a rheumatologist with ultrasound guidance for needle placement. After introduction of the needle into the joint cavity the ultrasound machine will be switched off so that the medication flowing into the joint is not identifiable on the screen, thus maintaining the study allocation concealment and blinding of study personnel.

Subjects will have the option of having local anesthetic (Gebauer's Pain Ease:1,1,1,3,3-Pentafluoropropane and 1,1,1,2-Tetrafluorothane) and / or Lidocaine Hydrochloride 1% percutaneously as clinically indicated. These agents will be admistered by the injecting physician. No anesthetic will be placed intra-articularly.

7.6 INVESTIGATIONAL MODE OF MEDICAL DEVICE

Although this study is not investigating the safety or effectiveness of any medical device, and although the GE Lunar Prodigy Advance medical device ("Prodigy") is cleared by the FDA, some parts of the study will entail use of software known as Knee Software as a secondary measurement tool. When the Knee Software is enabled on the Prodigy, technically the Prodigy is an investigational device. When the Knee Software is not enabled, the Prodigy is no longer an investigational device. When the Knee Software is enabled, a warning will appear that the Knee Software is not cleared by the FDA and that it is supplied for investigational purposes only. When the study is complete, the Knee Software will be disabled.

7.7 DURATION OF TREATMENT

Each randomized participant will receive a total of eight doses of intra-articular triamcinoline acetonide 40 mg/mL or placebo, one dose every twelve weeks over a two-year period. In this study a month is defined as 28 days.

7.8 WARNINGS AND PRECAUTIONS

7.8.1 Triamcinolone

Joint sepsis is a primary concern in relation to intra-articular injection. However, while its rate of occurrence is difficult to measure with certainty, it is clearly low. A 1999 estimate from a retrospective survey in France suggests a rate of 13 infections per million injections, lower with pre-packaged syringes[63]. Concordant with this low estimate, a survey of all cases of septic arthritis incident between 1982 to 1991 in Nottingham, UK (which had a base population of 600,000) identified only 3 cases attributable to IACS[64].

More frequent, but of less medical concern, are local aseptic inflammatory reactions to IACS injections[65]. These self-limiting events have been reported with rates up to 24% in some

series[45-47, 66]. The biologic basis for these reactions is uncertain but may relate to the crystalline nature of the injected corticosteroid formulations.

There is systemic absorption of steroid injected into a joint. Peak plasma levels after an intra-articular injection of 80 mg methyl prednisolone are in the order of 169 ng/ml, achieved 8 hours post injection[67]. A measurable transient effect on the pituitary axis is apparent with injections of 40 mg of methyl prednisolone[68] or >40 mg triamcinolone diacetate[69]. However, it seems unlikely that this has any long term effects at the frequency planned in this study (3 months). Problems with the pituitary axis were also apparently not encountered in an RCT in which IACS were administered every 3 months over a 24 month period[51]. While links have been made between IACS and various systemic features (immunosuppression[70], impaired diabetes control[71, 72], flushing, menstrual irregularities[73]) substantial data quantifying their frequency is generally lacking[56, 74]. One study provided data showing elevation in blood pressure and blood glucose levels following epidural corticosteroid injections, but the doses used were greater than those proposed in this study[71]. Anaphylaxis after IACS has been rarely reported[75, 76], as has steroid psychosis[77].

The most systematic evaluation of adverse effects from IACS was performed by Bellamy, in a Cochrane review of their efficacy for OA[57]. He found that side effects were uncommon and noted no reports of serious adverse events among the trials reviewed. Reported adverse events included post-injection flare, crystal-induced synovitis, tissue atrophy, fat necrosis, calcification, joint sepsis, steroid arthropathy, avascular necrosis, hematoma, fluid retention, hyperglycemia and hypertension.

Pregnancy Category: Rating C (FDA) Studies in animals have revealed adverse effects on the fetus (teratogenic or embryocidal or other) and there are no well-controlled studies in women, or studies in women and animals are not available. Drugs should be given only if the potential benefit justifies the potential risk to the fetus.

Animal studies have indicated that steroids may be teratogenic, therefore birth control for women of childbearing potential will be required for this study.

Participants will be given a handout post-injection of post-procedure care, as well as signs and symptoms that require medical attention.

7.8.2 0.9% Sodium Chloride (placebo)

Though reactions to a physiological fluid are not expected, adverse events could occur because of the technique of administration or contamination of the fluid. These could result in a febrile response, local tenderness, abscess, tissue necrosis, thrombosis or infection at the site of injection. Based on the data described above, the likely incidence of such events is extremely low.

Participants will be given a handout post-injection of post-procedure care and signs and symptoms that require medical attention.

7.8.3 MRIs and Women of Child Bearing Potential

MRIs should be avoided during the first trimester. Therefore, birth control for women of childbearing potential will be required for this study.

7.8.4 Uncontrolled HIV Infection

Patients who have been diagnosed as HIV + will be required to review the study with their infectious disease physician and provide a letter from their physician confirming his/her belief that the study is safe for the patient.

8. Procedures and Monitoring

8.1 RANDOMIZATION

Randomization will be stratified by radiographic severity of knee OA at baseline (Kellgren and Lawrence grade of 2 or 3) and gender. Specially designed software will be used to generate the random numbers. The statistician will review lists prior to release and will keep a copy of the randomization list in a locked filing cabinet. The randomization list will be released to the research pharmacist at Tufts Medical Center who will be unblinded to participants' treatment allocation / randomization.

To preserve the concealment of the randomized treatment allocation, only the statistician and research pharmacist will have access to the treatment assignments, and neither will have any interaction with study subjects. All other members of the research team are to remain blinded to treatment assignment to prevent unblinding of study personnel who interact with study subjects, and to prevent study personnel from selectively recruiting subjects due to advance knowledge of upcoming treatment assignments.

8.2 GOOD CLINICAL PRACTICE (GCP) AND MONITORING

This study will be performed under U.S Code of Federal Regulations (CFR 21), International Committee on Harmonization (ICH) and Good Clinical Practice (GCP) guidelines. The protocol, informed consent form (ICF), and recruitment materials will be submitted to the Institutional Review Board (IRB) of the Tufts Health Sciences Campus and Tufts Medical Center for review and approval. Annual continuing review of the project will be submitted to the IRB and any other governmental agencies as required.

All Unanticipated Problems and SAEs will be reported according the Tufts MC / TUHS Campus IRB policy. All Unanticipated Problems will be reported to the DSMB (via KAI) and Tufts IRB within 48 hours of knowledge of the event. All SAEs (regardless of being anticipated or unanticipated) will be reported to the DSMB's Safety Officer (via KAI) within 48 hours of knowledge of event. All anticipated SAEs that have been reported to the DSMB will be submitted to the Tufts IRB within 15 business days. All anticipated non-serious AEs that have been reported to the DSMB will be summarized on the TMC AE Summary Report Form and submitted to the Tufts IRB annually at the time of continuing review. Please see section 8.8 for additional details.

The investigator agrees to any inspections of study-related records by the National Institutes of Health (NIH), FDA, CDER, and / or Tufts Medical Center IRB and other regulatory bodies as required.

8.3 ELECTRONIC DATA CAPTURE

We will use electronic data capture (EDC) for Case Report Form (CRF) completion and most source documentation. We will use for this our secure website, which is hosted on a Tufts-owned server.

8.3.1 Security of the Electronic Data

Our web server is owned by Tufts Medical Center and so is inaccessible without an individual secure username and password provided to the study staff. Study participants will also receive secure usernames and passwords to enable them to answer website-based questionnaires only. The server is highly protected by its architecture, firewalls and anti-virus software, and user authentication protocols. Within this structure, our website will have an additional layer of user authentication using high level time-expiring passwords for study staff. We will also operate user-type permission levels to constrain access to the different functions and data (role-based security). We will encrypt in the database highly sensitive information such as passwords. As part of the institutional network protections and maintenance, our database will be backed up regularly (at least daily), which will ensure that any data can be recovered in case of erasure or other hardware loss. Finally, each software component will be updated regularly by our IT group. Patches and other updates on servers are done daily to ensure that Microsoft's vulnerabilities can be identified and solved quickly.

8.3.2 HIPAA

In order to be compliant with Health Insurance Portability and Accountability Act (HIPAA) requirements, we will operate the following additional procedures:

1. Database normalization. Using a practice known as standard normalization of data, participant names will not be stored in the same database table as their contact information. It is through this framework that two database tables will need to be queried in order to link a participant's name with their protected health information (PHI).
2. Use of encryption for sensitive information such as passwords.
3. Database audit trails. Deletions and major changes made to a participant's stored information will be tracked automatically.
4. Any paper records that we store (e.g. consent forms) will be kept in our secure office environment protected by two levels of lockage (e.g. in a locked filing cabinet in a locked office area).

8.4 CONSENTS

8.4.1 Informed Consent

Prior to study participation, each potential participant will be provided with an informed consent form (ICF) to read. At the first screening visit each potential participant will have a discussion about the study requirements and expectations with trained study staff. The principal investigator (PI) or a sub-investigator (Sub-I) will be available for additional questions. If the potential participant agrees to the study requirements and expectations then the ICF will be signed and dated by both the participant and the study staff. A copy of the signed ICF will be given to the participant and the original kept in the research files in the Division of Rheumatology.

Potential study participants who suffer visual or auditory impairment will be required to bring an unbiased designee to ensure their understanding of the consent documents. These participants will be subject to the same eligibility criteria as anyone else, provided that they are able to participate in all required aspects of the study (e.g. 20-meter walk, filling out questionnaires, etc.).Any amendments that warrant changes to the ICF will be submitted to the IRB for review and approval. Any new version of the ICF will be presented to the active participants at their next study visit and discussed. The participants will then decide if they want to continue their study participation. If they choose to continue to participate then the participants will sign the new ICF. A copy of the signed ICF will be given to the participant.

8.4.2 Research Authorization Form

To comply with the Health Insurance Portability and Accountability Act (HIPAA) of 1996 each participant will provide written permission to allow their personal/protected health information (PHI) to be disclosed within the context of this research project.

