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Developing anti-inflammatory therapeutics for patients with osteoarthritis

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Abstract

Osteoarthritis (OA) is the most common joint disorder in the world but there are no approved therapeutics to prevent disease progression. Historically, OA has been considered a wear and tear joint disease and efforts to identify and develop disease modifying therapeutics have predominantly focussed on direct inhibition of cartilage degeneration. However, there is now increasing evidence that inflammation is a key mediator of OA joint pathology and also that the link between obesity and OA is not solely due to excessive load-bearing, suggesting therefore that targeting inflammation in OA could be a rewarding therapeutic strategy. In this review we therefore re-evaluate historical clinical trial data on anti-inflammatory therapeutics in OA patients, highlight some of the more promising emerging therapeutic targets and discuss the implications for future clinical trial design.
Osteoarthritis and inflammation

Inflammation in OA has been overlooked for many years. However, histological analysis, ultrasound and MRI imaging have shown evidence of synovitis in OA joints [1] with increased cellular infiltration of activated B cells and T lymphocytes. Histological studies indicate that synovitis is present in 50% of patients with early OA, and in nearly all patients with late-stage OA [2]. Furthermore, synovial hypertrophy as detected by ultrasound has been shown to correlate to knee OA disease severity [3].

Several studies have reported elevated levels of a number of pro-inflammatory mediators (including TNF-α, IL-1β, IL-6, IL-15, IL-17, IL-18 and NO) in the serum or synovial fluid of OA patients [4, 5], compared to healthy patients (Figure 1). Furthermore, stimulation of cartilage tissue with pro-inflammatory cytokines mimics many of the structural changes associated with OA [6-8].

In light of this, it is perhaps surprising that clinical studies in OA patients with anti-inflammatory therapeutics have thus far disappointed. However, OA pathology is highly heterogeneous, with the degree of synovitis varying amongst patients. Indeed, synovitis is not present in half of patients with early OA [2]. Therefore, patient selection is an important consideration for such clinical studies, and unless taken into account could impact on whether the clinical end point is met. For this reason, we re-evaluate the clinical trial data of anti-inflammatory therapeutics in OA (Table 1), to highlight in particular the specific OA patient cohort studied, whether patients were selected for synovitis and the clinical end-points measured. This is not a systematic review. Instead, we have focussed on completed randomised control
trials (RCTs) conducted during the last 10 years in patients with hand, knee and hip OA, which have reported clinical end-points. Secondly, we explore the opportunities for the development of new targeted therapeutics (Table 2) and discuss the requirement to stratify patients for such approaches.

Anti-inflammatory therapeutics in OA patients

**Anti TNF-α therapies**

In OA joint tissues, synovial fluid, and serum, TNF-α has been reported to be elevated compared to healthy patients [9-11]. In rodent models of traumatic joint injury, TNF-α expression is induced [12] and correlates to joint space narrowing (JSN) [13]. Furthermore, overproduction of TNF-α induces NO [14], and upregulates the expression of MMPs [15], whilst TNF-α receptor antagonists block NO production in human cartilage tissue [16]. TNF-α has also been demonstrated to inhibit proteoglycan [17] and Type II collagen synthesis. However, despite these data and the proven successes in treating rheumatoid arthritis (RA) patients with anti-TNF-α therapeutics, clinical trials in OA patients have yielded conflicting and generally disappointing results.

Adalimumab (Humira; Abbott Laboratories, IL) is a human monoclonal antibody (mAb) bioengineered to bind to TNF-α and prevent receptor binding [18]. It has been shown to be efficacious in reducing the pathological symptoms of RA in several clinical trials (DE001/003, DE004, DE007, DE009, and DE010) [18]. However, its effect on OA disease modification has not been clinically proven.
In a PhII, 12 month RCT (NCT00296894), 60 patients with active erosive hand OA received Adalimumab (40mg) or placebo subcutaneously every two weeks. Progression from palpable soft tissue swelling to joint damage decreased 10-fold in the Adalimumab group compared to placebo [19], with a reduction in the proportion of patients experiencing a new erosive interphalangeal joint at 12 month follow-up (26.7% vs 40% placebo control). However these findings did not reach statistical significance and Adalimumab was deemed ineffective at reducing disease activity [19].

