Bone 51 (2012) 297–311

Contents lists available at SciVerse ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Review

Future therapeutics for osteoarthritis

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ARTICLE INFO

Article history:
Received 30 August 2011
Accepted 7 October 2011
Available online 17 October 2011

Keywords:
Osteoarthritis
DMOAD
Drug development
Treatment
Symptomatic drugs

ABSTRACT

Osteoarthritis (OA) is a disease of the joints that affects several million individuals worldwide. This disease, which involves mainly the diarthrodial joints, is chronic and develops slowly over decades, making it very difficult to precisely identify the different etiological and risk factors that influence its onset. At present, most therapies for OA are symptomatic. This review will focus on new OA therapeutics in development that are directed toward pain relief as well as others with the potential to reduce or stop the progression of the disease (DMOADs).
This article is part of a Special Issue entitled "Osteoarthritis".

Contents

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doi:10.1016/j.bone.2011.10.008

Introduction

The most common form of joint disease, osteoarthritis (OA) represents a major cause of morbidity and disability, particularly in the second half of life. The burden of this disease has been steadily gaining importance in the last few decades with the aging of the world's population and has become by far the most common musculoskeletal disease, imposing enormous costs and challenges on the healthcare system. The increasing number of patients seeking treatment for this disease could very well cause a global financial "tsunami" in the near future.

Although important advances have been made in understanding the pathophysiological processes of OA, today's treatments still focus mainly on improving the symptoms. As the relationship between the pathology and pain of OA has become better defined, new concepts have emerged allowing the development of new therapeutic approaches that are likely to improve symptomatic treatment. The newer agents of this category will be reviewed.

Even though in the last few decades, finding a disease modifying OA drug (DMOAD) has remained elusive, research into the development of such new and innovative agents continues. The progress made in the understanding of the pathophysiology of OA and the identification of risk factors has guided DMOAD development programs toward promising therapeutic targets. These, as well as new drugs and agents aiming at stopping the disease progression, will be reviewed.

Of note, this review may not be fully comprehensive of all development programs as this field is advancing rapidly.

Pathophysiology of osteoarthritis

Osteoarthritis, which mainly affects the diarthrodial joints, is a chronic disease that develops progressively over decades, making it very difficult to precisely identify the different etiological and risk factors that influence the onset of the disease. In the majority of patients, the disease is considered to be idiopathic.

It is now well recognized that OA is not a single disease entity, but rather the common final stage of joint failure. Osteoarthritis of the knee is a heterogeneous chronic disease involving all the joint tissues and the initial stages can be triggered by numerous causes and/or factors. Hence, it would seem logical that the disease be divided into subgroups, some of which may have a more biomechanical, biochemical, inflammatory or genetic signature. All these subgroups may eventually converge into a common phenotype.

It is well known that OA structural changes are mediated by a multitude of complex autocrine and paracrine anabolic and catabolic factors that act upon diverse cells from articular tissues. Studies have revealed that there is some continuity between the subchondral bone and cartilage as well as between the synovial membrane and cartilage, suggesting cross-talk between these tissues. However, the exact sequence of pathological events in OA remains unclear, as the temporal relationship between cartilage erosion, chronic synovial inflammation and subchondral bone remodeling has yet to be determined.

Symptomatic drugs for osteoarthritis (Table 1)

The most common treatment prescribed for OA is for symptomatic relief, to control the pain and improve joint function. Pain in OA is difficult to classify, as the factors contributing to its genesis such as inflammation and neuropathic and/or nociceptive pain components have yet to be fully identified. The specific sources of OA pain within the joint have also remained elusive and are likely to be multiple, including tissues of the joint such as the subchondral bone, the synovium, and the soft tissues. Although a number of local and systemic treatments have been developed that can provide patients with some degree of relief, thus improving quality of life, there remains a high level of unmet needs. Some of the therapeutics targeting disease symptom such as nonsteroidal anti-inflammatory drugs (NSAIDs) and narcotics have been found to cause significant side effects, which is a concern for the aging population. Likewise, acetaminophen, the first line agent recommended by most practice guidelines, has also demonstrated potential toxicity [1], and consequently authorities now recommend that it be taken with caution. Also frequently used for symptom relief are intra-articular injections of corticosteroids and hyaluronic acid, which have demonstrated their effectiveness and safety.