A valid Research Authorization Form (RAF) is an individual's signed permission that allows a covered entity, in this study Tufts Medical Center, to use or disclose the individual's PHI for the purposes and to the recipients stated in the RAF. When a research authorization is obtained, the Privacy Rule requires that it pertain only to a specific research study, not to future, unspecified study projects.

Once the RAF is signed, the participant will be given a copy of the signed RAF. Participants that choose not to sign a RAF will be excluded from the study. A copy of the RAF will be submitted to and filed in the IRB.

8.4.3 E-Mail

Research participants desiring to communicate with research team members during the study shall provide written consent as per Tufts Medical Center's guidelines for electronic mail communication of protected health information.

8.4.4 Photography/Video-Recording

Research participants who are willing to participate in the gait analysis portion of the study will be presented with a photography/video consent form.

Participants who do not wish to sign the photography/video-recording consent form will not be excluded from participation into the study.

8.4.5 Optional Tissue Banking

Research participants will be presented with a Tissue Banking ICF. All subjects will sign the optional tissue banking ICF noting which specimens (plasma, serum, buffy coat, urine, and/or synovial fluid), they consent to have banked.

Participants who do not wish to have specimens banked for future studies will not be excluded from participation in the main study.

8.5 OBSERVATIONS AND MEASUREMENTS

8.5.1 Study Visits

All efforts will be made to keep the time of participants' study visits consistent (i.e., all a.m. or all p.m.) throughout the study to minimize intra-patient variability. Additionally, keeping a participant's study visits at approximately the same time will be important to laboratory specimen collection and MRI scanning. Laboratory collections and MRI scans done at approximately the same time for each participant will decrease biorhythm variability in the laboratory samples and MRI scans. The time of blood draw and urine collection will be noted at each visit. Participants who will be having an MRI scan will be asked to refrain from strenuous physical activity the day before their scheduled MRI scan.

Subjects who are randomized will receive a stipend for their participation in the study. Subjects that complete the screening visit will not receive a stipend. Subjects will receive $100 upon completion of Visit Months 0,3,6,9,12,15,18, and 21 and $200 upon completion of Visit Month 24 for a total of $1000 over the 2 years in which they are in the study. Subjects have the option of receiving the study payment as a petty cash payment or Tufts debit Mastercard (Clincard) given at the end of each study visit or as a check for the total amount at the end of their study participation. Petty cash slips can be cashed out only at the Cashier's Office on the 1st Floor of the Proger Building at Tufts Medical Center.

Subjects who agree to participate in either of 2 MRI Ancillary studies (detailed in Section 8.5.6) will be compensated an extra $50 for each additional MRI. Thus, those who participate in MRI Ancillary Study #1 will receive $50 total extra compensation for a total of $1050 upon study completion; those who participate in MRI Ancillary Study #2 will receive $100 total extra compensation for a total of $1100 upon study completion.

Beginning at Month 3, the study visit window will be +/- 1 week; however, MRI will have up to 2 weeks prior to Month 0, 12, and 24. The visit schedule and procedures that will occur at each visit are summarized in Appendix B.

Month -1, Screening

This is the initial screening visit. Prior to any research procedures being done informed consent will be obtained. The remaining procedures may be done at this visit:

- Confirm that subject has had 48 hour wash-out of NSAIDs / Analgesics
- Vital signs (BP, temperature)
- Height and Weight
- Obtaining informed consent and research authorization (and other applicable consents, e.g., e-mail communication, photography/video-recording, optional tissue banking) from the potential participant
- Participant WOMAC© LK3.1 pain subscale – Right and Left knees
- Collection of demographic data
- Collect rheumatic history, include type, duration, and treatment history of OA as well as notation of any known complications of disease or therapies
- Medical & surgical history to include past medical history, secondary diagnoses, concomitant medications, all medications and treatments taken within the last two months
- Bilateral knee examinations
- Participant physical function test (20 meter walk)
- MRI coil fit check
- Knee x-rays – bilateral, weight bearing, semi-flexed, posteroanterior (PA)
- Bilateral knee ultrasound scans
- Laboratory studies (which will be analyzed prior to the first injection to determine eligibility)
  - Hemoglobin A1c
  - Rheumatoid factor, anti-CCP and hsCRP
  - Urine Pregnancy Test (only for women who have not been menopausal for 2 years or who have not undergone surgical sterilization)
- Provide and teach use of participant diary/calendar/journal
- Randomization

Month 0, Baseline (up to 21 days after Screening)

This is the first visit of the double-blind treatment period. Only those participants that fulfill inclusion/exclusion criteria will be randomized. The following procedures will be done at this visit:

- Confirm that subject has had 48 hour wash-out of NSAIDs / Analgesics
- Vital signs (BP, temperature)
- Review of informed consents – study requirements and expectations
- Height/Weight
- Participant WOMAC© LK3.1, SF-36, HAQ, VAS global assessment
  - *The Alignment questionnaire can be completed once at any visit, Baseline or after, at participant convenience
- Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences
  - Re-educate about diary/calendar/journal
  - Present and discuss calendar of target/ideal visit dates and times for Months 3-24 visits
- Knee examination
- Musculoskeletal examination
- Participant physical function tests (chair stand, 20 meter walk)
- Laboratory studies / collections

- Hemoglobin A1c
- Serum CrossLaps
- Serum for archive
- Plasma for archive
- Urine CrossLaps
- Urine for storage
- White Blood Cell (Buffy Coat) archive
- Synovial fluid (if obtained) for cell count, crystal exam
- Synovial fluid (if obtained) for archive

♦ MRI of study knee (this exam will be done up to two weeks prior to Month 0 and reviewed for any exclusionary criteria prior to first injection)
♦ *For 10 participants who volunteer, a 2nd MRI of study knee within 5 days of the first at the opposite MRI location
♦ *For 15 participants who volunteer, a 2nd MRI of study knee within 5 days of the first at the same MRI location
♦ DXA – bilateral hip, knee and lumbar spine
♦ Knee ultrasound exam
♦ Knee injection of triamcinolone or placebo by ultrasound guidance

Month 3 (+/- 1 week window)

The following procedures will be done at this visit:

♦ Confirm that subject has had 48 hour wash-out of NSAIDs / Analgesics
♦ Vital signs (BP, temperature)
♦ Participant WOMAC© LK3.1, VAS global assessment
♦ Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences
  - Analgesia/NSAID accounting
♦ Laboratory studies
  - Hemoglobin A1c
  - Synovial fluid (if obtained) for cell count, crystal exam
  - Synovial fluid (if obtained) for archive
♦ Knee examination
♦ Knee injection of triamcinolone or placebo by ultrasound guidance

Month 6 (+/- 1 week window)

The following procedures will be done at this visit:

♦ Confirm that subject has had 48 hour wash-out of NSAIDs / Analgesics
♦ Vital signs (BP, temperature)
♦ Participant WOMAC© LK3.1, VAS global assessment
♦ Participant physical function tests (chair stand, 20 meter walk)
♦ Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences
  - Analgesia/NSAID accounting
♦ Laboratory studies / collections
  - Hemoglobin A1c

- Synovial fluid (if obtained) for cell count, crystal exam, and/or culture analysis
- Synovial fluid (if obtained) for archive

♦ Knee ultrasound exam
♦ Knee examination
♦ Knee injection of triamcinolone or placebo by ultrasound guidance

Month 9 (+/- 1 week window)

The following procedures will be done at this visit:

♦ Confirm that subject has had 48 hour wash-out of NSAIDs / Analgesics
♦ Vital signs (BP, temperature)
♦ Participant WOMAC© LK3.1, VAS global assessment
♦ Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences
  - Analgesia/NSAID accounting
♦ Laboratory studies / collections
  - Hemoglobin A1c
  - Synovial fluid (if obtained) for cell count, crystal exam
  - Synovial fluid (if obtained) for archive
♦ Knee examination
♦ Knee injection of triamcinolone or placebo by ultrasound guidance

Month 12 (+/- 1 week window)

The following procedures will be done at this visit:

♦ Confirm that subject has had 48 hour wash-out of NSAIDs / Analgesics
♦ Vital signs (BP, temperature)
♦ Height/Weight
♦ Participant WOMAC© LK3.1, SF-36, HAQ, VAS global assessment
♦ Participant physical function tests (chair stand, 20 meter walk)
♦ Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences
  - Analgesia/NSAID accounting
♦ Knee examination
♦ Laboratory studies / collections
  - Hemoglobin A1c
  - hsCRP
  - Serum CrossLaps
  - Serum for archive
  - Plasma for archive
  - Urine CrossLaps
  - Urine for storage
  - White Blood Cell (Buffy Coat) archive
  - Synovial fluid (if obtained) for cell count, crystal exam
  - Synovial fluid (if obtained) for archive
♦ MRI of study knee (*this exam will be done up to two weeks prior to Month 12*)
♦ DXA – bilateral hip, knee and lumbar spine

- Knee ultrasound exam
- Knee injection of triamcinolone or placebo by ultrasound guidance

Month 15 (+/- 1 week window)

The following procedures will be done at this visit:

- Confirm that subject has had 48 hour wash-out of NSAIDs / Analgesics
- Vital signs (BP, temperature)
- Participant WOMAC© LK3.1, VAS global assessment
- Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences
  - Analgesia/NSAID accounting
- Laboratory studies / collections
  - Hemoglobin A1c
  - Synovial fluid (if obtained) for cell count, crystal exam
  - Synovial fluid (if obtained) for archive
- Knee examination

Knee injection of triamcinolone or placebo by ultrasound guidance

Month 18 (+/- 1 week window)

The following procedures will be done at this visit:

- Confirm that subject has had 48 hour wash-out of NSAIDs / Analgesics
- Vital signs (BP, temperature)
- Participant WOMAC© LK3.1, VAS global assessment
- Participant physical function tests (chair stand, 20 meter walk)
- Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences
  - Analgesia/NSAID accounting
- Laboratory studies / collections
  - Hemoglobin A1c
  - Synovial fluid (if obtained) for cell count, crystal exam
  - Synovial fluid (if obtained) for archive
- Knee ultrasound exam
- Knee examination
- Knee injection of triamcinolone or placebo by ultrasound guidance

