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Effects of intraarticular IL1-Ra for acute anterior cruciate ligament knee injury: a randomized controlled pilot trial (NCT00332254)

V.B. Kraus †*, J. Birmingham †, T.V. Stabler †, S. Feng ‡, D.C. Taylor §, C.T. Moorman III §, W.E. Garrett §, A.P. Toth §

† Department of Medicine, Duke University Medical Center, Durham, NC, USA
‡ Biostatistics and Bioinformatics, Duke University Medical Center, Durham, NC, USA
§ Orthopaedic Surgery, Duke University Medical Center, Durham, NC, USA

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SUMMARY

Objective: To evaluate the clinical effectiveness of intraarticular IL-1 receptor antagonist (IL-1Ra) for anterior cruciate ligament (ACL) tear.

Methods: Eleven patients with acute ACL tear confirmed by magnetic resonance imaging (MRI) were randomized to receive a single intraarticular injection of IL-1Ra (anakinra 150 mg, $n = 6$) or equal volume of saline placebo (1 ml, $n = 5$). The double-blinded treatment was administered a mean 2 weeks after injury. Synovial fluid (SF) ($n = 9$ patients) and sera (all patients) were available at baseline (prior to injection) and immediately prior to surgery (mean 35 days later) and analyzed for SF IL-1α, IL-1β, IL-1Ra and serum hyaluronan (HA), an indicator of synovial inflammation. The primary outcome, standardized Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire, was obtained at 0 (baseline), 4, and 14 days after injection.

Results: Compared with placebo, the IL-1Ra group had substantially greater improvement in key outcomes over 14 days (KOOS pain $P = 0.001$; activities of daily living $P = 0.0015$; KOOS sports function $P = 0.0026$; KOOS quality of life (QOL) $P = 0.0048$; and total KOOS $P < 0.0001$). There were no adverse reactions in either group. SF IL-1α ($P = 0.05$) and serum HA ($P = 0.03$), but not IL-1β, or IL-1Ra, decreased significantly in the IL-1Ra but not the placebo treated patients. Compared with placebo, IL-1α was borderline significantly different in the IL-1Ra treated group ($P = 0.06$).

Conclusions: Administered within the first month following severe knee injury, IL-1Ra reduced knee pain and improved function over a 2-week interval. This promising proof of concept study provides a new paradigm for studies of acute joint injury and suggests that a larger follow-up study is warranted.

Introduction

Estimates suggest that approximately 12% of the overall prevalence of symptomatic OA is due to joint injury[1,2]. On average, 50% of individuals develop radiographic osteoarthritis (OA) approximately 10–20 years after anterior cruciate ligament (ACL) or meniscal knee injury[3]. However, it is increasingly recognized that biochemical abnormalities within the joint precede radiographic abnormalities of OA by as much as decades[4–7]. This not only provides the opportunity for early diagnosis of joint metabolic abnormalities prior to their culmination in radiographic OA, but the prospect of early intervention and treatment monitoring as well. In contrast to idiopathic OA, post-traumatic arthritis is initiated by intraarticular processes with a known date of onset, namely the date of joint injury. This makes it much more amenable to early intervention and early monitoring than idiopathic OA whose onset is not clearly definable at this time.

After joint injury, it is proposed that abnormal joint motion and loading lead to a biological response dominated by catabolic activity[8]. There is considerable variation in response to surgical treatment and a lack of objective evidence to support a protective role of repair or reconstructive surgery of the ACL or meniscus against OA development[3,9]. In part, we hypothesize that this may be due to the damage accrued by the joint during the first month after injury, traditionally a time for bracing and analgesics prior to surgical intervention. Hemarthrosis often occurs in the acute phase of joint injury, is highly toxic to the joint, and contributes to cartilage catabolism with inhibition of proteoglycan synthesis after only a 4-day exposure to mononuclear cells and erythrocytes in concentrations equivalent to

* Address correspondence and reprint requests to: V.B. Kraus, Box 3416, Duke University Medical Center, Durham, NC 27710. Tel: 1-919-681-6652; Fax: 1-919-684-8907.
E-mail address: vbk@duke.edu (V.B. Kraus).

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those in whole blood[10]. We hypothesized that the early biological response of the injured joint has a crucial impact on the long-term health consequences of the joint organ, and therefore, that the early phase of acute injury may represent a window of opportunity for providing treatment to promote healing and to prevent the subsequent cascade of joint destructive processes following injury that often culminate in arthritis.