In a PhI/II study of 17 patients with knee OA (NCT00686439), Adalimumab (40mg, every 2 weeks) resulted in significant improvements in joint pain at 12 weeks with WOMAC 20% and WOMAC 50% responses in 70% and 40% of patients respectively [20]. However, this study did not incorporate measures of disease modification including JSN, so it is unknown whether prolonged treatment with Adalimumab in this patient cohort would ultimately be disease-modifying. Furthermore, Adalimumab failed to reach its primary end-point of improving pain in a PhII study (NCT00597623) in patients with hand OA refractory to standard-of-care analgesics [21].

It is clear that anti-TNF-α therapeutics are far less efficacious in improving OA pain and joint mobility compared to the outcomes achieved in RA patients. Indeed, RA is a more inflammatory condition with greater evidence of TNF-α and immune cell involvement. However, some positive results have been reported with Adalimumab in patients with knee OA and in patients with erosive hand OA. It should be stressed that these clinical studies did not measure disease progression, and likely involved highly heterogeneous OA patient cohorts. Despite several pilot studies (NCT01144143)
[22], and small RCTs (NCT00819572) [23], larger placebo RCTs to determine the efficacy of other anti-TNF-α therapies such as infliximab and DLX105 (TNF-α mAb) have not yet been undertaken to fully evaluate the potential of TNF-α therapeutics in OA [22, 24].

**IL-1β signalling inhibitors**

IL-1β has been purported to be a driver of OA pathology [25] by mediating damage to articular cartilage tissue [6, 26, 27] and inhibiting the anabolic processes of articular chondrocytes [28]. Found in its active form in cartilage, synovial fluid and synovium, IL-1β inhibits type II collagen [8], aggrecan [8] and proteoglycan synthesis [29] and stimulates production of pro-inflammatory cytokines, MMPs and PGE2. Increased abundance of IL-1β has been reported in synovial fluid of both knee OA and ACL patients, compared to healthy individuals [30-33], and in the synovial membrane expression correlates with OA grade [9].

Diacerein, a small molecule IL-1β inhibitor, reduces the number of IL-1 receptors resulting in a reduction in functional IL-1 heterodimer receptor complexes [34]. It has demonstrated “anti-arthritic” properties both in vitro [35] and in vivo in a murine model [36] through the inhibition of MMPs [37] and NO production [38]. In a 3yr RCT (NCT00451360), 507 hip OA patients received either Diacerein or placebo (2x50mg orally) daily. Although pain and functional impairment associated with OA remained unchanged, Diacerein significantly reduced JSN compared to placebo [39]. However, gastrointestinal adverse effects with Diacerein were a common, cause of treatment discontinuation, restricting its usage. It is not recommended for individuals aged over 65 years.
Alternative IL-1 pathway inhibitors such as the human mAb AMG108 have been designed to directly target the IL-1 receptor. In a RCT\(^{(NCT00110942)}\) of 160 patients with knee OA, AMG108 was well tolerated independent of dosage or route of administration. However, although marginal improvements in pain were noted in the AMG108 group, the difference was statistically insignificant and the clinical endpoint was not achieved \([40]\).

Given the limited clinical efficacy with subcutaneous administration of IL-1β inhibitors and the potential for adverse systemic events, recent studies have focussed on intra-articular (I.A) administration \([41]\). A RCT\(^{(NCT00110916)}\) involving 170 patients with painful knee OA administered either 50mg or 150mg of Anakinra (a IL-1β receptor antagonist), or placebo control. Unfortunately, no improvement in WOMAC score or cartilage turnover was reported after 4 weeks \([42]\). However, patients were not pre-selected on the basis of any confirmed synovitis. Thus it is possible that the study population contained a heterogeneous mix of responders and non-responders.