Month 21 (+/- 1 week window)

The following procedures will be done at this visit:

- Confirm that subject has had 48 hour wash-out of NSAIDs / Analgesics
- Vital signs (BP, temperature)
- Participant WOMAC© LK3.1, VAS global assessment
- Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences
  - Analgesia/NSAID accounting
- Laboratory studies / collections

- Hemoglobin A1c
- Synovial fluid (if obtained) for cell count, crystal exam
- Synovial fluid (if obtained) for archive

♦ Knee examination
♦ Knee injection of triamcinolone or placebo by ultrasound guidance

Month 24 (+/- 1 week window)

Month 24 is the final visit of the double-blind treatment period. The following procedures will be done at this visit:

♦ Confirm that subject has had 48 hour wash-out of NSAIDs / Analgesics
♦ Vital signs (BP, temperature)
♦ Height/Weight
♦ Participant WOMAC© LK3.1, SF-36, HAQ, VAS global assessment
♦ Participant physical function tests (chair stand, 20 meter walk)
♦ Collect and review diary/Issue new diary
  - Concomitant medications
  - Adverse experiences
  - Analgesia/NSAID accounting
♦ Laboratory studies / collections
  - Hemoglobin A1c
  - hsCRP
  - Serum CrossLaps
  - Serum for archive
  - Plasma for archive
  - Urine CrossLaps
  - Urine for storage
  - White Blood Cell (Buffy Coat) archive
  - Synovial fluid (if obtained) for cell count, crystal exam
  - Synovial fluid (if obtained) for archive
♦ MRI of study knee (this exam will be done up to two weeks prior to Month 24)
♦ *For 15 participants who volunteer, a 2nd MRI of study knee within 5 days of the first at the same MRI location
♦ Knee x-rays – bilateral, weight bearing, semi-flexed, posteroanterior (PA)
♦ DXA – bilateral hip, knee and lumbar spine
♦ Knee ultrasound exam

Premature Termination / Early Termination

A participant may choose with withdraw his or her study participation, or be withdrawn from the study by the investigator. The following procedures, as applicable, should be done:

♦ Confirm that subject has had 48 hour wash-out of NSAIDs / Analgesics
♦ Vital signs (BP, temperature)
♦ Height/Weight
♦ Participant WOMAC© LK3.1, SF-36, HAQ, VAS global assessment
♦ Participant physical function tests (chair stand, 20 meter walk)
♦ Collect and review diary
  - Concomitant medications
  - Adverse experiences

    - Analgesia/NSAID accounting
- Knee examination
- Laboratory studies /collections
    - Hemoglobin A1c
    - Serum CrossLaps
    - Serum for archive
    - Plasma for archive
    - Urine CrossLaps
    - Urine for storage
    - White Blood Cell (Buffy Coat) archive
    - Synovial fluid (if obtained) for cell count, crystal exam
    - Synovial fluid (if obtained) for archive
- MRI of study knee
- Knee x-rays – bilateral, weight bearing, semi-flexed, posteroanterior (PA)
- DXA – bilateral hip, knee and lumbar spine
- Knee ultrasound exam

Interim Visit

A participant may need to be seen in between scheduled visits. The following procedures, as applicable, should be done:

- Confirm that subject has had 48 hour wash-out of NSAIDs / Analgesics
- Vital signs (BP, temperature)
- Height/Weight
- Participant WOMAC© LK3.1, SF-36, HAQ, VAS global assessment
- Participant physical function tests (chair stand, 20 meter walk)
- Collect and review diary
    - Concomitant medications
    - Adverse experiences
    - Analgesia/NSAID accounting
- Knee examination
- Laboratory studies /collections
    - Hemoglobin A1c
    - Serum CrossLaps
    - Serum for archive
    - Plasma for archive
    - Urine CrossLaps
    - Urine for storage
    - White Blood Cell (Buffy Coat) archive
    - Synovial fluid (if obtained) for cell count, crystal exam
    - Synovial fluid (if obtained) for archive
- MRI of study knee
- DXA – bilateral hip, knee and lumbar spine
- Knee ultrasound exam

Safety Follow-Up

One month (28 days) following final study visit, certain participants will be contacted to follow-up on any adverse experiences that were noted at Month 24 (or premature termination) visit.

8.5.2 Blood Pressure Measurements

Blood pressure will be measured at every visit throughout the study. As recommended by National Heart, Lung, and Blood Institute[188], the auscultatory (manual) method of measurement should be used. Patients will be asked to sit quietly in a chair with feet on the floor for five minutes prior to measurement. Two measurements will be made, and the average computed and reported as the visit blood pressure.

We will use JNC 7 guidelines (Appendix J) in order to monitor those patients with elevated blood pressures and to determine whether patients exhibit "new hypertension" for study purposes. Per the American Heart Association, the study will define "new hypertension" as an average measurement (of two readings, at approximately 5 minutes apart) of 140 or greater systolic or 110 or greater diastolic on three separate occasions. Furthermore, the AHA does not specifically define "worsening hypertension". For this study, we will utilize the following definition, based on the JNC 7 and AHA guidelines described above: "Worsening hypertension" is an average measurement (of two readings, at approximately 5 minutes apart, on three separate occasions) of a systolic or diastolic blood pressure falling into a JNC category of at least "Stage 1 Hypertension" (>139 systolic or >90 diastolic) and higher than that which the patient has established in the study. The Screening visit measurement will serve as the initial "established" hypertension category for patients. For example, a patient with previously established Stage 1 hypertension whose Month 3 readings average to Stage 2 hypertension will be asked to self-monitor in the interim between his Month 3 and Month 6 visits.

"Three separate occasions" will be defined as 1-month intervals between readings. Therefore, patients who are asked to self-monitor between visits based on the criteria outlined above will be asked to report at-home measurements at 1 month and 2 months following the visit. Study staff will proactively call patients at these intervals to collect blood pressure readings. Patients whose readings remain elevated at all three measurements (study visit, study visit + 1 month, and study visit + 2 months) will be encouraged to consult with their primary care physician. We feel that monitoring of hypertensive thresholds is important considering the age demographics of our study population and the recognized potential hypertensive effects of the study drug as noted in Section 2.7 above.

8.5.3 Knee Exams

Bilateral knee exams will be performed at Screening and Baseline visits. The Screening exam will be completed by a Research Assistant/Coordinator, while the more comprehensive Baseline (musculoskeletal) exam will be completed by a study physician. The purpose of both exams is to ensure patient eligibility and rule out any forms of arthritis other than OA. Brief knee exams will also be performed on the participant's index knee by the injecting study physician prior to administering each injection to ensure the health of the knee for injection.

8.5.4 Laboratory

Tufts Medical Center will analyze blood samples for Hemoglobin A1c, Rheumatoid factor, anti-CCP and hsCRP. Pregnancy tests will be performed for all women of child-bearing potential prior to exposure to any radiation.

8.5.5 Radiology

Bilateral knee radiographs will be collected on all potential participants at Screening and at Month 24 (or Early Termination, if warranted). Participants will be screened for evidence of tibiofemoral OA with a Kellgren and Lawrence (K/L) score of 2 or 3, as evidenced on bilateral, PA semi-flexed, weight bearing knee x-rays. X-rays are obtained using a Synaflexor Positioning Frame (Synarc,

Inc. San Francisco, CA). X-rays will be evaluated for K/L score and joint space width (JSW) measurements.

8.5.6 Knee MRI

MRIs for this protocol will be done at either Tufts Medical Center or the Center for Biomedical Imaging (CBI) at Boston University Medical Campus. Tufts Medical Center radiologist Dr. R. Ward and CBI director Dr. Ronald J. Killiany. PhD, will develop the sequences and will supervise scanning. The protocol will utilize a Philips Achieva X-Series 3.0 Tesla scanner. This scanner includes the SmartKnee technology, an automated algorithm that ensures replication of the original parameters and positioning (and hence reproducibility) of repeat scans[189]. Participants will have three MRIs of their study knee; at Month 0, 12, and 24 (or ET if indicated).

8.5.6a Inter-Scanner Reliability Testing

Because the study is utilizing two separate facilities for MRI scans, we will employ a reliability test between the scanners at each site (Tufts MC and BU CBI). Each individual participant will be scanned at the same site at Baseline, 12, and 24 Months. However, while the MRI scanner will be consistent across time for a particular participant, multiple MRI scanners may introduce an additional source of between-subject error in MRI-based measurements. In consultation with the Data and Safety Monitoring Board we have opted to conduct an assessment of inter-scanner reliability between the Philips Achieva 3X-Series 3.0 Tesla scanners at Tufts Medical Center and Boston University. Ten participants will be recruited at Baseline and asked to volunteer to undergo two Baseline MRI scans: they will attend their standard MRI scan at Tufts Medical Center or Boston University, and within 5 days attend a second session to be scanned at the other site. The time between scans will be minimized to ensure that structural changes do not occur between scans. The additional Baseline MRI scan will require the participant to attend an extra 40 to 60 minute visit. Participants will receive additional compensation to cover the extra time and travel expense. The patient's follow-up scans will take place as normal at the location of the first Baseline scan. Data from the Tufts Medical Center and Boston University MRI scanners will be compared to determine inter-scanner reliability and agreement.

8.5.6b Assessment of Smallest Detectable Differences

Recent recommendations for assessing structural progression suggest that it is advantageous to present clinical outcomes at an individual level (e.g., percent progressors) based on a cut-off defined by change in outcomes greater than a smallest detectable difference[190]. Unfortunately, smallest detectable differences are poorly defined for MRI-based outcomes and may be very specific to a study population. Therefore, we will perform repeat MRI scans to determine smallest detectable differences in our study population. Fifteen participants will be recruited to undergo 2 Baseline (Month 0) and 2 24-month MRI scans at Boston University. We will make an effort to ensure that repeat scans during each time period (Baseline or 24-month follow-up) will be performed within 5 days in order to be certain that structural changes do not occur between repeat scans.