IL-1 levels are elevated in patients with ACL rupture and correlate with severity of chondral damage[11]. In this pilot study, we therefore chose to test the effectiveness of early intervention with an anti-cytokine therapy, interleukin-1 receptor antagonist (IL-1Ra), to reduce knee pain and inflammation and improve function. A wealth of preclinical (summarized by Calich *et al.* [12]) and human clinical data support the anti-arthritic therapeutic potential of IL-1 inhibition. Preclinical data include efficacy in collagen induced arthritis in mice[13–17], and therapeutic efficacy of IL-1Ra in OA models in rats[18–20], dogs[21,22], rabbits[23], and horses[24]. Low innate production of cytokines (IL-1beta, IL-1Ra and IL-6) upon whole blood LPS stimulation are associated with low body burdens of OA[25]. In addition, polymorphisms in genes encoding IL-1 receptor antagonist[26,27] and IL-1 receptor[28] and the IL-1 gene cluster[29–31] are associated with OA. Based on these data we chose, in this pilot study, to investigate the effects of IL-1 inhibition on symptoms and functioning following joint injury.

IL-1Ra is a naturally occurring IL-1 inhibitor that binds the IL-1RI receptor without triggering an agonist response and thus functions as a receptor antagonist. The IL-1Ra approved for human use is a recombinant, non-glycosylated version of human IL-1Ra. It consists of 153 amino acids and differs from native human IL-1Ra only in the addition of a single methionine residue on its amino terminus. The drug is sold under the tradename Kineret® (Biovitrum AB, Stockholm) and generic name anakinra. Anakinra is Federal Drug Administration (FDA) approved for daily subcutaneous (SC) injection (100 mg/day) for the treatment of rheumatoid arthritis (RA). Delivery by intraarticular injection is an attractive alternative considering it has the potential to minimize systemic side effects of a biological agent. Two clinical trials of IL-1Ra for established knee OA used single intraarticular injections and resulted in short-term benefit and no safety concerns[32–34]. IL-1Ra has also been shown to be beneficial for erosive hand OA[35]. Informed by these trials of IL-1Ra for OA, we designed and conducted a randomized placebo-controlled pilot trial of IL-1Ra for acute knee injury involving ACL tear.

Methods

Trial design

This was an investigator-initiated, placebo controlled, randomized, double-blinded proof of concept pilot trial of intraarticular IL-1Ra (anakinra) for acute knee injury with ACL tear. The Duke Institutional Review Board (IRB) approved this study. The investigation was conducted in accordance with the principles expressed in the Declaration of Helsinki. Upon review by the FDA, the study was deemed exempt from the requirements of Part 312 of the Investigational New Drug (IND) regulations as it met all of the requisites set forth at 21 CFR 312.2(b)(1), namely that the route of administration, dose and patient population did not significantly increase the risks or decrease the acceptability of the risks, informed consent was to be obtained, the study would be IRB reviewed and approved prior to initiation, the study was not intended to be used to support a new indication or change in labeling of a drug product, and the drug would not be commercially represented as safe or effective for the purpose for which it was under investigation. Randomization was computer-generated and performed by a physician unconnected with the study. The physician study coordinator (JB) enrolled the participants. The Duke Research Pharmacy assigned participants to the intervention based on the randomization schedule. The trial NCT00332254 "Study to Prevent Cartilage Damage Following Acute Knee Injury" was registered with ClinicalTrials.gov on 5.30.2006 and was conducted between July 2006 and April 2007. The trial is summarized in the CONSORT Flow Diagram (Fig. 1).

Participants

To be considered for inclusion in this trial, the following requirements had to be met: onset of injury less than 4 weeks prior to evaluation; severe knee injury expected to require surgery, Magnetic Resonance Imaging (MRI) confirmed ACL tear; age 18–30 years; negative serum pregnancy testing (βHCG) at time of entry and on follow-up evaluation for women of childbearing potential with their agreement to use some form of contraception during the study period; and ability to provide informed consent. In addition, all participants had to be non-obese to minimize risk of pre-existing OA. Exclusion criteria for this study were: prior signal joint injury requiring medical evaluation; history of arthritis or rheumatic disease; history of intraarticular corticosteroid in the index joint; septic joint; evidence of chronic joint disease by plain radiograph; or fracture. Study participants were identified through the Duke University Sports Medicine clinic and by screening MRIs for acute knee injury.

Intervention

A total of 11 patients were treated within the first 30 days of acute knee injury: six patients were randomized to intraarticular IL-1Ra (anakinra 150 mg) and five patients to intraarticular saline placebo (1 ml). This dose was chosen on the basis of an *in vivo* phase I, intraarticular dose-escalating study by Chevalier *et al.*[32] wherein the maximum possible intraarticular dose used, 150 mg, was administered without adverse events; this dose was based on previous maximum dose administered subcutaneously for RA[32,36]. The Duke Research Pharmacy supplied the drug or placebo in solutions of equal volume (1 ml volume), color and consistency in syringes ready for injection, making the interventions indistinguishable to patients and study personnel in charge of administering the agents. In addition, prior to surgery, all subjects were prescribed standard conservative therapy that included knee bracing ($n = 6$), non-steroidal anti-inflammatory drugs (NSAIDs) (as needed Ibuprofen $n = 5$), analgesics (acetaminophen $n = 2$, hydrocodone $n = 1$), ice ($n = 3$), and range of motion exercise and quadriceps strengthening ($n = 6$). These interventions did not differ by treatment group (Table I).