Table 1
Trials on compounds targeting osteoarthritis symptoms excluding NSAIDs and COX-2 specific inhibitors.

Category	Example	Mode of action	Highest phase	Target joint/disease	NIH-registration
Antidepressants	Duloxetine	Serotonin norepinephrine reuptake inhibitor	III	Knee OA	NCT00945945, NCT00408421, NCT00433290, NCT01018680
Anti-nerve growth factor (NGF)	Tanezumab	Monoclonal antibody against NGF	III	Knee and hip OA	NCT01089725, NCT00809354, NCT00864097, NCT00744471, NCT00863304, NCT00830063, NCT00733902, NCT00809783, NCT00985621, NCT00399490
	JNJ-42160443	Monoclonal antibody against NGF	II	Knee or hip OA	NCT00973141, NCT01094262
	REGN-475	Monoclonal antibody against NGF	II	Knee OA	NCT01239017, NCT00944892
	MEDI-578	Monoclonal antibody against NGF	I	Knee OA	NCT01072591
	PG110	Monoclonal antibody against NGF	I	Knee OA	NCT00941746
Cannabinoids	GW842166	Noncannabinoid CB2 agonist	II	Knee OA	NCT00447486, NCT00479427
	PF-04457845 (fatty acid amide hydrolase (FAAH) inhibitor)	Inhibition of endocannabinoid degradation	II	Knee OA	NCT00981357
Kainate receptor antagonists	LY545694 (synthetic iGluR5 antagonist)	Blocking kainate receptor iGluR5	II	Knee OA	NCT00790790
Capsaicin analogs	Adlea (ALGRX-4975, highly purified injectable capsaicin)	TRPV1 receptor agonist	III	End stage knee and hip OA	NCT00683267, NCT00681356
	Civamide cream (Zucapsaicin, topical)	TRPV1 receptor agonist	III	Knee OA	NCT00995306, NCT00077935
Bradykinin antagonists	MEN16132 (injectable)	Anti-algogenic and -inflammatory	II	Knee OA	NCT01091116

Table based on the NIH registry for clinical trials www.clinicaltrials.gov and MEDLINE. NGF, nerve growth factor; OA, osteoarthritis.

Drug therapy for musculoskeletal pain, however, is changing and most of the current research in the area of OA pain arises from broader research efforts with some of the drug developers repurposing drugs from other indications to OA. Indeed, in recent years, antidepressants have been introduced for chronic pain management, some of which have demonstrated an analgesic effect independent of their effect on depressive status. Moreover, although OA pain is mainly considered to be nociceptive, neuropathic symptoms can also be found in patients [2,3]. Data on some of the new antidepressants assessed for chronic pain in rheumatic conditions, including OA, reported that the improvement of depression extended beyond the reduction of depressive symptoms to a decrease in pain and improved function and quality of life [4,5]. These therapies include selective serotonin norepinephrine reuptake inhibitors. One of them, duloxetine, has demonstrated analgesic properties and, interestingly, in a study on knee OA pain, from which patients with significant psychiatric co-morbidities were excluded, the analgesic effects were not related to an action on depressive symptoms [6]. This drug is already marketed for acute and maintenance treatment of major depressive disorders, diabetic peripheral neuropathic pain, fibromyalgia, and generalized anxiety disorder. In 2010, it was approved by the USA Food and Drug Administration (FDA) for generalized chronic musculoskeletal pain including OA and chronic lower back pain.

Other new pain agents that have been investigated for their effect on OA symptoms are described below, some of which are in ongoing clinical trials.

Pain related agents under study

Nerve growth factor (NGF)

Nerve growth factor (NGF) belongs to the neurotrophin family of structurally related secreted proteins that include brain-derived neurotrophic factor and neutrophin 3 and 4. Mature neurotrophins bind to two types of receptors: $p75^{NTR}$ and members of the Trk family of receptor tyrosine kinases, of which TrkA is the receptor for NGF [7]. NGF levels are increased in arthritis conditions and animal model studies have shown that the inhibition of NGF reduces pain and hyperalgesia [8]. The initial proof-of-concept trial was based on preclinical studies showing that NGF regulates the structure and function of responsive sensory neurons, including small diameter nociceptive afferents.