Fifteen participants will be recruited with anticipation that up to 3 participants may be lost at follow-up. With a sample size of 12 participants we will calculate smallest detectable differences for our study population. These participants will be expected to complete 2 extra MR scans, but to minimize participant burden the repeated scans will only include two sequences: one for measuring cartilage volume (3D WATSc) and a second for quantifying bone marrow lesions (sagittal PDW HR SPAIR). These extra visits will take 20

to 30 minutes each. Participants will receive additional compensation to cover the extra time and travel expense.

8.5.7 Knee Ultrasonography

Ultrasound examination of the knee will be performed in the Rheumatology Outpatient Clinic at Tufts Medical Center under the direction of Dr. J. Yinh. Participants will have six ultrasound knee exams of their study knee; at Month –1 (screening), 0 (baseline), 6, 12, 18, and 24 study visits. All IA injections will be done under ultrasound guidance to verify needle placement in the joint space.

8.5.8 DXA (Bone Density Testing)

Bilateral hip, study knee, lumbar spine, and total body composition DXA scans will be done in the Rheumatology Outpatient Clinic at Tufts Medical Center. These measurements will be obtained using the GE Lunar Prodigy Advance medical device dedicated for this study. The software applications for hip and spine DXA are provided by GE and are cleared by the FDA. The knee software is provided by GE, but is investigational in nature because it has not received FDA clearance. The knee software is not being used as a primary measurement tool, but as a secondary tool. Randomized participants will undergo three DXA studies; Month 0, 12, and 24. The weight and height measurements will be obtained on the same day as each DXA exam.

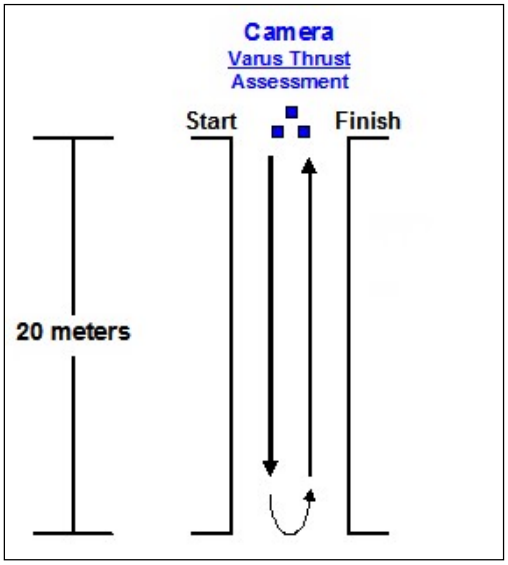
8.5.9 Physical Function Tests

Physical function will be assessed using the 20-meter walk test (to measure walking speed) and/or the chair stand test (to measure lower extremity strength and balance).

8.5.10 Observed Gait Analysis

In order to obtain dynamic gait assessments, including varus thrust, we will perform an observational gait analysis (OGA). We will video record the participants walking barefooted 20 meters down a corridor and turning around to walk another 20 meters back, at the participant's chosen speed. (See below for diagram.)

OGA will be obtained on consenting participants at Month 0, 12, and 24 study visits. Subjects who do not want to be videotaped may opt out of this portion of the protocol. Similarly, participants who require the use of a walking aid will be excluded from this portion of the protocol while still being able to participate in the remainder of this study.



8.6 PARTICIPANT COMPLIANCE

8.6.1 Investigational Product

Compliance of the IP will be ensured as the participant is either receiving the IACS or the IA placebo of Normal Saline at each study visit.

8.6.2 Study Visits

It is expected that randomized participants will complete all study visits. Participants missing visits will be contacted to reschedule missed appointments.

8.7 CONCOMITANT MEDICATIONS

The use of concomitant medications is allowed, **except** for the following:

- Other investigational drugs
- MMP-like medications (i.e., tetracyclines or related compounds e.g., Doxycycline)
- Chronic glucocorticoid use
- Glucocorticoid intra-articular injections
- Hyaluronic acid formulation intra-articular injections

At the screening visit all concomitant medications (prescription, over-the-counter, and complementary alternative medicines (CAMs)) taken within the previous 90 days will be recorded. The name, dose, frequency, and start/stop dates will be noted. Throughout the study all concomitant medications will be recorded in similar fashion.

8.7.1 Washout Period

Participants will be asked to discontinue use of any analgesics (NSAIDs, opioids) two full days prior to each pain assessment (including the baseline eligibility evaluation) and take only acetaminophen (up to 3 grams/day) if needed. If acetaminophen is consumed in the two days prior to the study visit, participants will be asked to record this in their study calendars.

8.8 UNANTICIPATED PROBLEMS AND ADVERSE EXPERIENCES/EVENTS (AE)

All Unanticipated Problems and Adverse Events will be reported according to Tufts Medical Center/Tufts University Health Sciences Institutional Review Board (IRB) and study DSMB guidance. The following protocol has been adapted from the Tufts IRB policies.

8.8.1 Definitions

Unanticipated Problem (UP)

Definition: An *Unanticipated Problem* is an incident, experience, or outcome that meets all of the following criteria:

1. The nature, severity, or frequency is unexpected for the subject population or research activities as described in the current IRB approved protocol, supporting documents, and the ICF(s).
2. It is related or possibly related to participation in the research.
3. It suggests the research may place the subject or others at a greater risk of harm than was previously recognized.

Adverse Event (AE)

Definition: An *AE* is any untoward or unfavorable medical occurrence in a human subject, including any abnormal physical exam or laboratory finding, symptom, or disease, temporally associated with a subject's participation in the research. Every *AE* is classified as:

1. *Non-serious* or *Serious*
2. *Related* (includes both definite and probable relationships), *Possibly Related*, or *Unrelated* to participation in the research.
3. *Expected* or *Unexpected* based on the known:
    a. Risks associated with drugs, devices, or other protocol activities described in the IRB approved protocol, supporting documents, and ICFs, or
    b. Natural progression of an underlying illness, or
    c. Health characteristics of the study population.

Serious Adverse Event (SAE)

Definition: A *SAE* is any *AE* that:

1. Results in *death*, or
2. Is *life-threatening*, or
3. Results in *hospitalization* or *prolongation of existing hospitalization*, or
4. Results in a *persistent or significant disability/incapacitation*, or
5. Results in a *congenital anomaly/birth defect*, or
6. May *jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed above*.

Determination of whether an Adverse Event is an Unanticipated Problem

An Unanticipated Problem is a major concern of an investigator and the IRB as it generally requires actions such as modification or suspension of the protocol, or informing subjects. **All three** of the criteria in the definition above must be met to be an *Unanticipated Problem*. This means that not all *Unexpected Serious Adverse Events* are *Unanticipated Problems* since some of them may, for example, not be related to the participation in the research study. Likewise, there does not have to be an *AE* to be an *Unanticipated Problem*.

For example, an event may be observed which is unexpected, related to participation in a study, but did **not** result in harm to the subject. If, however, it is determined that the subject (or others) are at an **increased risk** for harm, this would be an *Unexpected Problem*. *Unanticipated Problems* do not only include risks of physical harm, but also psychological, economic, or social harm. Please see the flow chart in Appendix A for more detail, and Appendix B for specific examples.

All *Unanticipated Problems* must be promptly reported to the IRB. *Unanticipated Problems* may also require prompt reporting to the appropriate institutional officials, the study sponsor or funding source (if applicable), the FDA (for drug associated events), and/or OHRP.

8.8.2 UP and SAE/AE Reporting

Unanticipated Problems

Each ***Unanticipated Problem*** requires immediate action by the principal investigator (PI) as follows:

1. Immediate corrective action must be taken to eliminate or minimize risk to enrolled subjects. This could necessitate a voluntary hold on further enrollment and/or research activities for already enrolled subjects. If subjects are at immediate risk, these corrective actions must be initiated immediately, and if necessary for subject safety, simultaneous with completion of reporting requirements. In such an instance, the PI should immediately call the IRB office.
2. Enrollment of new subjects should be voluntarily stopped until a revised protocol and/or ICF(s) are reviewed and approved by the Tufts Medical Center/TUHS IRB. In some situations enrollment may continue, provided new subjects are not at risk, and the PI provides the IRB the necessary documentation in support of continuation of enrollment. It may also be necessary for the IRB to formally suspend a study under certain conditions.
3. The problem must be promptly reported to the Tufts Medical Center/TUHS IRB, and the DSMB (via KAI).
    a. An initial report to the Tufts MC/TUHS IRB and DSMB must be submitted in writing no later than two (2) business days after the PI/study team become aware of the problem. This report is to briefly summarize the nature of the event, summarize the corrective action plan as developed and initiated at that time, and clarify whether subject enrollment is continuing. In the rare circumstance where an original written report cannot be submitted directly to the IRB office, it may be faxed within 2 business days (617-636-8394). The IRB office may be contacted by phone at 617-636-7512 for necessary guidance, and PIs are encouraged to do so.
    b. An *Event Reporting Form* must be completed with accompanying documentation addressing each item in this list and submitted to the Tufts MC/TUHS IRB and DSMB no later than five (5) business days after the PI/study team became aware of the problem.

Serious Adverse Events (SAE)

Reporting requirements vary for *SAEs* as follow:

1. A *SAE* that is *Related* or *Probably Related* and *Unexpected* meets criteria for an *Unanticipated Event* and should be acted upon as outlined above.
2. An *Internal SAE* not meeting criteria for an *Unanticipated Problem* must be reported to the DSMB within 48 hours and to Tufts Medical Center/TUHS IRB within fifteen (15) business days of the PI/research team learning of the event; the *SAE/UP Reporting Form* must be used.
    a. If changes are required to the protocol and/or ICF(s), subject enrollment and study activities related to the AE, and not necessary for subject safety, cannot continue until the changes have been reviewed and approved by the Tufts Medical Center/TUHS IRB.