Clinical outcomes

The designated primary outcome was change over time in standardized Knee Injury and Osteoarthritis Outcome Score (KOOS) scores obtained at baseline (0), 4, 14, and 28 days after study enrollment. The KOOS scores were normalized as specified in the KOOS investigators instructions found at www.koos.nu/KOOSGuide2003.pdf. The higher KOOS scores represent less pain and better function. The majority of participants (8/11) underwent surgical repair prior to the 28 day post-enrollment timepoint so 28-day KOOS data were not available for analysis.

Sample collection

Blood for serum was collected at baseline ($n = 11$) and at the time of ACL reconstructive surgery ($n = 11$) and stored at −80°C

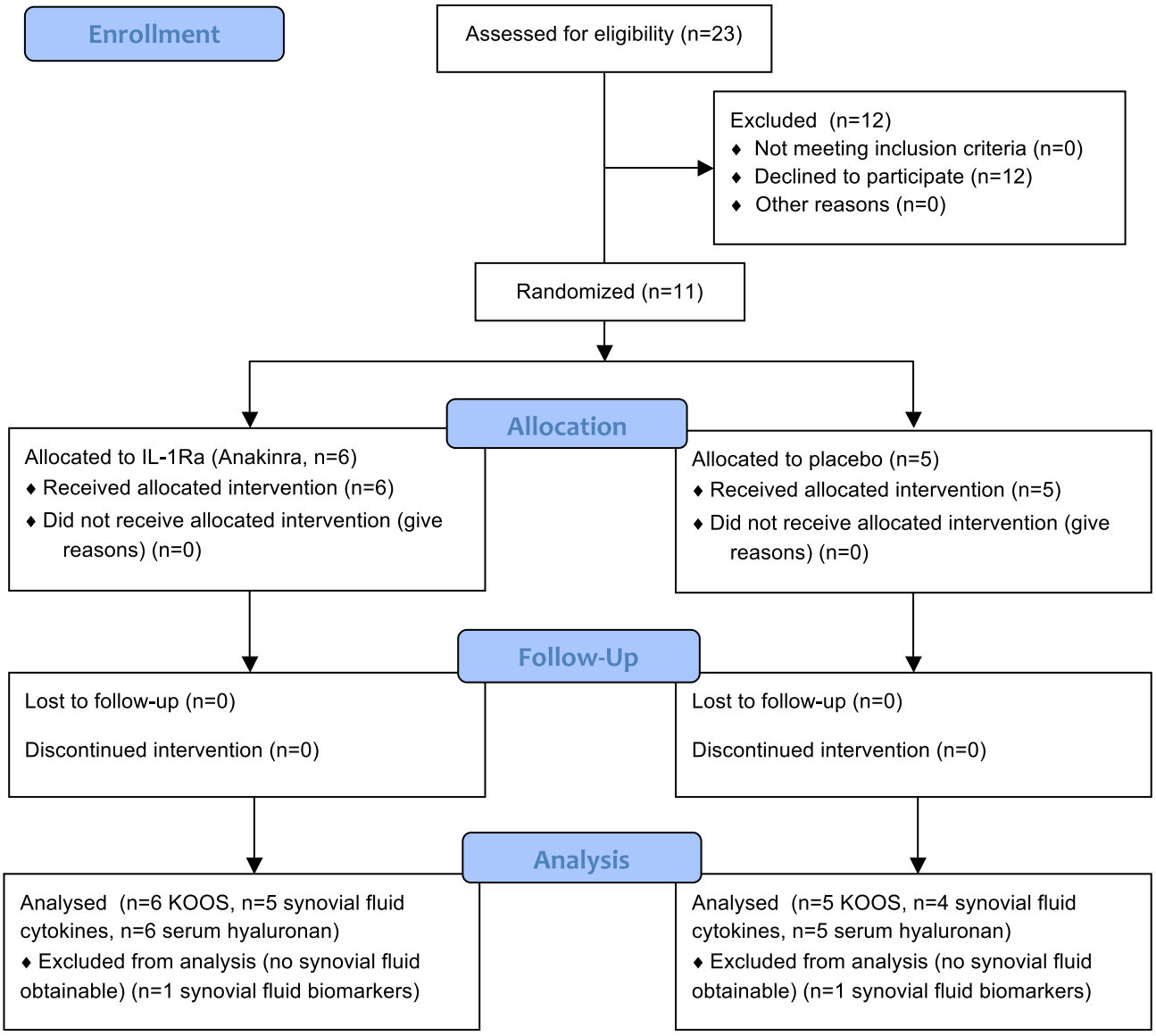

Fig. 1. Consort Flow Diagram. This shows the flow of participants through each stage of the randomized pilot trial of intraarticular IL-1Ra (anakinra) vs saline for acute ACL tear injury.