There have been a number of Phase II and III clinical trials with tanezumab, a monoclonal antibody raised against NGF and a Phase I study with a fully humanized anti-NGF monoclonal antibody, PG110. The Phase II knee OA study showed that tanezumab reduced pain during walking in moderate to severe knee OA and was associated with improvement in function [9]. However, these results in terms of efficacy (higher than with NSAIDs) are challenged by the neurological side effects seen in all the studies conducted with tanezumab, of which the most common was paresthesia. Two other Phase III trials in knee [10] and hip OA patients [11] confirmed the results of the previous Phase II study. All Phase III trials with tanezumab were recently halted by the FDA because of an unexpected increase in the need for total joint replacement with this drug. Although there are several tentative explanations for such events, it seems likely that drug-induced osteonecrosis and an accelerated progression of OA possibly due to joint overuse could be responsible for such events. The dossier is currently under review by the FDA.

Cannabinoids

Cannabis has been used as a medicinal plant for thousands of years for a variety of illnesses including pain syndromes. In animal studies, it was shown to reverse inflammation-induced allodynia, to block the development of experimental hyperalgesia, and to enhance morphine-induced anti-nociception. Moreover, in different models of arthritis pain, cannabinoid agonists were found to suppress nociceptive transmission and to inhibit pain related behavior.

Two cannabinoid receptors, cannabinoid receptor 1 (CB1) and CB2, have been cloned and characterized. The CB1 receptor is present in the central and peripheral nervous system while the CB2 receptor is predominantly associated with the immune system. CB2 was also shown to regulate the inflammatory response in various settings.

Current cannabinoid pain therapies are frequently limited by central nervous system-mediated side effects. However, selective CB2 receptor agonists have been shown to be devoid of such effects, thus they have been further studied for their potential anti-inflammatory and analgesic effects in various pain models. In brief, CB2-selective agonists display anti-nociceptive activity in models of acute, persistent inflammatory, postoperative, cancer, and neurotic pain [12,13].

Recently, CB2 receptor expression has been demonstrated in the synovium of OA patients [14], supporting a potential role for CB2-selective ligands in the treatment of arthritis pain. Phase II studies evaluating the efficacy and safety of such a compound, GW842166, in patients with knee OA have been completed but data are not yet reported.

The fatty acid amide hydrolase (FAAH) is an integral membrane enzyme that hydrolyzes the fatty acid amide family of lipid transmitters including the endogenous cannabinoid. In animal models it has been found that inhibition of FAAH displayed analgesia, anxiolysis, and antidepression effects without the untoward side effects observed with direct CB1 agonists. Therefore, the selective pharmacological inhibition of FAAH has emerged as a potential strategy to retain the beneficial effects of cannabinoid receptor activation, while avoiding the undesirable effects of direct CB1 agonists. One such drug, PF-04457845, has recently completed a Phase IIa study aimed at investigating its effectiveness at treating pain in patients with knee OA.

Kainate receptor antagonists

(S)-Glutamic acid (Glu) is the major excitatory neurotransmitter in the mammalian central nervous system, activating the plethora of glutamate receptors. The physiological and pathological actions of Glu are mediated by activation of a range of excitatory amino acid transporters, ionotropic glutamate receptors (iGluRs, ligand-gated ion channels), and the metabotropic glutamate receptors (mGluRs, G-protein-coupled). Within the iGluRs, five subtypes (KA1, KA2, and iGluR5–7) show high affinity and express full agonist activity upon binding of the naturally occurring amino acid, kainic acid (KA) [15]. Although the complex roles of the iGluRs are far from being understood, a number of studies have indicated the involvement of kainate receptors in nociception. The iGluRs play a principal role in spinal cord nociceptive transmission and the iGluR5 subunit is the most readily detectable at presynaptic sites in the dorsal root ganglion. A Phase II trial with an iGluR5 antagonist, LY545694, in the treatment of knee OA pain has been completed.