Non-serious Adverse Events

1. All clinically significant *Internal Non-serious Adverse Events* not meeting criteria of an *Unanticipated Problem* can be summarized and submitted to the DSMB and Tufts Medical Center/TUHS IRB at the time of the continuing review, or when the PI terminates the study if this occurs before the date of the next continuing review.

Table 1. Guidelines for reporting *Unanticipated Problems* and *Adverse Events* to the Tufts MC/TUHS IRB.

Unanticipated Problem: *Internal*	Immediate reporting as described above Completed *SAE/UP Reporting Form* with supporting documents submitted to IRB within 5 business days
SAE: *Internal*, *Related* or *Probably Related, Unexpected*	Meets criteria for an ***Unanticipated Problem*** and is to be reported as such
SAE: *Internal*, all other situations	Complete *SAE/UP Reporting Form* and submit to IRB within 15 business days but notify the DSMB within 48 hours.
Non-Serious AE: *Internal*, all situations not considered an *Unanticipated Problem*	Using the *Summary Reporting Form* for clinically significant *AEs* may be summarized at the time of Continuing Review, or study termination if before the next scheduled Continuing Review

8.8.3 UP/AE/SAE Monitoring

An independent Data Safety Monitoring Board (DSMB) will be assembled under the direction of KAI Research, Inc. (under contract with NIAMS). KAI will assemble the DSMB and the protocols for AE/SAE reporting and monitoring.

Study participants will be monitored for occurrence of adverse events per the protocol described above. This will be done proactively by (i) posing structured and open-ended questions at each visit to obtain information on undesirable experiences and (ii) by exerting surveillance of all laboratory tests. Also, a telephone number will be provided to participants to enable them to report any such experiences at any time to study personnel. Any participants who experience an adverse event between the hours of 5 PM and 8 AM that they feels requires medical attention should call the hospital page operator (617-636-5000) and ask to speak to the rheumatology fellow on call. If there is a life-threatening emergency, the participant should call 911 and go to the nearest emergency room.

8.9 EARLY WITHDRAWAL/TERMINATION OF PARTICIPANTS

Every effort will be made to keep enrolled participants in the study since it has an 'intent-to-treat' design and withdrawals / early terminations / 'drop-outs' will not be replaced.

Participants may be prematurely terminated from the study for the following reasons:

- An adverse experience with such severity that continuation in the study is unsafe
- Failure to keep study appointments
- Relocation such that the participant is not able to travel to Tufts Medical Center
- Participant personal reasons, including withdrawal of consent
- Lost to follow-up after all reasonable attempts to locate the participant for early termination visit
- Joint replacement surgery, osteotomy, arthroscopy, arthrocentesis or other invasive procedure on the study knee

9. Computational Analyses

Hypotheses 1a,b: Knees with OA and synovitis treated with IACS for 2 years will exhibit (a) slower rates of cartilage volume loss (our primary *structural* outcome) (b) less progression in bone marrow lesions and subchondral bone attrition.

Hypotheses 2a,b: The treated knees will exhibit less change in (a) tibial subchondral bone mineral density and (b) apparent bone volume fraction.

Hypotheses 3a,b,c: The group assigned to IACS will have less (a) overall pain (our primary *clinical* outcome), (b) decline in physical function and (c) healthcare utilization

We will perform descriptive analyses of baseline characteristics of the treated and placebo groups to permit assessment of generalizability and success of randomization. All analyses will be stratified by the factors used to stratify the randomized treatment assignment: radiographic severity of knee OA at baseline (Kellgren and Lawrence grade of 2 or 3) and gender. We will use either linear regression analyses (stratified by radiographic severity and gender) or rank-based stratified Wilcoxon tests (also known as van Elteren's test) whenever appropriate. Our primary analytic approach will be to use intention-to-treat (ITT) analyses with mixed effects regression models for the longitudinal repeated measures data. We will also evaluate the dropout and adherence patterns of trial subjects to see if these issues may change the assessment of treatment effect.

Table 4. Conceptualization of the study measures

	Structural outcome	***Clinical outcome***	***Covariate***
Primary	cartilage volume	WOMAC© LK3.1 pain	knee alignment
Secondary	BML Score subchondral attrition tibial medial:lateral BMD ratio tibial subchondral aBVF tibial BMD femoral neck BMD	WOMAC© LK3.1 function Physical function healthcare utilization	femoral neck BMD (synovitis; US and/or MRI)

Our analyses will evaluate the cartilage volume and WOMAC© LK3.1 pain scores over time using mixed effects regression models (incorporating both fixed and random effects) for longitudinal repeated measures to see if there is a difference in the trajectory of the structural and clinical outcome measures over time between the treatment groups[191]. The two co-primary endpoints are (i) cartilage loss (structural) and WOMAC© LK3.1 pain (clinical). The models will include a random intercept for each subject, 3 dummy variables corresponding to the 4 randomization strata (gender and radiographic severity of KOA at entry to the trial (Kellgren and Lawrence grades 2 vs. 3)), use of analgesia (acetaminophen) at the times of outcome measurement, a time effect, an effect of treatment, and the interaction between time and treatment that will provide the basis for testing hypothesis 1 (i.e. testing if the trajectories are different between treatment groups). The effect of time will be assumed to be linear in this model unless preliminary data analysis suggests that the cartilage volume or WOMAC© LK3.1 score have a distinctly non-linear trend over time. The compound symmetry variance structure will be assumed for the correlation between repeated measures over time within a subject. If the likelihood cannot be maximized for this model, then the covariance structure will be simplified by removing the random intercept. In secondary analyses we will evaluate whether the clinical conclusions drawn from this trial are sensitive to the choice of different modeling methods.

For secondary analyses, we will employ linear regression analyses and the rank-based stratified Wilcoxon test (van Elteren's test), both stratified by radiographic severity and gender, to evaluate the difference between treatment groups in the change in WOMAC© LK3.1 scores or cartilage volume, as

that is how these outcomes have often been analyzed. Multiple imputation will be used to address missing data due to dropout from the trial in the secondary analysis of change as well as to provide an alternative analysis of the longitudinal repeated measures from the primary analysis. This will allow us to gauge the sensitivity of the primary analysis to the dropout from the trial. Additional secondary analyses will use alternative regression modeling approaches, including ordinal logistic regression[192], and regression tree models[193].

The influence of exposures that might exert intermediary or confounding effects on the outcomes (e.g. knee OA severity, radiographic knee alignment, use of analgesia, and BMI) will be tested by performing stratified analyses and by entering them as covariates in the linear regression models (Table 4). The ways in which all these variables might interact are complex so we will interpret our results in respect of differentiating confounding from an intermediary role on the basis of biologically based a priori hypotheses.

10. Investigator's Regulatory Obligations

10.1 INSTITUTIONAL REVIEW BOARD

The study protocol, participant informed consent form, and any recruitment materials will be reviewed and approved by the Tufts Medical Center IRB prior to the start of participant recruitment.

10.2 TUFTS MEDICAL CENTER RHEUMATOLOGY OUTPATIENT CLINIC

The study will occur at the Tufts Medical Center's Adult Rheumatology Outpatient Clinic, Tufts Clinical and Translational Research Center and the Boston University Center for Biomedical Imaging.

10.3 DATA AND SAFETY MONITORING BOARD (DSMB)

NIAMS has developed guidelines for data safety monitoring board activities for NIAMS funded studies and has appointed KAI to assemble and administer a DSMB for this trial. Tufts Medical Center Division of Rheumatology will work with NIAMS and KAI Research, Inc in the formation of and procedures for data and safety monitoring.

DSMB meeting minutes will be copied and forwarded to the Tufts Medical Center IRB.

10.4 RECORDS/DATA RETENTION

Research records and data will be kept for a minimum of 7 years after study completion. This requirement is as outlined in the Policies and Operations Manual for the Institutional Review Board of Tufts Medical Center and Tufts University Health Sciences (Version date January 7, 2009).

11. References

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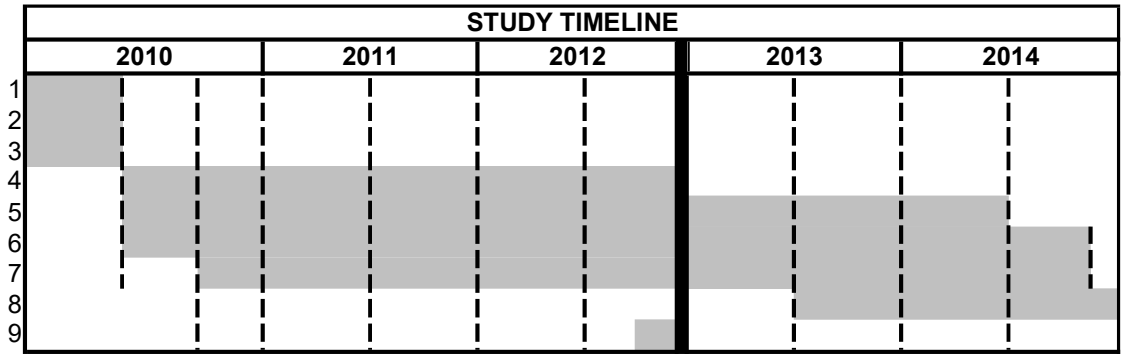
Appendix A - Study Timeline



1. Development and testing of the website and electronic data capture systems
2. Construction of the manual of operations, assembly of DSMB, start-up DSMB meeting
3. Acquisition and deployment of study materials (e.g. injection kit, ultrasound scanner, medication)
4. Advertising and recruitment
5. Conduct of the clinical trial
6. Quality control of study DXA scans and MRIs
7. Interpretation, scoring of paired MRIs
8. Data processing, cleaning & analysis; presentation of findings; manuscript preparation
9. **Adaptive interim analysis**. Timeline for subsequent study activities will be truncated if the study is stopped early.