Table I
Clinical characteristics by intervention group

Characteristic	IL-1Ra ($n = 6$)	Placebo ($n = 5$)
Mean (SD) age years	22 (3)	24 (4)
Female	2	3
Intra-articular pathology by clinical MRI	Two isolated ACL tears; two ACL tears with partial medial collateral ligament tears; two ACL tears with meniscal tears	One isolated ACL tear; one ACL tear with partial medial collateral ligament tear; two ACL tears with meniscal tears; and one ACL tear with medial collateral ligament and meniscal tears
Standard care	Knee bracing ($n = 3$), NSAIDs (as needed Ibuprofen $n = 2$), ice ($n = 2$), and range of motion exercise and quadriceps strengthening ($n = 2$).	Knee bracing ($n = 3$), NSAIDs (as needed Ibuprofen $n = 3$), analgesics (acetaminophen $n = 2$, hydrocodone $n = 1$), ice ($n = 1$), and range of motion exercise and quadriceps strengthening ($n = 4$).
Mean/median (SD) days from injury to enrollment	13/14 (6)	17/17 (9)
Mean/median (SD) days between baseline and follow-up sample collection	20/22 (9)	53/40 (40)
Mean/median (SD) days between injury and surgery	33/34 (12)	70/67 (46)

until analysis. In addition, neat synovial fluid (SF) was aspirated at baseline from the injured knee ($n = 11$), the syringe was changed and the drug or placebo was injected. Neat SF was also aspirated from the injured knee at the time of surgical reconstruction ($n = 9$). SF was centrifuged at 3500 rpm for 15 min and the supernatants stored at −80°C until analysis.

Biomarker assays

Secondary exploratory outcomes included analysis of SF concentrations of IL-1α, IL-1β, IL-1Ra and serum hyaluronan (HA) in baseline samples and samples obtained on the day of surgery. A total of nine pairs of SFs and eleven pairs of sera were available for biomarker analyses. IL-1α, IL-1β, and IL-1Ra were quantified by sensitive commercially available sandwich ELISAs (R&D Quantikine). Manufacturer reported intra- and inter-assay CVs were: 2% and 4% for serum IL-1α; 4% and 7% for IL-1β; and 5% and 9% for IL-1Ra. The minimal detectable concentration per the manufacturer's instructions was 3.9 pg/ml for IL-1α, however we were able to markedly improve the sensitivity of the IL-1α immunoassay by using an extended initial incubation time (overnight at 4°C with shaking) coupled with an expanded standard curve in the low range, which we found to be linear. With these modifications, the minimum detectable concentrations were ≤0.05 pg/ml (IL-1α); 1.6 pg/ml (IL-1β); and 6.26 pg/ml (IL-1Ra). Serum levels of HA were quantified using a commercially available assay (Corgenix). Intra- and inter-assay CVs were 2% and 3%, respectively. Biomarker measurements were performed blinded to treatment group assignment.

Statistical analysis

The trial was unblinded after 11 patients were treated to determine whether the results merited seeking external funding and proceeding with a larger trial. Analysis of Covariance (ANCOVA) was used to compare the regression models of clinical KOOS outcomes over time (over first 14 days) in different treatment groups (drug vs placebo). The 95% confidence intervals (CIs) for the slope of response over time for each treatment and KOOS (total and subdomain) were computed. The non-parametric Wilcoxon Signed–Rank sum test was used to evaluate the change in biomarkers over time within a group and to compare the change in biomarker concentrations between groups (drug vs placebo). All analyses were by pre-assigned group. A P value ≤0.05 was considered significant. Based on the fixed sample size of 11, a *post hoc* power calculation with the type I error rate set at 5% shows the study had 80% power to detect an effect size of 0.4 in KOOS outcomes.

Results

Participant characteristics

Patients were recruited a mean 15 ± 7 (SD) days from injury (range 6–27 days post-injury). The mean age of participants (six male, five female; two African American) was 24 ± 4 (SD) years. The clinical characteristics of each group are summarized in Table I. For this pilot study, enrollment was limited to patients under 30 years of age to try to ensure the lack of an underlying pre-existing arthropathy. Participants suffered acute knee injuries secondary to skiing (3), soccer (2), basketball (1), ultimate Frisbee (1), rugby (1), flag football (1), running (1), and a fall while cheerleading (1). Clinical knee MRIs were performed revealing ACL tears in all patients with the following additional intra-articular pathologies: three patients with isolated ACL tear only; three patients with medial collateral ligament and ACL tears; four patients with meniscal and ACL tears; and one patient with medial collateral ligament, meniscal and ACL tears. These combination pathologies were equally distributed by group and the types of lesions in each group are summarized in Table I.