**Arachidonic acid pathway inhibitors**

Non-steroidal anti-inflammatory drugs (NSAIDs) are often prescribed to reduce OA pain \([43]\). Pharmacological they inhibit cyclooxygenase (COX) enzymes in the arachidonic acid pathway, leading to a reduction in prostaglandin synthesis, which are known mediators of pain and inflammation. Non-selective NSAIDs (nsNSAIDs) (e.g Ibuprofen, naproxen and Diclofenac), inhibit both COX-1 and COX-2 enzymes, and their efficacy in reducing pain, inflammation and swelling in patients with OA has been demonstrated in several clinical studies \(^{(NCT01461369, NCT01860833)}\) \([44-46]\).
Critically, long-term administration of nsNSAIDs is associated with significant adverse events from GI perforation, ulceration and bleeding [47] due to the reduction of GI tract protective prostanoids; a process mediated by COX-1 [48]. Due to these issues, selective COX-2 inhibitors have been developed, including Celecoxib and Valdecoxib.

In a RCT (NCT00650624), Valdecoxib (5mg, 10mg, and 20mg daily dosage) was compared to the nsNSAID naproxen (500mg twice daily dosage), in patients with moderate/severe knee OA. At 5mg and 10mg doses, the Valdecoxib group exhibited significantly reduced GI toxicity compared to the naproxen group [49]. Unfortunately, although clinical trials demonstrated a reduction in GI complications [50, 51], an increased risk of adverse cardiovascular events were noted [52]. This is attributed to the mode-of-action of selective COX-2 inhibitors which decrease prostacyclin production without altering thromboxane levels.

An alternative approach is dual inhibition of leukotrienes and prostaglandins, by targeting both 5-lipoxygenase (LOX) activity and COX-2. One such COX/LOX inhibitor is Licofelone (Merckle GmbH). In preclinical models of OA, Licofelone has shown disease-modifying efficacy in reducing synovium hypertrophy, cartilage lesions and production of pro-inflammatory PGE2 and leukotrienes B4 [53, 54].

Licofelone was shown to significantly reduce cartilage volume loss (vs nsNSAID alone) in a recent RCT of patients with knee OA [55]. Specifically, 355 knee OA patients received twice daily naproxen (500mg) or licofelone (200mg). Cartilage
volume and JSN was measured at baseline, 6, 12 and 24 months [55]. Global cartilage loss was significantly less in the licofelone group compared with naproxen [55]. Critically, no reported GI or CV toxicity at efficacious concentrations were recorded [56].

Despite these promising results, Licofelone has not yet been submitted for regulatory approval. There is evidence to suggest that the rate of OA progression may increase in patients receiving long-term NSAID treatment. Radiological assessment of knee and hip OA progression found an increase in the risk of OA progression in individuals on long-term Diclofenac treatment (>180 days) compared to individuals on short-term treatment (1-30 days) [57]. Indeed, in contrast to their efficacy in blocking pro-inflammatory responses, COX2 inhibitors have been reported to delay the resolution of inflammation in chronic inflammatory preclinical models [58]. Whether these data are the reason why Licofelone has not progressed since its positive PhIII data is unclear.

**NO inhibitors**

NO plays an important role in inflammatory processes and is considered a key mediator of cartilage destruction in OA [59]. NO and the inducible NO synthase (iNOS) are upregulated in preclinical animal models of OA [60] as well as in human OA chondrocytes [61], and mediate the release of pro-inflammatory cytokines. Indeed, NO can mediate many of the pathogenic effects of IL-1β through the activation of MMPs, the inhibition of proteoglycan and collagen synthesis and the enhancement of inflammation [62]. Preclinical studies have shown that iNOS KO mice are resistant to development of OA [63], and that pharmacological inhibition of
iNOS reduces OA progression and pain in the MIA rodent of OA [64] and in a canine model of OA [65].

A recent clinical trial investigated the safety and efficacy of a novel irreversible iNOS inhibitor (SD-6010) on slowing OA progression in a cohort of overweight and obese patients with knee OA\(^{(\text{NCT00565812})}\). Disappointingly, although the iNOS inhibitor was well tolerated, the drug failed to slow the rate of JSN versus placebo over a course of 96 weeks [66]. However, additional iNOS inhibitors are in development, including S-Methylisothiourea, which in preclinical models is effective in reducing NO and disease progression in the MIA rodent model of OA [67]. Whether this promising preclinical data translates into human efficacy is a critical question, but these are encouraging studies.