Transient receptor potential vanilloid subfamily 1 (TRPV1) agonists

The transient receptor potential (TRP) belongs to a superfamily of ion channels. The vanilloid receptor TRPV1 is a non-selective cation channel expressed in the nociceptors. TRPV1 agonists and antagonists were both shown to relieve pain behaviors in preclinical animal models including in inflammation and OA [16,17]. TRPV1 is not limited to neural cells; it is also found in articular cells including chondrocytes, osteoclasts, osteoblasts, and synovial fibroblasts [18–20]. A recent study reported an association between the TRPV1 gene and risk of symptomatic knee OA [21]. Civamide cream has recently received approval in Canada for treatment of knee OA pain. Another compound, ALGRX-4975 (Adlea), showed in Phase II clinical trials that a single injection significantly reduced pain levels in patients following total knee arthroplasty (TKA) or bunionectomy, and reduced pain in patients with OA or Morton's neuroma [22]. Phase III trials have been completed in patients who are undergoing total hip arthroplasty or arthroscopic shoulder surgery and in patients with knee OA.

Bradykinin B2 receptor antagonists

Bradykinin is a peptide known to participate in both acute and chronic inflammation. It is a vasodilator and inflammatory nonapeptide, and has been found in OA synovium [23–27]. It contributes to the initiation and maintenance of inflammation, to exciting and sensitizing sensory nerve fibers thus producing pain, and to activating synoviocytes and chondrocytes. Moreover, bradykinin synergistically potentiates the effects produced by some pro-inflammatory cytokines. It exerts its actions by selectively activating the receptor B2. There are two main actions of bradykinin that suggest B2 receptor blockade as a therapeutically relevant target for local treatment of OA: its algogenic effect by activating nociceptors which innervate the capsule and the synovium, and its inflammatory effects, actions that are involved in producing the pain and synovitis of knee OA. A study was conducted with the peptidic B2 receptor antagonist, Icatibant (previously known as HOE140), administered intra-articularly in patients with knee OA [28]. Data indicate that this antagonist is capable of producing an analgesic effect in OA patients and, interestingly, this effect was reported to be more significant on the pain perceived during activity than on pain at rest. In a subsequent study, it was also reported that Icatibant induced pain relief in patients with knee OA, and has a rapid onset of action which can last for up to three months following treatment. The analgesic effect was achieved with no gastrointestinal or cardiovascular side effects [29]. However, a few months after the development of Icatibant, it was unexpectedly discontinued because of "lack of efficacy" [29]. Unfortunately, the reasons leading to the suspension of clinical development of Icatibant for knee OA treatment have not been disclosed, although problems linked to the ADME (absorption, distribution, metabolism, and excretion) profile of this antagonist may be argued. Presently, another such compound, MEN16132 in an injectable form, has completed a Phase II trial in knee OA.

Disease modifying osteoarthritis drugs (DMOADs)

The ultimate vision for the treatment of OA has been to find agents that can reduce or stop the progression of the disease. Thus far no such treatment has been approved by regulatory authorities as meeting the appropriate guidelines and criteria. However, a number of agents present several interesting properties including an extremely low incidence of side effects, a carryover effect of several weeks, and some have an additive effect with NSAIDs. These include chondroitin sulfate [30,31], glucosamine sulfate [32,33], and diacerein [34] (Table 2). Using X-rays, the two first agents were shown to effectively reduce the joint space narrowing in knee OA patients and diacerein reduced the progression of hip OA. Recently, in a knee OA cohort, chondroitin sulfate was shown by MRI to decrease the cartilage volume loss and bone marrow lesions [35]. However, there has been disagreement over the interpretation of the results of some of the studies using X-rays pertaining to issues regarding sample size, sensitivity and reliability of imaging technology, and the patient population studied.

A number of recent trials specifically investigating DMOADs have been disappointing, failing these primary objectives mainly because of the lack of efficacy or safety. New mediators and pathways relevant to OA are continually being discovered, bringing new hope into the field. These factors will be reviewed and broadly divided as follows: genetics, cartilage catabolism and anabolism, synovial inflammation, and remodeling of subchondral bone.