Primary clinical outcomes

Improvements over time in all five subscales of the KOOS were evident in the IL-1Ra treated but not the placebo treated patients (Fig. 2). The IL-1Ra group had substantially greater improvement (~1–3 standard deviations) in key outcomes whereas the placebo group had much less improvement (~1/4 of a standard deviation on most outcomes). Relative to the placebo treated group, there was a statistically significant improvement in the anakinra treated group in KOOS pain, KOOS function in daily living (ADL), KOOS Sports Function, KOOS knee related quality of life (QOL) and total KOOS (Table II). In the IL-1Ra treated group, the extent of improvements (increases in KOOS subscale scores relative to

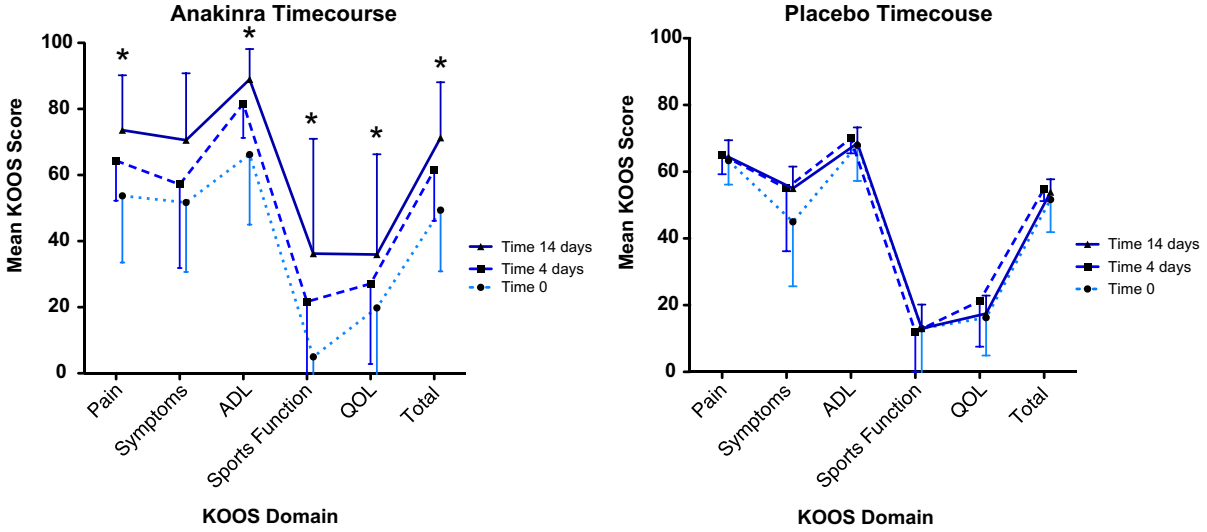

Fig. 2. Clinical KOOS outcomes. The panels show mean (error bars are standard deviations) of KOOS outcomes at baseline (time 0), and 4 and 14 weeks after intraarticular injection of the IL-1 receptor antagonist anakinra (left panel) and saline placebo (right panel). The asterisks denote KOOS domains that showed significant improvement over time in the anakinra compared with the placebo treated group.

Table II
KOOS clinical outcomes

KOOS Subscale†	Between Group p value*	Mean (SD) baseline	Mean (SD) 4 days	Mean (SD) 14 days	95% CIs for slope of the response over time	Mean (SD) baseline	Mean (SD) 4 days	Mean (SD) 14 days	95% CIs for slope of the response over time
		Anakinra treatment ($n = 6$)				Placebo treatment ($n = 5$)			
Pain	***P* = 0.0011**	**53.7 (20.2)**	**64.3 (12.1)**	**73.6 (16.6)**	**(5.5141, 15.0626)**	63.3 (7.2)	65.0 (5.8)	64.4 (11.1)	(−4.0008, 5.1128)
Other Symptoms	$P = 0.4540$	51.8 (21.0)	57.1 (25.3)	70.5 (20.3)	(2.1869, 13.5031)	45.0 (19.3)	55.0 (18.8)	55.0 (14.6)	(−0.3847, 10.3847)
ADL	***P* = 0.0015**	**66.2 (21.2)**	**81.6 (10.4)**	**89.0 (9.2)**	**(6.1131, 17.5111)**	67.9 (10.7)	70.0 (4.5)	68.5 (10.6)	(−5.1625, 5.7525)
Sports Function	***P* = 0.0026**	**5 (6.3)**	**21.7 (24.6)**	**36.3 (34.7)**	**(7.3955, 22.8768)**	13.0 (18.6)	12.0 (12.0)	13.0 (16.0)	(−7.3985, 7.3985)
QOL	***P* = 0.0048**	**19.8 (26.9)**	**27.1 (24.3)**	**35.9 (30.4)**	**(3.5742, 12.4005)**	16.3 (11.4)	21.3 (13.7)	17.5 (12.0)	(−3.5691, 4.8191)
Total	***P* < 0.0001**	**49.4 (18.6)**	**61.5 (15.4)**	**71.3 (16.8)**	**(7.1387, 14.5874)**	51.7 (9.8)	54.8 (3.7)	53.9 (8.6)	(−2.4133, 4.6753)