Emerging anti-inflammatory drug targets

Since efforts to develop effective disease modifying OA therapeutics against prime inflammatory drug targets have yet to bear fruit, ongoing research to better understand the inflammatory response in OA joint tissues is critical so that new drug targets can be identified. There are a number of different therapeutic approaches being investigated which may ultimately provide an effective anti-inflammatory therapeutic for OA patients, including non-pharmacological approaches such as nutraceuticals and gene therapy [68]. Below we summarise some of the promising emerging targets, based on their disease linkage to OA and available preclinical data.

Synovitis associated cytokines
In addition to TNF-α and IL-1β, many additional cytokines and chemokines, detectable in both the synovial fluid and in serum, have also been associated with OA [69]. One notable example is IL-15 which has been associated with RA pathology. However, IL-15 may also play an important role in early OA, since IL-15 expression in synovial fluid [70], and in serum are associated with the progression of early knee OA [71] and pain [72]. Similarly, IL-7 has been implicated in joint inflammation [73] since studies have shown it can mediate TNF-α production in the RA joint [74]. Importantly, recent studies have shown that IL-7 is expressed by articular chondrocytes and that IL-7 stimulation of cartilage induces MMP13 production and proteoglycan loss [75].

Critically, whether these inflammation-associated cytokines are central regulators of early OA disease pathology or purely biomarkers of an inflammatory tissue is an important question. This will require target validation studies in appropriate and translatable preclinical models, as well as in human ex-vivo tissues. One of the prime candidates for such studies is IL-17 since IL-17A polymorphisms correlate to OA susceptibility in particular patient cohorts [76]. In knee OA, IL-17 is elevated in both the serum and synovial fluid, and correlates with KL grade [77]. In vitro human chondrocyte studies have shown IL-17 stimulates the production of NO and MMPs [78], and decreases TIMP-2 and TIMP4 expression [79]. Given these data, studies that aim to modulate IL-17 expression or its signalling activity in appropriate models are an important next step.

**p38 pathway inhibitors**
There is good evidence that the p38 MAPK signalling pathway is activated in OA, and is a prominent pathway which mediates pro-inflammatory cytokine signal transduction. In human OA articular cartilage, p38 is phosphorylated by several putative drivers of OA including IL-1β, TNFα and damage associated molecular patterns (DAMPs) such as fibronectin fragments [80, 81]. Furthermore, p38 inhibitors have shown efficacy in reducing cartilage degeneration in both the MIA OA model [82-84], and reducing pain in RA animal models [85].

Clinically, a p38 inhibitor (PH-797804) is currently being tested for its analgesic efficacy when administered orally in patients with knee OA KL grade <2 (NCT01102660). However, due to the cell-type-dependent pro-inflammatory and anti-inflammatory functions of p38 MAPK [86], systemic delivery of p38 inhibitors may not be appropriate. An alternative approach is to develop therapeutics that target molecules downstream in the p38 MAPK pathway, thus providing a more selective targeting approach.

MAPKAPK2 (MK2) is one of several kinases directly activated by p38 [87], and is known to post-transcriptionally regulate transcripts containing adenine rich elements [88] including TNF-α and MMP-13. We have previously shown that MK2 is active in areas of cartilage damage, and that MK2 inhibition is as effective as p38 inhibition in preventing the IL-1β-mediated secretion of PGE2 and MMPs [89]. The in vivo efficacy of MK2 inhibition in OA preclinical models has not yet been reported, but MK2 inhibitors have shown efficacy in an inflammatory bowel murine model [90].
Toll-like receptors

Several DAMPs are associated with OA including cartilage extracellular matrix fragments and plasma proteins that enter the synovial fluid. Binding of DAMPs to pattern recognition receptors such as Toll-like receptors (TLRs) can activate NFκB-mediated inflammatory signalling. Articular chondrocytes have been shown to express TLR1-9 [91], but studies on TLR4 have so far provided the strongest link to OA. In humans, TLR4 expression is associated with areas of cartilage damage [92], JSN [93], and the expression of pro-inflammatory cytokines including IL-1β, TNFα, PGE₂ and NO [94]. Multiple agents that either bind to TLR4 agonists (e.g. Pep-1), or modulate TLR4 signalling (e.g. PPARdelta and BMP-7) can inhibit pro-inflammatory and pro-catabolic effects in chondrocytes [91, 95] and prevent cartilage loss in a murine model of OA [96]. Therefore, development of specific pharmacological TLR4 inhibitors may be of therapeutic value.