Genetics

In addition to some already known heritable genes, the genome-wide association (GWA) studies recently showed that currently only three loci have alleles demonstrating truly compelling association with OA across a broad range of ethnic groups. Factors related to these alleles could thus be targeted. The three loci are the 7q22 [36], the *GDF5*[37], and the *DIO2* [38].

The chromosomal region of the 7q22 is located within a large (500 kb) LD block which contains six genes: *PRKAR2B* (protein kinase, cAMP-dependent, regulatory, type II, β), *HBP1* (HMG-box transcription factor 1), *COG5* (component of oligomeric Golgi complex 5), *GPR22* (G protein-coupled receptor 22), *DUS4L* (dihydrouridine synthase 4-like), and *BCAP29* (B cell receptor-associated protein 29) which were reported to have a biological function during chondrogenesis (*COG5* and *DUS4L*) or cartilage metabolism (*BCAP29*, *COG5*, *DUS4L* and *HBP1*). The gene expression data support the epidemiological findings indicating that any of the genes at the 7q22 region may confer risk for knee OA [39].

The growth differentiation factor 5 (GDF5), also known as cartilage-derived morphogenetic protein 1, is a member of the transforming growth factor superfamily and participates in the development, maintenance, and repair of bone, cartilage, and other tissues of the joint [40]. The OA risk mediated by this locus is not restricted to cartilage, as many other soft tissues of the joint showed a consistent allelic expression imbalance of *GDF5*.

The third one, *DIO2*, codes for iodothyronine-deiodinase enzyme type 2 (D2), a selenoprotein that converts intracellular inactive thyroid hormone into its active form. D2 is a provider of local bioactive thyroid hormone and is active during normal skeletal morphogenesis as well as in mature adult tissues. Moreover, in OA the polymorphism in other genes whose proteins regulate thyroid hormone activation, the *DIO3*, has recently been suggested to also modulate OA disease risk [41].

Targeting cartilage catabolism and anabolism

Blocking MMPs and ADAMTS

In OA cartilage there is now clear evidence that the earliest histopathological lesions, which are a depletion of proteoglycans and a breakdown of the collagen network, result from increased synthesis and/or activity of proteolytic enzymes. This appears to be due to the involvement of the matrix metalloproteinase (MMP) family and to some members of the adamalysin family, the ADAMTS. For two decades, considerable attention has been devoted to developing strategies to reduce their levels and/or activity in arthritic joints.

MMPs are enzymes implicated in the natural turnover of the extracellular macromolecules and collectively they can degrade all the major macromolecules of the extracellular matrix. Some of these enzymes are synthesized as pro-enzymes, and must be activated by proteolytic cleavage. Generally, they are present as soluble forms, but some are membrane-bound. The activation of the latent secreted

Table 2
Trials that have proven a DMOAD effect on cartilage.

Category	Example	Mode of action	Highest phase	Target joint/disease	NIH registration
SYSADOA	Chondroitin sulfate	Inhibition of proteases and cytokines	III	Knee, hand OA	NCT00604539
	Glucosamine sulfate	Inhibition of proteases and cytokines	III	Knee OA	–
IL-1β inhibitor	Diacerein	Inhibition of IL-1β production and proteases	III	Hip OA	NCT00451360
Tetracycline	Doxycycline	Inhibition of collagenase and gelatinase	III	Knee OA	NCT00000403

Table based on the NIH registry for clinical trials (www.clinicaltrials.gov) and MEDLINE. DMOAD, disease modifying osteoarthritis drug; IL-1, interleukin 1; SYSADOA, symptomatic slow acting drug for osteoarthritis; OA, osteoarthritis.

enzyme results from the proteolytic cleavage of the pro-peptide domain from the N-terminus of the enzyme.