KOOS Subscales: Pain; Other Symptoms (such as knee swelling, clicking catching and stiffness); ADL = Function in Daily Living.
Sports Function = Function in Sport and Recreation; QOL = knee related Quality of Life; and Total = combination of all five subscales.
* *P* value by Repeated Measures ANCOVA (analysis was by assigned group). Values in bold are significant at the $p<0.05$ level.
† Normalized KOOS scores (%).

baseline) over 14 days were: Pain of 37%, ADL of 34%, Sports Function 626%, QOL 81%, and total KOOS of 44%; in contrast the placebo group changed only 2%, 1%, 0%, 1% and 4% in these outcomes respectively. There were no adverse events associated with the injections or drug treatment and no discernible difficulties or delays in wound healing following surgical repair.

Secondary biomarker outcomes

To explore the possible biological impact of a single IL-1Ra intraarticular injection, we measured SF IL-1α, IL-1β, and IL-1Ra, and serum HA, an indicator of synovitis, in samples obtained prior to study drug injection and on the day of surgery. Pre- and post-treatment SF samples were available from five IL-1Ra treated patients and four placebo treated patients. The mean (SD) SF volumes (ml) obtained were similar in both treatment groups: baseline 14.9 ± 4.3 and 13.0 ± 9.9 for anakinra and placebo respectively; follow-up 21.1 ± 16.4 and 16.0 ± 9.9 for anakinra and placebo respectively. SF analyte concentrations exceeded serum concentrations (mean ± SD pg/ml): IL-1α SF 0.24 ± 0.25 vs serum 0.05 ± 0.11; SF IL-1β 0.77 ± 0.51 vs serum 0.20 ± 0.10; and SF IL-1Ra 5429 ± 11305 vs serum 408 ± 156. The change with treatment (Time 1 to Time 2) in SF IL-1α constituted the most intriguing and promising cytokine result from this pilot trial (Fig. 3). IL-1α increased over time in all the placebo treated patients ($n = 4$ pairs of samples available), whereas IL-1α decreased in 4 of 5 of the IL-1Ra treated patients ($n = 5$ pairs of samples available). Relative to the placebo treated group, the change in SF IL-1α from baseline to follow-up in the anakinra group was of borderline significance ($P = 0.06$). The change in IL-1β and IL-1Ra were not significant in either treatment group. Pre- and post-treatment serum samples were available from all six IL-1Ra treated patients and the five placebo treated patients. Serum HA decreased from baseline to follow-up in all the IL-1Ra treated patients (Fig. 3, $P = 0.03$) but did not change significantly in the placebo treated group and was not significantly different between treatment groups.

Discussion

As shown by this small, randomized pilot trial, intraarticular IL-1Ra decreased pain and improved function (ADL, Sports Function and QOL) following acute knee injury. A minimal perceptible clinical improvement of 9–12 mm (based on a 100 mm Visual Analogue Scale (VAS)) on Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scales of pain, physical function and stiffness has been established as a perceptible change to patients with hip and knee OA[37]. Since the KOOS dimension of ADL is equivalent to that of Function in the WOMAC Osteoarthritis Index LK 3.0[38], the WOMAC metric for clinically important difference can be applied directly to the normalized ADL scores achieved with KOOS. We observed a mean (SD) change (improvement) of 22.2 (11.8) points in KOOS ADL scores over 14 days in the IL-1Ra treated group compared with mean (SD) change of only 0.6 (14.4) points in the placebo treated group. Thus this trial exceeded the minimal perceptible clinical improvement in ADL that has been established for the WOMAC. A change in normalized KOOS scores of 8–10