A number of studies have also examined the role of TLR2 in OA. Similarly to TLR4, its expression is associated with lesional areas of cartilage damage [92], and several reports have demonstrated that TLR2 stimulation induces the production of pro-inflammatory cytokines [97-99] and cartilage degradation [92]. However, in vivo modulation of TLR2 in arthritic joint models has revealed conflicting results. Of concern, TLR2 knockout mice develop a more severe OA pathology [100], which could be due to compensatory developmental effects which have yet to be established. More promisingly, TLR2 mAbs have been shown to inhibit both the production of pro-inflammatory cytokines and reduce the development of arthritis in the collagen-induced arthritis (CIA) model [99]. The therapeutic utility of such TLR2 mAbs in OA specific models has not yet been reported.
Adipose secreted cytokines

The discovery that adipose tissue secretes cytokines (adipokines) has led to the current understanding of adipose tissue as an endocrine organ [101]. Adipokine release has been mechanistically linked to metabolic complications and the metabolic syndrome [102, 103] by contributing to the low-level pro-inflammatory state seen commonly in obese individuals [104].

Critically, adipokine signalling is now receiving much attention in relation to OA joint pathophysiology largely due to the association between obesity and OA [104, 105] in both weight-bearing and non-weight bearing joints (e.g hands) [106]. Furthermore, differential expression of particular adipokines have been reported in OA serum and synovial fluid, which are capable to modulating cartilage catabolic and anabolic pathways [105].

One of the most widely studied adipokines is leptin, a product of the ob gene, which is known to regulate metabolism and appetite [107]. Leptin is increased in OA synovial fluid compared to non-OA controls [108]. Recombinant leptin, either alone or synergistically with IL-1β, induces MMP-1 and MMP-13 expression in primary human chondrocytes [109] and increases the production of inflammatory mediators including IL-1β, IL-6, IL-8 and PGE2 [110]. Due to the pro-inflammatory nature of leptin and its potential to drive cartilage catabolism, the recent development of peptide-based and antibody-based leptin antagonists could provide an effective therapeutic [111, 112].
RNA interference (RNAi)-based therapeutics such as siRNAs represents a relatively new and largely untapped avenue of therapeutic potential [113]. Relatively cheap to design and produce, this class of therapeutic is highly attractive to drug discovery [114].

One of the greatest hurdles facing siRNA therapeutics is achieving successful in vivo delivery to the site of action. Unless protected, for example by complexing with cationic lipids, siRNAs rapidly degrade in serum [115]. Successful preclinical studies using naked siRNAs have predominantly been achieved where systemic delivery can be avoided, such as in airway disease models [116], or in ocular conditions where the siRNA can be delivered directly to the site of action [117].

Systemic in vivo delivery of anti-inflammatory siRNAs has produced positive results in preclinical models of inflammatory joint disease where cationic lipids have been utilised as delivery vehicles. For example, reduced arthritis disease severity has been reported in the CIA murine RA model using cationic lipids to deliver siRNA targeting TNFα [118] and siRNA targeting a panel of pro-inflammatory mediators including TNFα, IL-1β, IL-6, IL-18 and cytosolic phospholipase A₂α [119]. In addition, IA delivery of virally packaged siRNAs targeting TNFα have shown disease-modifying efficacy in the murine CIA RA model and similarly siRNA targeting NFκB in the rodent meniscus tear OA model [120].

Optimisation of the delivery of RNAi-based therapeutics into the joint is required, together with a greater understanding of their specificity before their utility as OA
therapeutics can be fully realised. However, given that a limited proportion of targets are considered tractable with either small molecule or mAb entities, this field represents a highly important one. Furthermore, since Next Generation Sequencing has now identified multiple families of non-coding RNAs (e.g. microRNA and LincRNAs) [121], the number of potential targets for therapeutic intervention is set to markedly increase. Importantly, several of these non-coding RNAs have already been shown to regulate chondrocyte inflammatory responses.