MMP genes are expressed in all tissues of the joint and generally in low levels. Their gene transcription induced by factors such as pro-inflammatory cytokines (interleukin-1β [IL-1β], tumor necrosis factor-α [TNF-α]) and some growth factors (epidermal growth factor [EGF], platelet-derived growth factor [PDGF], basic fibroblast growth factor [bFGF], and transforming growth factor-β [TGF-β]) [42]. The secreted pro-MMPs are activated by a number of physiological activators. In turn, some physiological agents can bind their active site thus inhibiting their catalytic activity. Among these natural factors are the tissue inhibitors of metalloproteinase (TIMPs). TIMPs are expressed in high levels in cartilage and the balance between the level of activated MMPs and the available TIMP determines the net enzyme activity and is a key determinant of extracellular matrix turnover. Increasing the local synthesis of TIMP has been suggested to be an effective way of preventing articular tissue turnover and OA progression. Although the major TIMP found in articular tissues is TIMP-1, recent investigations have highlighted the therapeutic potential of TIMP-3, as it regulates the pro-MMP-2 activation and demonstrates high affinity inhibition of both ADAMTS-4 and ADAMTS-5 [43] and MT3-MMP [44]. An in vivo study in mice has demonstrated that TIMP-3 deficiency results in cartilage degradation similar to OA [45].

However, TIMPs have narrow application as a therapeutic agent, mainly because of their limitation regarding the administration of proteins. Based on knowledge of the TIMP/MMP complex structure, researchers have looked at engineering the TIMP molecule to be selective to a specific MMP [46–48]. This concept of engineering TIMPs to increase the binding affinity and specificity for individual MMPs remains to be further developed, involving additional optimization and engineering.

There have also been a large variety of synthetic approaches to controlling the level of MMP synthesis/activity. Developing protease inhibitors that are therapeutically active is very challenging as, in addition to ensuring that the molecule has the required potency, it must also be bioavailable, orally active, specific to the targeted enzyme family, and have no significant toxicity.

The first rational approach to the design of synthetic inhibitors targeted collagenase. Synthetic MMP inhibitors are low-molecular-weight compounds that are divided into two categories: peptidomimetics and non-peptidomimetics. Peptidomimetics are designed to mimic the structure of collagen at the site where MMPs bind and cleave, whereas non-peptidomimetics allow for the development of highly selective inhibitors. Different chelating moieties were tested, including thiols, carboxyl-alkyls, phosphonic acids, phophonamides, and hydroxamate groups [49]. The hydroxamate-based compounds are potent MMP inhibitors. They interact with the active site of the MMP molecule and bind the zinc molecule, thus inactivating the enzyme. Thiols and carboxyl-alkyls have a similar mode of action. A number of these compounds have already been tested in clinical trials (Table 3), and data have shown that MMP inhibitors may produce musculoskeletal side effects characterized by joint stiffness, joint fibroplasias and accumulation of type I collagen in the affected joints, as well as mild anemia and elevated levels of liver enzymes [50,51]. This was suggested to be due to low MMP selectivity, and recently it has been speculated that sheddase inhibition (specific inhibition of TACE/ADAM-17) may be responsible for the observed side effects. Another collagenase compound tested in Phase III clinical trials for rheumatoid arthritis was halted not because of musculoskeletal side effects but presumably due to a lack of clinical benefit [52,53].

To achieve tight enzyme binding, inhibitors have been designed to be dependent on the use of a strong zinc metal chelator. However, a common structural feature shared among all MMPs is a conserved zinc-binding catalytic domain. As a result, these inhibitors possess low selectivity and inhibit non-targeted MMPs. In turn, inhibiting non-targeted MMPs is of concern and adverse side effects are exhibited because some MMPs are essential for normal joint physiology, cellular processing, and homeostasis. For instance, MMP-3 and ADAM15-deficient mice [54,55] develop OA more readily compared with their wild-type counterparts. Therefore, a different approach has been the use of semi-synthetic forms of the antibiotic tetracycline (doxycycline and minocycline). These compounds possess significant inhibitory properties against MMP (collagenase, gelatinase) activity and the synthesis of inducible nitric oxide synthase (iNOS) [56–59]. Their action is mediated by chelating the zinc present in the active site of MMPs. Tetracycline is a weak inhibitor of MMPs, whereas semi-synthetic homologs are more potent, making them more attractive. A clinical trial (Table 2) exploring the therapeutic efficacy of doxycycline in knee OA patients showed that it can reduce the rate of joint space narrowing in established OA [60]. However, it did not reduce the mean severity of joint pain and had no effect on the contralateral knee [60]. Its lack of effect on the contralateral knee, where the disease is less severe, suggests that pathogenetic mechanisms in that joint were perhaps different from those in the index knee. Nonetheless, this study provides the first proof of concept of the effectiveness of anti-MMP strategies for developing DMOADs.