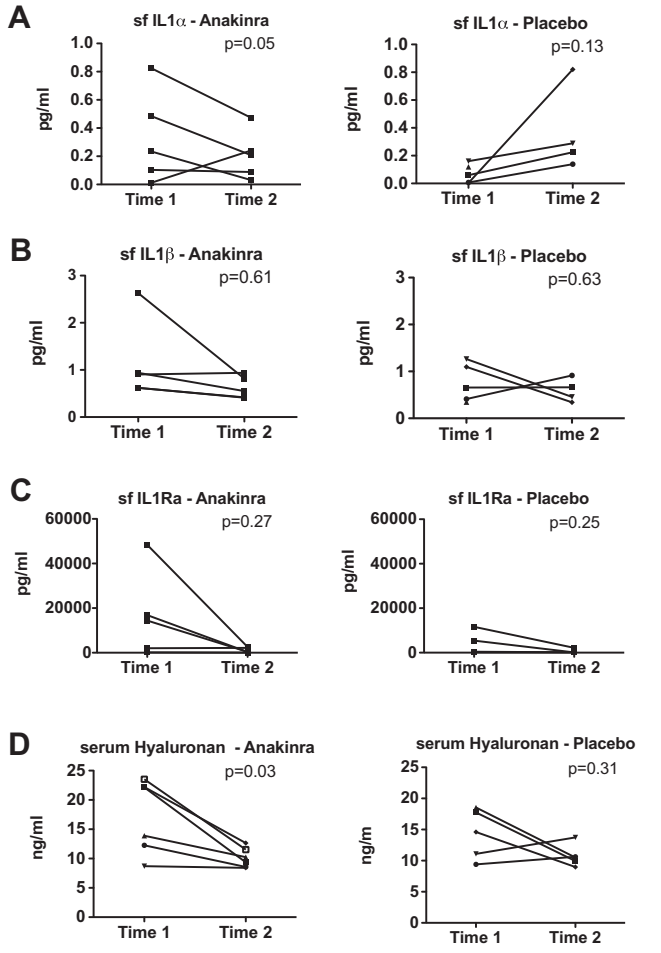

Fig. 3. Biomarker analyses. Biomarker results at baseline and time of surgery in the intraarticular IL-1Ra treatment group (left panel) and the intraarticular saline placebo treatment group (right panel). A) SF IL1α, B) SF IL1β, C) SF IL1-Ra, D) serum HA. Non-parametric Wilcoxon Signed–Rank sum tests were used to evaluate change scores within a group (*P* values shown in figure), and change scores between groups (drug vs placebo): IL-1α $P = 0.06$, IL1β $P = 0.61$, IL1-Ra $P = 0.27$ and HA $P = 0.29$.

points has also been suggested to represent a minimal perceptible clinical improvement following ACL reconstruction[38]. In this pilot study we observed this level of change in normalized KOOS scores in all the KOOS subscales in the IL-1Ra treated group (range mean 15.2–30 point change from baseline to 14 days) but only in symptoms (mean 10 point change) in the placebo treated group (remaining subscales yielded only mean changes ranging from 0.6 to 2.3 points in the placebo treated group). Thus, based on either metric, the magnitude of change in KOOS subdomains achieved by IL-1Ra would indicate a clinically meaningful improvement in this small pilot study.

In the prior Phase I, dose-escalating study of IL-1Ra (anakinra) for knee OA[32], a significant response (responder defined as having a 50% reduction from baseline) was observed at 1 month following a single intraarticular injection in the VAS pain outcome in 7 of 13 patients, and in the global WOMAC outcome in 10 of 13 patients. By 3 months post injection, there were six responders for each outcome. There were no acute reactions and no injection site reactions. One patient had a sterile effusion (150 cells/mm^3) within 3 days post injection without pain that resolved spontaneously; this episode was deemed by the study investigator to most likely be unrelated to the drug. Following the pilot study they performed a randomized controlled trial ($n = 170$)[39] in which no safety concerns were raised. It did not replicate the clinical benefit reported in the initial pilot study based on the main outcome of pain at 1 month; however, there was apparent improvement in the 150 mg group at Day 4[34]. They interpreted these results to be in keeping with the short half-life of IL-1Ra suggesting that IL-1 inhibition may be therapeutically relevant, and postulated that longer term therapy might be required for a more sustained effect. Peak plasma concentrations of anakinra are reached 3–7 hours after subcutaneous (SC) injection with a half-life of 4–6 h[40]. After IL-1Ra is discontinued, simulations indicate that it takes approximately 3 days for the IL-1Ra concentration to decline to a level below the lower limit of quantification. Compared with the saline treated group, the anakinra group showed steady improvement in KOOS outcomes during the 14 days following a single intraarticular injection suggesting that early intervention, even with a short-acting agent, may result in clinical benefit in the setting of acute knee injury Table II.