**Stratification of OA patients for anti-inflammatory therapeutics**

Critically, for clinical trials of anti-inflammatory therapeutics, synovitis is not reported to be present in all patients with early OA. However, one cohort where synovitis is believed to play a central role is post-traumatic OA, which is attributed to 12% of symptomatic OA of the hip, knee and ankle [122]. Electron microscopy analysis suggests that trauma induces a differential synoviocyte configuration compared to primary OA synovitis [123], and increased cytokine expression has been documented after joint trauma in chondrocytes, synoviocytes, infiltrating immune cells, and synovial fluid [30, 124, 125].

Gender may also be an important factor to consider for trials of particular OA anti-inflammatory therapeutics. There are no large cohort studies that have reported differences in the degree of synovitis between male and female patients with OA using modern imaging techniques. However, such imaging techniques cannot determine differences in individual inflammatory mediators. For example, free levels of leptin have been reported to be higher in the joints of females with knee OA,
compared to males [126]. This finding could explain why the association between obesity and OA risk is reported to be greater in females [127], and suggests that therapeutics targeting leptin signalling could be more efficacious in female OA patients. Furthermore, a longitudinal prospective follow-up study found that females exhibited a greater incidence of poly-articular OA, more rapid structural progression, and a more severe symptomatic disease [128]. The higher incidence of multi-joint in females with hip OA could indicate the presence of a systemic inflammatory OA driver which is not present to the same degree, in males [128]. In addition, the increase in incidence of OA in females rises dramatically post-menopause [129, 130], with retrospective case-controlled studies demonstrating a protective and anti-inflammatory role of estrogen in OA [131-133]. Indeed, in vitro studies have shown that estrogens enhance glucosaminoglycan synthesis [134], inhibit COX2 mRNA expression [135] and protect against TNF-α, oncostatin-M [136], and ROS induced damage [137]. Furthermore, modulation of estrogen signalling is itself a potential pipeline for developing a therapeutic; however, estrogen receptor α or β agonists have failed to demonstrate anti-inflammatory efficacy in patients with RA [138].

This knowledge of the diverse OA patient population necessitates the requirement for clear patient stratification in clinical trials. The impact of not selecting the appropriate patient population was illustrated by clinical trials with the drug Iressa (AstraZeneca), an EGFR-targeted therapy for non-small cell lung cancer patients. Iressa originally failed to meet its clinical end point during a PhII trial. However, subsequent analysis showed those patients with EGFR mutation [139] had improved survival. Not selecting the appropriate patient population masked the beneficial effect of the drug in the original clinical study. Iressa was eventually approved 5
years after the original PhIII trial and several large costly follow-up clinical studies [139]. An important step towards identifying such clinical patient-selection biomarkers for clinical trials of OA anti-inflammatory therapeutics was recently made by Ruston and colleagues who identified a subgroup of hip OA patients that are epigenetically and transcriptomically predisposed to a cartilage inflammatory phenotype due to demethylation of the promotors of inflammatory/immunity-related genes [140].

Conclusions
Increasing evidence that inflammation contributes to OA joint pathology, particularly in specific patient cohorts, places great importance on both the identification of new drug targets that mediate inflammation in the joint and the requirement to re-evaluate historical clinical trial data. Preclinical studies have identified several promising targets for potential therapeutic development, and further opportunities will likely arise in the near future from new drug classes (such as RNAi therapeutics) and from the re-purposing of candidate drugs from other therapeutic research areas.

Critical in the development of any future anti-inflammatory therapeutic will be the need to identify and select the appropriate patient cohort. Our review of the historical clinical trial data of anti-inflammatory therapeutics in OA patients reveals that trials have not selected or stratified patients on the basis of synovitis or other inflammatory biomarker measurements. As such, some of these reagents may be worth revisiting in the clinic in the context of an appropriately selected patient cohort. Ideally in such cases a specific genomic or proteomic patient-selection biomarker would be identified closely linked to the mechanism of action of the therapeutic being tested.
the absence of such a specific biomarker, it would appear pertinent to select patients on the basis of overt synovitis from either MRI or imaging analysis of the joint. Such an approach will result in smaller and cheaper clinical trials, with a much greater chance to observe beneficial effects, and ultimately is the route to the development of a personalised OA therapeutic.