Appendix B - Schedule of Visits

When→ What ↓	Month -1 Screen	Month 0	Month 3	Month 6	Month 9	Month 12	Month 15	Month 18	Month 21	Month 24	Early Term (ET)*
Consents	x										
Screening ultrasound	x										
Medical-Surgical history	x										
Blood tests	x	x	x	x	x	x	x	x	x	x	x
Vitals (BP, temp)	x	x	x	x	x	x	x	x	x	x	x
Review all medicines	x	x	x	x	x	x	x	x	x	x	x
Review of adverse events / side effects		x	x	x	x	x	x	x	x	x	x
Review your study calendar/diary/journal		x	x	x	x	x	x	x	x	x	x
Knee exam	x	x	x	x	x	x	x	x	x		x
MRI coil fit test	x										
Questionnaires	x	x	x	x	x	x	x	x	x	x	x
Physical function tests	x	x		x		x		x		x	x
Observed Gait Analysis (optional)		x				x				x	
Knee X-rays	x									x	x
Knee synovial fluid for storage		x	x	x	x	x	x	x	x	x	x
Blood collection (serum, plasma, and white cell) for storage		x				x				x	x
Urine collection for storage		x				x				x	x
Bone density test		x				x				x	x
Height & weight	x	x				x				x	x
Knee ultrasound		x		x		x		x		x	x
Knee study injection		x	x	x	x	x	x	x	x		
MRI of study knee ‡		x				x				x	x

*Early Termination (ET) visit is performed only if the participant withdraws from the study prior to completion.

‡ Patients volunteering to participate in Inter-Scanner Reliability Testing will undergo 2 MRIs not more than 5 days apart between the Screening and Month 0 visits. Patients volunteering to participate in MRI Assessment of Smallest Detectable Differences will undergo 2 MRIs, not more than 5 days apart, at Boston University CBI both between the Screening and Month 0 visits and at Month 24.

Appendix C - ACR Criteria

[1986] Criteria for Classification of Idiopathic Osteoarthritis (OA) of the Knee*.

Clinical and laboratory	**Clinical and radiographic**	**Clinical†**
Knee pain	Knee pain	Knee pain
+ at least 5 of 9:	+ at least 1 of 3:	+ at least 3 of 6:
- Age > 50 years	- Age > 50 years	- Age > 50 years
- Stiffness < 30 minutes	- Stiffness < 30 minutes	- Stiffness < 30 minutes
- Crepitus	- Crepitus + Osteophytes	- Crepitus
- Bony Tenderness		- Bony Tenderness
- Bony enlargement		- Bony enlargement
- No palpable warmth		- No palpable warmth
- ESR <40 mm/hour		
- RF <1:40		
- SF OA		
92% sensitive	91% sensitive	95% sensitive
75% specific	86% specific	69% specific

* ESR = erythrocyte sedimentation rate (Westergren); RF = rheumatoid factor; SF OA = synovial fluid signs of OA (clear, viscous, or white blood cell count <2,000/mm^3).

† Alternative for the clinical category would be 4 of 6, which is 84% sensitive and 89% specific.

R. Altman, E. Asch, D. Bloch, G. Bole, D. Borenstein, K. Brandt, et al. The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the knee. Arthritis Rheum 1986;29:1039--1049.

Appendix D - K/L Grading System

Kellgren and Lawrence Radiographic Grading System for Osteoarthritis

Grade	Classification	Description
0	Normal	No features of OA
1	Doubtful	Minute osteophyte, doubtful significance
2	Minimal	Definite osteophyte, unimpaired joint space
3	Moderate	Moderate diminution of joint space
4	Severe	Joint space greatly impaired with sclerosis of subchondral bone

Appendix E - Questionnaires

WOMAC® LK3.1 Questionnaire

The Western Ontario and McMaster Universities OA Index (WOMAC® LK3.1), is a tri-dimensional, self administered, patient-centered health status questionnaire. Its item inventory has been designated to capture the essential elements of pain, stiffness, and physical disability in patients with OA of the knee and/or hip joints. It contains twenty-four items (5 pain, 2 stiffness, and 17 physical function) and takes approximately ten minutes for the participant to complete.

The WOMAC® LK3.1 Index has been subject to more than 30 studies examining its basic clinimetric properties and has been translated into over 70 different language forms. Its ability to detect change in health status has been demonstrated following patient exposure to a variety of different interventions - NSAIDs, COX-2 Inhibitors, Analgesics, Viscosupplements, Physiotherapy and Orthopaedic surgery - making WOMAC® LK3.1 a standard instrument utilized in clinical studies.

WOMAC® LK3.1 scoring will be done according to User Guide IX (©2009) instructions, for which the study has an established User Agreement in place. Based on conversations pertaining to the potential for change in the WOMAC® LK3.1 in the interim between the Screening and Month 0 visits, we will use the Screening WOMAC® LK3.1 score as the baseline score for statistical analysis purposes.

SF-36v2™

The SF-36 is a multi-purpose, short-form health survey consisting of 36 questions. It yields an 8-scale profile of functional health and well-being scores as well as psychometrically-based physical and mental health summary measures and a preference-based health utility index. It is a generic measure, as opposed to one that targets a specific age, disease, or treatment group. Accordingly, the SF-36 has proven useful in surveys of general and specific populations, comparing the relative burden of diseases, and in differentiating the health benefits produced by a wide range of different treatments.

PROMIS Health Assessment Questionnaire

The PROMIS Health Assessment Questionnaire (HAQ) is a well-validated and widely used instrument that originated from work on rheumatic disease populations and collects data on domains of disability, pain, adverse treatment effects and healthcare costs[196]. The elements that we will utilize for this study include the H*AQ Disability Index,* which assesses a patient's level of functional ability, as well as questions about drug toxicity, and medical cost data both direct (e.g. physician visits, medications) and indirect (e.g. loss of productivity).

Global Knee Pain Severity Scale (VAS Global Assessment)

Knee pain severity (not activity-specific) during the past 30 days is assessed using a 0-10 numerical rating scale. The validity of numerical rating scales has been well documented, they are easy to administer and score, and can be used with a greater variety of participants than can a visual analog scale. Numeric rating scales can also be administered over the telephone to participants who become unable to visit the clinic.

Alignment Questionnaire

To assess history of malalignment during young adult years, each participant will complete at their convenience, one survey with visual depictions of various knee alignments that has been previously validated. [197, 198]The participants will be asked both about their current alignment as well as their recalled alignment during their 20s.

Appendix F - BU/CBI MRI Screening Form

Subject Name:_____

For staff only:	
Session #:	**IRB #:**
Operator name and #	

BU Safety Screening Form

1. Do you have a problem with claustrophobia (fear of closed spaces?)
   No_____ A little _____ Pretty much _____ Severe _____
2. Do you have a heart pacemaker or defibrillator or other implanted devices? If yes, describe.
   No _____ Yes _____
3. Have you ever had an operation? If yes, Investigator to fill out Page 2.
   No _____ Yes _____
4. Have you ever been injured by metallic foreign body which was not removed?
   No _____ Yes _____
5. Do you wear braces on your teeth, or do you have false teeth or removable bridgework? Do you have any unremovable body piercings?
   No _____ Yes _____
6. Do you have any tatoos? If yes, describe their location.
   No _____ Yes _____
7. (Females only): Is there any possibility that you are pregnant?
   No _____ Yes _____
8. Please list medications you took today or are taking regularly.
   (try to include the name of the medicine, dose, how often, and time of last dose).
9. Have you ever had any previous studies (MRI, CT or other)? If yes circle on list.
   No _____ Yes _____
10. Do you have a breathing disorder or movement disorder? If yes describe.
    No _____ Yes _____
11. Weight: (lbs)_____ Birthdate:___/___/____

_____ Date: / /
Signature of Person Completing Page 1

1

Investigator to complete if Item #3 on Page 1 is Yes.

Some of the following items may be hazardous to your safety and some can interfere with the MRI examination. Please check the correct answer for each of the following. Do you have any of the following:

o Yes o No	Cardiac pacemaker
o Yes o No	Implanted cardiac defibrillator
o Yes o No	Aneurysm clip(s)
o Yes o No	Carotid artery vascular clamp
o Yes o No	Neurostimulator
o Yes o No	Insulin or infusion pump
o Yes o No	Implanted drug infusion device
o Yes o No	Bone growth/fusion stimulator
o Yes o No	Cochlear, otologic, or ear implant
o Yes o No	Any type of prosthesis (eye, penile, etc.)
o Yes o No	Heart valve prosthesis
o Yes o No	Artificial limb or joint
o Yes o No	Electrodes (on body, head, or brain)
o Yes o No	Intravascular stents, filters, or coils
o Yes o No	Shunt (spinal or intraventricular)
o Yes o No	Vascular access port and/or catheter
o Yes o No	Swan-Ganz catheter
o Yes o No	Any implant held in place by a magnet
o Yes o No	Transdermal delivery system (Nitro)
o Yes o No	IUD or diaphragm
o Yes o No	Tattooed makeup (eyeliner, lips, etc)
o Yes o No	Body piercing(s)
o Yes o No	Any metal fragments
o Yes o No	Internal pacing wires
o Yes o No	Aortic clip
o Yes o No	Metal or wire mesh implants
o Yes o No	Wire sutures or surgical staples
o Yes o No	Harrington rods (spine)
o Yes o No	Metal rods in bones
o Yes o No	Joint replacement _____
o Yes o No	Bone/joint pin, screw, nail, wire, plate
o Yes o No	Hearing aid ***(Remove before MRI)***
o Yes o No	Dentures ***(Remove before MRI)***

NOTE: YOU ARE REQUIRED TO WEAR EARPLUGS OR EARPHONES DURING THE MRI EXAMINATION.

---------- Date: / /

Signature of Investigator Completing Page 2

Tufts Medical Center

Department of Radiology

MRI OUTPATIENT SCREENING PATIENT INFORMATION

2-Hole 1/4 2 3/4 - 3-Hole 1/4 4 1/4

1) Patient Name: _____ Hospital #: _____

2) Has placement of an intravenous line been difficult in the past? ☐ Yes ☐ No If yes, describe: _____

3) Do you have an intravenous access device? ☐ Yes ☐ No If yes, which? ☐ Broviac ☐ Hickman ☐ Infusaport

4) Do you have any allergies to food, medications or latex? ☐ Yes ☐ No If yes, list

5) Please list medications, including over the counter medications, with the doses that you currently take.