Taken together with the wealth of preclinical and clinical data, this study increases confidence in IL-1 as a promising target for symptom and potential disease modification following severe joint injury. We speculate that repeated injections of a short-acting IL-1 inhibitor or a longer-acting agent may be required to optimally block cartilage degradation and ultimately lessen the risk of arthritis development. With respect to anakinra, the main barrier to overcome is drug retention in the joint. Means of overcoming this problem (summarized by Martel–Pelletier *et al.*[41]) include: the use of repeated intraarticular injections of anakinra such as on a weekly basis; combining anakinra with a sustained release system[19], and exploration of the effectiveness of SC injections of the drug (the FDA approved method of delivery for the treatment of RA)[41]. The challenge will be to deliver an agent with a long enough half-life to minimize the number of joint injections and maximize the prospect of blocking joint degradation during the critical immediate post-joint injury period and possibly immediate post-surgical repair period of inflammation without increasing the risk of infections in the setting of surgery.

The delay in surgical reconstruction of the ACL is typically predicated on the attempt to prevent arthrofibrosis. This approach is supported by evidence that performing surgery within 4 weeks of ACL injury is a risk factor for post-operative development of arthrofibrosis[42–44]. Mayr *et al.* found that the presence of knee irritation pre-operatively (swelling, effusion, hyperthermia) correlated ($P = 0.001$) with development of arthrofibrosis[45]. Furthermore, they found that if joint irritation persisted after 4 weeks, the risk of arthrofibrosis remained elevated. They concluded that it is inflammation, and not timing of the surgery, which predicts development of arthrofibrosis post-operatively. Therefore, attempts to reduce preoperative inflammation were recommended as the critical intervention in preventing this difficult post-surgical complication. At present, there are no specific therapies for the reduction of pre- and peri-operative joint inflammation in humans beyond general measures such as NSAIDs, icing, and bracing[46]. However, in antigen-induced arthritis in rabbits, IL-1Ra had a profound antifibrotic effect[47]. In this model, the synovial fibrosis was not only halted by administration of IL-1Ra but it was reversed[47].

SF IL-1α decreased over 1 month in response to a single IL-1Ra intraarticular injection in our pilot trial. The decrease in SF IL-1α in the IL-1Ra treated patients likely represents downregulation of IL-1α expression by synovium in response to IL-1Ra therapy. This mechanism of action of IL-1Ra is supported by *in vitro* work by Haupt and Evans *et al.*[48]. IL-1 is particularly important at the local level and more potent than tumor necrosis factor alpha in stimulating matrix metalloproteinases and specifically impeding cartilage repair[49]. It is known[50] that IL-1α binds the membrane bound form of IL-1RI in preference to the soluble form. Moreover, IL-1Ra binds IL-1RI nearly irreversibly. In contrast, the binding of IL-1β to the decoy receptor, IL-1RII, is nearly irreversible. The known differential binding affinities of these receptors for the IL-1 family of cytokines have great relevance for this study because they make it clear that IL-1Ra has a preference for IL-1RI receptor and therefore would preferentially block IL-1 from signaling through the functional receptor. These data also suggest that IL-1α, despite low absolute concentrations in the SF in acute joint injury may have great functional relevance by virtue of its binding affinity for the membrane bound functional receptor. The promising results with SF IL-1α suggest that IL-1 inhibition in the immediate post-injury period may reduce joint destructive inflammation. Therefore, optimization of this approach may, in future, allow for earlier surgical intervention without increasing the risk for arthrofibrosis.

This study has several limitations including the small study size, and the short half-life of the compound used. Since this study was conducted, two longer acting anti-IL-1 agents, Rilonacept and Canakinumab (a fully humanized anti-IL-1beta monoclonal antibody[51]), received FDA Orphan Drug approval for treatment of rare human autoimmune cryopyrinopathies, with 7-day and one-month half-lives respectively; these might provide a viable longer acting alternative to IL-1Ra. Other anti-IL-1 strategies that might also be considered include sustained intraarticular delivery of IL1-Ra[19] (not yet approved for human use), Orthokine®[52–54], and intraarticular gene therapy delivery of IL-1Ra (used without adverse events in RA)[55,56]. This study was also limited by the difference in follow-up times between groups that would have impacted the SF biomarkers but not the clinical outcome data; this would be expected to have resulted in a naturally lower level of inflammatory markers in the placebo group and biased against finding a difference in biomarkers due to drug treatment. Despite these limitations, this study was designed as a proof of concept trial and in that regard it has succeeded in providing a paradigm by which the utility of biological agents for acute joint injury may be tested. Together these two approaches, biological therapies to minimize joint destruction and inflammation and allowing for the possibility of earlier surgical repair, constitute a new treatment paradigm that deserves to be stringently tested to determine whether this approach could decrease the incidence of post-traumatic arthritis. Analogous to the treatment paradigm for acute myocardial infarction, in future, an acute joint injury might be

treated emergently to prevent joint damage in order to preserve long-term full joint organ function.

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Contribution statement

All authors contributed to the conception and design, acquisition of data or analysis and interpretation of data; V Kraus drafted the article and all authors revised it critically for important intellectual content and provided final approval of the submitted version.

Conflicts of interest
No authors had any conflict of interest.

Supplementary material

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