Key Messages

1) Inflammation is a contributor to OA joint pathology, particularly in specific patient cohorts.
2) Preclinical studies have identified several promising targets for potential therapeutic development.
3) Clinical trials have not detailed any patient selection based on synovitis or inflammatory biomarker measurement.

Acknowledgment

Figure 1 was produced using Omnigraffle 6. Clinical trials were identified using www.clinicaltrials.gov.

Disclosure statement

The authors declare no conflict of interest

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Interleukin-17F affects cartilage matrix turnover by increasing the expression of collagenases and stromelysin-1 and by decreasing the expression of their inhibitors and extracellular matrix components in chondrocytes. *Cytokine* 2011, 56(2):376-386.


Toll-like receptors and chondrocytes: the lipopolysaccharide-induced decrease in cartilage matrix synthesis is dependent on the presence of toll-like receptor 4 and...


### Table 1: Completed RCTs of anti-inflammatory therapeutics in OA

<table>
<thead>
<tr>
<th>Target Class</th>
<th>Mode-of-action</th>
<th>Drug</th>
<th>Clinical Trial Data</th>
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<tr>
<td><strong>TNFα inhibition</strong></td>
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<td></td>
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<tr>
<td></td>
<td>Adalimumab</td>
<td>PhII (NCT00296894) [19]</td>
<td>Hand OA (60 patients)</td>
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<tr>
<td></td>
<td></td>
<td>PhII/II (NCT00686439) [20]</td>
<td>Knee OA (17 patients)</td>
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<tr>
<td></td>
<td></td>
<td>PhII (NCT00597623) [21]</td>
<td>Refractory hand OA (99 patients)</td>
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<tr>
<td></td>
<td>DLX105</td>
<td>PhII/IIa (NCT00819572)</td>
<td>Painful knee OA (27 patients)</td>
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<td><strong>IL1β inhibition</strong></td>
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<tr>
<td></td>
<td>Diacerein</td>
<td>PhII (NCT00451360) [39]</td>
<td>Hip OA (507 patients)</td>
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<td></td>
<td></td>
<td>PhIV (NCT00685542) [141]</td>
<td>Hand OA (86 patients)</td>
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<td></td>
<td>AMG108</td>
<td>PhII (NCT00110942) [40]</td>
<td>Knee OA (160 patients)</td>
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<td></td>
<td></td>
<td>PhII (NCT00110916) [42]</td>
<td>Painful knee OA (170 patients)</td>
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<td><strong>Arachidonic acid pathway inhibitors</strong></td>
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<td></td>
<td>Valdecoxib</td>
<td>PhII (NCT00650624) [49]</td>
<td>Symptomatic OA of the knee (1019 patients)</td>
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<td></td>
<td>Lumiracoxib</td>
<td>PhIII (NCT00154219) [142]</td>
<td>Primary Hip OA (1262 patients)</td>
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<td></td>
<td>Licofelone</td>
<td>Unknown [55]</td>
<td>Knee OA (355 patients)</td>
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<td><strong>NO inhibitors</strong></td>
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<td></td>
<td>SD-6010</td>
<td>PhII/III (NCT00565812) [66]</td>
<td>Overweight patients with knee OA (1457 patients)</td>
</tr>
</tbody>
</table>
Abbreviations: Ph, Phase; n/s, non-significant; WOMAC, Western Ontario and McMaster Universities Arthritis Index; OA, osteoarthritis; JSN, joint space narrowing; VAS, Visual Analogue Scale; NO, Nitric Oxide; IL1β, Interleukin-1 Beta; TNFα, Tumour Necrosis Factor alpha; COX-2, cyclooxygenase-2; AUSCAN, Australian/Canadian Hand Osteoarthritis Index.
Table 2. Promising emerging targets for development of a DMOAD