6) Please list surgical operations that you have had

7) Do you have any of the following medical problems?

Asthma or Shortness of breath:	☐ Yes	☐ No	Sickle cell anemia:	☐ Yes	☐ No
Hypertension:	☐ Yes	☐ No	Multiple myeloma:	☐ Yes	☐ No
Diabetes:	☐ Yes	☐ No	Pheochromocytoma:	☐ Yes	☐ No
Kidney failure:	☐ Yes	☐ No	Heart disease:	☐ Yes	☐ No

8) Have you eaten in the last four hours? ☐ Yes ☐ No

9) Are you pregnant? ☐ Yes ☐ No ☐ Do not know Date of last period? _____

10) Patient Weight _____ lb

11) Have you had a prior MRI exam(s)? ☐ Yes ☐ No If Yes, where and date _____

MRI: MR Safety - Please check the appropriate box if you have any of the following:

	YES	NO		YES	NO
Cardiac pacemaker, pacer wires			Renal transplant clips		
EKG leads			Insulin pump		
Transdermal medication patches			Transcutaneous nerve simulator		
Implantable Device or ICD			Biostimulator		
Intracranial aneurysm clips			Cardiac arrhythmias		
Vascular clips			Kidney problems		
History of metal in eyes or metal work, Shrapnel			Back or neck surgery		
Heart valve prosthesis			Back or neck pain		
Joint or limb prosthesis			Numbness or tingling		
Joint rods, screws, pins, etc.			Hair Wig		
Middle ear prosthesis			Claustrophobia		
Eye/Orbital prosthesis			Dentures, retainers or braces		
Hearing Aid			Metal IUD		
Body Piercing Date/Location			Tattoo Date/Location		

Have you ever had an IV injection of Gadolinium (MRI IV Contrast)? ☐ Yes ☐ No

If yes, did you experience any difficulty with it? (please explain) _____

Technologist signature completing medication reconciliation _____ Date _____

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Appendix H - Lab Testing

Laboratory Testing TMC Central Lab

Hemoglobin A1c – Collect in 3 mL EDTA (lavender top).

Rheumatoid Factor – Collect in 5 mL SST (gold top).

anti-CCP – Collect in 5 mL SST (gold top).

hsCRP – Collect in 5 mL SST (gold top).

Note: All 3 are collected in the same 5 mL tube.

Urine Pregnancy Test – Collect in 120 mL urine collection cup.

Laboratory for Storage

Plasma – collected in 10 mL EDTA (lavender top).

Buffy Coat – collect white blood cell layer from plasma tube.

Serum – collected in 10 mL SST (tiger top).

Urine – random urine sample to store 5 mL of urine.

Synovial Fluid (knee) – any synovial fluid drained from the knee at any visit will be stored.

SAE and UAP Report Form

Tufts Medical Center/Tufts University Health Sciences Institutional Review Board (IRB):
SERIOUS ADVERSE EVENT and UNANTICIPATED PROBLEM REPORTING FORM

This form is to be typed and submitted to Box 817. Please complete each field and indicate "N/A" as needed; do not leave fields blank. The form is designed to be completed in Microsoft Word with self-expanding text boxes. Please print after completion, sign and date, and submit with accompanying documentation to the IRB. When completing this form, refer to the IRB *Unanticipated Problem and Adverse Event Reporting Policy*. When completing this form please contact the IRB Office at (617) 636-7512 with questions.

This form is for Tufts MC/TUHS IRB reporting only. Reporting requirements for outside agencies (for example, study sponsor, funding agency, FDA) are the responsibility of the Principal Investigator (PI).

IRB #: Principal Investigator:

Protocol Title:

Study Subject Identification (Initials/Study ID#)

Report Type (check all that apply): ☐ Internal ☐ External ☐ Initial ☐ Follow-up

Date of Event: Date of Report:

Please provide a summary description of the event. Attach additional pages, as needed, along with other supporting documentation.

A. ***Serious Adverse Event (SAE).***

Nature of event (check all that apply):

- ☐ resulted in *death*
- ☐ was *life-threatening*
- ☐ resulted in *hospitalization* or *prolongation of existing hospitalization*
- ☐ resulted in a *persistent or significant disability/incapacitation*
- ☐ resulted in a *congenital anomaly/birth defect*
- ☐ may *jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed above.*

☐ Is one or more of the above 6 boxes checked? If yes, check this box; **event is an *SAE*.**

B. ***Unanticipated Problem.***

Event (check all that apply):

- ☐ was *Unexpected*
- ☐ was *Related* or *Probably Related* to participation in the study

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☐ may place subject(s) or others at greater risk of harm than *previously* recognized

☐ Are all 3 of the above boxes checked? **If yes, check this box; event is an *Unanticipated Problem***

C. Status of research activities.

Please check all that apply:

☐ No change in research activities

☐ All research activities have been temporarily and voluntarily stopped for all subjects

☐ Partial voluntary hold on some research activities for all subjects (please detail below)

☐ Voluntary hold on new subject enrollment only

If event is an *Unanticipated Problem*, please describe how subject safety for continuing research activities is being ensured, and describe corrective actions already taken to ensure safety of currently enrolled subjects:

D. Study protocol and informed consent form(s) (ICF).

Please check all that apply:

Study protocol

Requires changes as a result of the event* ☐ Yes ☐ No

ICF(s)

Requires changes as a result of the event* ☐ Yes ☐ No

If yes, do currently enrolled subjects require notification or re-consenting as a result of the event*? ☐ Yes ☐ No

If yes, detail when and how notification and/or re-consent will occur (*e.g.*, at next clinic appointment, immediate telephone notification to subjects):

* All protocol and ICF revisions or other new materials (*e.g.*, a letter of subject notification) must be approved by the IRB before study enrollment can proceed, and, when indicated, subject participation in study activities may continue.

If protocol changes, ICF changes, subject notification, or subject re-consent are not required, please provide a brief justification:

Report prepared by: _____ Date:
Please print name

_____
Please sign

SAE and UAP Report Form

PI attestation:

I have reviewed all of the information included in this report and confirm it is accurate based on review of all available information concerning the reported event.

Principal Investigator: _____ Date:
Please print name

_____
Please sign

IRB Office Review – Office use only:

☐ Report reviewed; no further action required at this time.
☐ Report and proposed corrective actions approved; protocol and ICF changes to be reviewed separately.
☐ Report and proposed corrective actions reviewed; recommend further review by convened IRB.
☐ Report and proposed corrective actions reviewed; additional information required from PI:

_____ _____
Signature of Reviewer Date

_____
Printed name of Reviewer

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Appendix J – NHLBI JNC 7 Blood Pressure Classification Chart

CLASSIFICATION OF BLOOD PRESSURE (BP)*			
CATEGORY	SBP MMHG		DBP MMHG
Normal	<120	and	<80
Prehypertension	120–139	or	80–89
Hypertension, Stage 1	140–159	or	90–99
Hypertension, Stage 2	≥160	or	≥100

* See *Blood Pressure Measurement Techniques* (reverse side)
Key: SBP = systolic blood pressure DBP = diastolic blood pressure

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health
National Heart, Lung, and Blood Institute

The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Bethesda MD: National Heart, Lung, and Blood Institute (US) 2004.

- Protocol: Summary of Changes –

Protocol Version 1 (April 12, 2010)

- Original version (within document)

Protocol Version 2 (June 18, 2010)

- Clarified the amount patients will be paid per visit in ICF.
- Described in more detail serious medical conditions that would exclude subjects from study participation.
- Expanded to include that women of child-bearing potential will be required to use birth control.

Protocol Version 3 (June 29, 2010)

- Payment section added to the protocol
- Updated ICF to include randomization and possibility of receiving placebo in "purpose of study" section (already in later pages of ICF)
- Revised Research Authorization Form for Limited Release of Protected Health Information
- Revised study advertisements

Protocol Version 4 (October 8, 2010)

- Updated and expanded abbreviations and definitions in ICF
- Updated exclusion criteria

Protocol Version 5 (February 16, 2011)

- (New and updated) Abbreviations page in protocol
- ACR Criteria included in Eligibility Criteria
- Exclusion criterion "prior osteonecrosis" updated to specify the index knee
- Evidence of other inflammatory joint disease added to exclusion criteria
- Hemoglobin A1c to be used instead of random glucose
- Added option for local analgesia

Protocol Version 6 (April 11, 2011)

- Clarified inclusion criteria to include Screening WOMAC instead of Baseline WOMAC
- Added physical function testing to Screening

Protocol Version 7 (August 3, 2011)

- Clarified visit windows beginning at Month 3
- Added Boston University as an MRI site
- Added body composition
- Added Boston Medical Center and Tufts Medical Center MRI safety questionnaires to appendix

Protocol Version 8 (January 25, 2012)

- Added hyaluronic acid injection within last 6 months prior to Baseline as exclusion criterion
- Added uncontrolled HIV as exclusion criterion
- Removed language regarding data capture of subject meal times since there is no fasting blood test for this study
- Added pre-paid debit card as payment method
- Added ancillary MRI studies, per DSMB request
- Added hsCRP blood test to Months 12 and 24
- Added knee ultrasound exam to Month 24
- Clarified procedure for conducting blood pressure tests per AHA guidelines
- Added clarification of pregnancy test timing with regard to radiation exposure

Protocol Version 9 (May 2, 2012)

- Added knee alignment self-report questionnaire

Protocol Version 10 (April 15, 2014)

- Removal of Section 10: Adaptive Interim Monitoring

-Original statistical analysis plan-

We will perform descriptive analyses of baseline characteristics of the treated and placebo groups to permit assessment of generalizability and success of randomization. We will use either Student t-tests or rank-based nonparametric tests whenever appropriate. Our primary analytic approach will be to use intention- to-treat (ITT) analyses with mixed effects regression models for the longitudinal repeated measures data. We will also evaluate the dropout and adherence patterns of trial subjects to see if these issues may change the assessment of treatment effect. The likelihood based mixed effects model has been shown to be less biased in the analysis of longitudinal data from clinical trials with dropouts than either deleting subjects who withdraw or imputing results using last observation carried forward7. The specific endpoints that we will test and their conceptualization as outcomes or intermediaries are presented in Table 4. In section E16 we propose use of an adaptive method for interim monitoring that will use these analyses performed on the first half of the trial subjects, and, if necessary, separately on the second half of the subjects as well.

Our analyses will evaluate the cartilage volumes and WOMAC pain scores over time using mixed effects regression models (incorporating both fixed and random effects) for longitudinal repeated measures to see if there is a difference in the trajectory of the structural and clinical outcome measures over time between the treatment groups249. The two co-primary endpoints are (i) cartilage loss (structural) and WOMAC pain (clinical). The models will include a random intercept for each subject, 3 dummy variables corresponding to the 4 randomization strata (gender and radiographic severity of KOA at entry to the trial (Kellgren and Lawrence grades 2 vs. 3)), use of analgesia (acetaminophen) at the times of outcome measurement, a time effect, an effect of treatment, and the interaction between time and treatment that will provide the basis for testing hypothesis 1 (i.e. testing if the trajectories are

different between treatment groups). The effect of time will be assumed to be linear in this model unless preliminary data analysis suggests that the cartilage volumes or WOMAC score have a distinctly non-linear trend over time. The compound symmetry variance structure will be assumed for the correlation between repeated measures over time within a subject. If the likelihood cannot be maximized for this model, then the covariance structure will be simplified by removing the random intercept. In secondary analyses we will evaluate whether the clinical conclusions drawn from this trial are sensitive to the choice of different modeling methods.

For secondary analyses, we will employ the Student-t test and the rank-based Wilcoxon test to evaluate the difference between treatment groups in the change in WOMAC scores or cartilage volume, as that is how these outcomes have often been analyzed. The LOCF approach will be used to address dropout from the trial in these analyses of change over the duration of the trial. Additional secondary analyses will use alternative regression modeling approaches, including ordinal logistic regression250, and regression tree models.

The influence of exposures that might exert intermediary or confounding effects on the outcomes (e.g. knee OA severity, serum 25(OH)D, radiographic knee alignment, use of analgesia, and BMI) will be tested by performing stratified analyses and by entering them as covariates in the linear regression models (Table 4). The ways in which all these variables might interact are complex so we will interpret our results in respect of differentiating confounding from an intermediary role on the basis of biologically based a priori hypotheses.

For interim monitoring, we will use the adaptive approach due to Bauer and Köhne6. This approach to interim monitoring is based on the use of Fisher's method of combing p-values from separate experiments252 rather than repeated analyses of accruing data, such as the Peto method. Fisher's method rejects a common null hypothesis based on two independent experiments with significance of 0.05 if $p1*p2 \leq 0.0087$, where this cutoff is based on the chi-square distribution with 4

degrees of freedom. In this case, the p1 would be the p-value for the null hypothesis of no treatment effect in the first half of the subjects, and p2 would be the same for the second half of the trial subjects. Using this relationship, Bauer and Köhne develop cutoffs for p1 would that would allow early stopping while maintaining a significance level of 0.05 for the product of the p-values. Using the values from their Table 1 we will stop the trial for success if $p1 \leq 0.0233$. The trial would be stopped for futility if $p1 > 0.5$. If p1 satisfies neither of these conditions, then the trial would continue, and the second half of the subjects would be analyzed separately at the end of the trial for p2; then the product of p1 and p2 would be

compared to 0.0087. This adaptive method for interim monitoring allows us to use the mixed effects analysis as outlined in section E.15 for the primary outcome of cartilage volume; this will be performed on the first half of the trial subjects at the interim analysis, and, if the trial continues, separately on the second half of the subjects at the trial's end.

Bauer and Köhne note that the power loss due to the adaptive interim analysis is quite small when compared to the best single test on the complete data when the sub-samples are the same size, and when stopping for futility is only done when p1 is at least as large as 0.5. In this setting they found 77.8% power for trials where a single complete analysis would have 80% power. In our simulations presented in section E.17, we found a slightly larger, but manageable, loss of power due to the adaptive interim analysis. The gain from this interim analysis is from the possibility of early stopping of the trial, which would significantly reduce the number of MRIs that need to be acquired and read in the second half of the subjects, and the truncation of their treatment and follow-up. It would also allow early closing of the study and earlier dissemination of study results. In 60-70% of the simulations presented in section D.17, the studies would have been stopped early.

The interim analysis of cartilage volume (pain will not be evaluated in the interim analysis) will be conducted by the study statistician and presented to the DSMB for the trial. The DSMB will evaluate these results in light of the stopping rules above and advise whether the trial should continue or stop.

Table 4. Conceptualization of study measures

	Structural outcome	Clinical outcome	Covariate
Primary	Cartilage volume	WOMAC pain	Knee alignment Analgesic use
Secondary	BML score Subchondral attrition Tibial medial: lateral BMD ratio Tibial subchondral aBVF tibial BMD femoral neck BMD	WOMAC function Physical function Healthcare utilization	Femoral neck BMD Serum 25 (OH)D (synovitis; US and/or MRI) Analgesic use

-Final statistical analysis plan-

Analytic plan

Our co-primary outcomes were change in knee cartilage volume (assessed using the CDI and mean cartilage thickness) and change in pain (WOMAC pain subscale). We used intention-to-treat analyses for all outcomes. All models were adjusted by Kellgren-Lawrence score and gender; and, when appropriate, for use of rescue analgesia. We performed multiple imputation to fill in missing values for structure, symptom and function outcomes. We used mixed effects regression models with a random intercept for longitudinal repeated measures, and used repeated measures GEE analyses for use of rescue analgesia. The chi-square test was used to analyze adverse events at the person level, while negative binomial regression was used to analyze whether the total count of AEs per person differed across groups. All analyses were performed in SAS 9.4 (Cary, NC).

- Analysis Plan: Summary of Changes –

- We did not perform an interim analysis. Please see letter to the DSMB for our reasoning, and their approval letter (both attached)
- Due to the null results, we did not perform the secondary analyses, including alternative modeling strategies and stratified analyses examining potential confounders
- We performed multiple imputation to estimate missing outcomes and perform the ITT analysis
- Analyses of adverse events were not specified in the initial analysis plan, and were added to the final analysis plan

Thursday, March 27, 2014

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Dear Dr. Jordan,

I am writing in regards to a planned interim analysis of data from the ongoing clinical trial of intra-articular corticosteroids for knee OA (R01 AR057802). This plan was proposed in the resubmission of the grant in response to a critique that called for a more innovative / adaptive design. Unfortunately, with hindsight, an interim analysis with potential stopping rules seems ill-conceived and wasteful of participant effort and data. Therefore, we are requesting permission from the DSMB and NIH to amend the protocol to eliminate the interim analysis; and to take the trial to full completion.

The plan for the interim analysis, including the decision rules for stopping the study, are described mainly in Sections A.4 and E.16 of the revised grant application (and highlighted in the attached document). Briefly, the approach allows termination of the trial at the point at which the first half of participants have completed the intervention, based on a statistical indication of either efficacy or futility for the primary structural outcome, cartilage volume.

The first obstacle to this plan relates to feasibility of completing the cartilage measurements in sufficient time for its deployment. During the course of the trial we found that precise quantification of change in this measure requires both baseline and follow-up image sets to be available contemporaneously. In other words, the original expectation that we could enhance the efficiency of performing this measurement by reading the baseline MRIs prior to acquiring the follow-up images turned out to actually increase workload. Therefore, this baseline analysis has not been performed. However, the

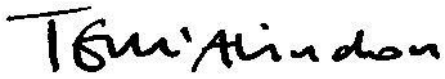
interim analysis plan requires that we read and quality-check all MRI pairs from the first 52 completed participants. Analysis of those image sets will take between 2 to 6 months depending on whether we apply full segmentation measurement or a parsimonious approach that we have been developing.
Either way, by the time these measurements are completed, a substantially larger number of participants will have completed the study intervention, diminishing any value of premature cessation of the trial. The last injection is performed at the month 21 visit and the last MRI occurs at the month 24 visit. If it takes 2 months to complete MRI measurement on the first half of the participants, only 28 people will NOT have received their last injection. If it takes six months, only 4 participants will NOT have received their last injection. The last study injection will be given on or about September 24, 2014 and the last study MRI will occur in December, 2014. Therefore stopping the trial to avoid study drug exposure (or lack thereof) on the basis of futility or efficacy will have very minimal benefit to ongoing participants.

Another concern is that a stopping rule based only on the primary outcome risks foregoing a great deal of valuable secondary information - for example, the effects of intra-articular corticosteroids on pain and function, as well as on other important structural processes such as effusion and subchondral bone changes. In addition, statistical power (already borderline for some outcomes) will be further compromised.

Our view is that an interim analysis will not benefit the study. There would be a loss of information if the study stops early – we will get more precise estimates of the efficacy and safety of IACS if the entire study is fully completed. Also, because of the long follow-up period for the study protocol on each subject (2 years) there is little ethical or practical benefit from stopping early. All of the study subjects have already been randomized and exposed to study treatment and MRI assessments, and even the subjects who are in the *interim analysis termination group* (the second half of subjects randomized) would have almost have completed the full follow-up. Most of these subjects would only be spared from the 24-month follow-up visit, a visit that does not involve intra-articular injection and during which we obtain the final primary outcome data point. Therefore, eliminating these visits would do very little to lessen participant burden, but would have a significant impact on the primary outcome and other important data points.

If you agree, we will proceed with a protocol amendment to remove the interim analysis from the plan.

Sincerely,



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cc: Gayle Lester, Ph.D.