<table>
<thead>
<tr>
<th>Target Class</th>
<th>Target</th>
<th>Disease Linkage</th>
<th>Preclinical Validation</th>
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<td><strong>In vitro</strong></td>
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<td><strong>In vivo</strong></td>
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<tr>
<td>Cytokines and chemokines</td>
<td>IL7</td>
<td>Expressed by articular chondrocytes [75]</td>
<td>Induces MMP13 and proteoglycan loss in cartilage [75]</td>
</tr>
<tr>
<td></td>
<td>IL-15</td>
<td>Elevated in synovial fluid of patients with early OA [143]. Serum levels associated with knee OA progression [71] and pain [72].</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL-17</td>
<td>Polymorphisms correlate to OA susceptibility [76]. Elevated in both serum and synovial fluid in knee OA, and correlation to disease severity [77].</td>
<td>Stimulates production of NO and MMPs in human chondrocytes [78], and reduced TIMP expression [79].</td>
</tr>
<tr>
<td>p38 pathway inhibitors</td>
<td>p38 MAPK</td>
<td>P38 activated by OA relevant pro-inflammatory cytokines</td>
<td>p38 inhibition blocks cytokine mediated production of PGE2, MMP3 and MMP13 in human chondrocytes [89]</td>
</tr>
<tr>
<td></td>
<td>MAPKAP K2 (MK2)</td>
<td>Active phospho MK2 in areas of OA cartilage damage.</td>
<td>MK2 inhibition blocks cytokine mediated production of PGE2, MMP3 and MMP13 in human chondrocytes [89]</td>
</tr>
<tr>
<td>Toll-like receptors</td>
<td>TLR2</td>
<td>Activated by DAMPS in OA synovial fluid. Expression high in areas of cartilage damage [92].</td>
<td>TLR2 induces cytokine production [97-99] and cartilage damage [92].</td>
</tr>
<tr>
<td></td>
<td>TLR4</td>
<td>Activated by DAMPS in OA synovial fluid. Expression high in areas of cartilage damage [92], and increased with JSN progression [93].</td>
<td>Activation promotes cytokine production in human chondrocytes [144]. Non-specific TLR4 inhibitors reduce pro-catabolic effects in chondrocytes [91, 95]</td>
</tr>
<tr>
<td>Adipokines</td>
<td>Leptin</td>
<td>Elevated in OA serum or synovial fluid [108]. Correlation between serum levels and cartilage loss at 2 years [145].</td>
<td>Induces production of MMPs [109] and pro-inflammatory cytokines [110] in chondrocytes.</td>
</tr>
<tr>
<td>RNAi therapeutics</td>
<td>TNFa (siRNA)</td>
<td>Elevated levels of TNFa in OA serum and synovial fluid [9-11]</td>
<td>Inhibition of TNF-a production</td>
</tr>
<tr>
<td></td>
<td>Pan-cytokine (siRNA)</td>
<td>Elevated levels of pro-inflammatory cytokines in OA synovial fluid [4, 69]</td>
<td>Inhibition of TNF-α, IL-1β, IL-6, IL-18 and A2α production [118]</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>siRNA reduced disease score in CIA murine model [118]</td>
</tr>
</tbody>
</table>
Abbreviations: MMP, matrix metalloproteinase; OA, osteoarthritis; MAPK, mitogen activated protein kinase; DAMPS, damage associated molecular pattern molecules; siRNA, small interfering RNA; TLR, toll like receptor; KO, knockout; NO, nitric oxide; JSN, joint space narrowing.
Cartilage degradation and fragmentation causes synovial inflammation, and increases production of pro-degradative (MMPs) and pro-inflammatory (IL-6, IL7, IL1β, IL-15, IL-17, TNFα) proteins. Synovium immune cell infiltration and obese adipose tissue contribute towards increased production of pro-inflammatory cytokines and thus increase the production of proteolytic enzymes which drive further cartilage breakdown. Blue ovals represent current and potential drugs and their associated targets. ▲ = Immune cells including B cells, T cells, and macrophages. Abbreviations; IL, interleukin; MMP, matrix metalloproteinase; NO,
nitric oxide; OA, osteoarthritis; PGE2, prostaglandin E2; TNF, tumour necrosis factor; TLR: Toll–like receptor.