Drug development programs are now exploring the use of selective inhibitors of proteases rather than broad protease inhibition. The main reason is based on the hypothesis that by doing so, a certain

Table 3
Trials on compounds with potential DMOAD effect on cartilage as measured either by X-ray or MRI.

Category	Example	Mode of action	Highest phase	Target joint/disease	NIH registration
MMPs	Broad MMP range (PG-530742, PG-116800)	Inhibition of MMPs	II	Knee OA	NCT00041756
	Aggrecanase (AGG-523)	Inhibition of ADAMTS-4/-5	I	Knee OA (mild to moderate)	NCT00427687
	Aggrecanase (AGG-523)	Inhibition of ADAMTS-4/-5	I	Knee OA (end stage)	NCT00454298
Nitric oxide	SD-6010	Inhibition of iNOS	III	Knee OA	NCT00565812
Growth factors	rhFGF-18	Fibroblast growth factor, increase the production and development of chondrocytes and osteoblasts	I	Knee OA	NCT00911469, NCT01033994
			II	Acute injury of the knee	NCT01066871
	Osteogenic protein-1 (OP-1 or BMP-7)	Pro-anabolic and anti-catabolic properties	I	Knee OA	NCT00456157
	38A BMP-7		I	Knee OA	NCT01133613
	BMP-7		II	Knee OA	NCT01111045
ASU (avocado oil and soybean unsaponifiables)	Piascledine 300	Inhibition of proteases	III	Hip OA	NCT01062737

Table based on the NIH registry for clinical trials (www.clinicaltrials.gov) and MEDLINE. ADAMTS, A Disintegrin and Metalloproteinase with Thrombospondin Motifs; BMP, bone morphogenetic protein; DMOAD, disease modifying osteoarthritis drug; MMP, matrix metalloproteinase; OA, osteoarthritis.

number of side effects can be avoided. However, among the MMPs, compounds selective for a single MMP member have been hard to achieve. Opinions differ as to the best MMP to target. One option points to stopping the degradation of the collagen network, as it has been shown that its loss leads to irreversible damage. With regard to collagenase inhibition, the main candidate at this time is MMP-13 as it is the most potent peptidolytic enzyme of the three known collagenases and, secondly, because it is present in only a few normal human tissues and therefore its inhibition should not be harmful to their function [61]. Its main role appears to be related to the remodeling process of cartilage in the early stages of OA, in addition to its role in the degradation of other cartilage components.

Recently, a new class of potent MMP-13 inhibitors was identified. Some of these selective MMP-13 inhibitors are non-hydroxamic acid-containing compounds and include pyrimidine dicarboxamides with a novel binding mode and carboxylic acid derivatives which were shown to reduce proteoglycan release in a rat model of MMP-13-induced cartilage degradation [62,63]. Similarly, an orally active MMP-13 inhibitor was shown in vivo in a rat meniscectomy model to exert chondroprotective effects and, interestingly, without observable musculoskeletal toxicity [64]. At about the same time, another class of MMP-13 inhibitors was discovered, one of which was demonstrated in the same rat model to reduce cartilage degradation biomarker (TIINE) levels and exert cartilage protective effects [65].

Osteoarthritis is also associated with increased loss of aggrecan fragments due to the activity of some MMPs as well as members of the ADAMTS family, ADAMTS-4 and ADAMTS-5 (also named aggrecanases). Although MMP-3 is well-known for its property of cleaving aggrecan, aggrecanases appear to be responsible for the aggrecan fragments identified in synovial fluid from OA patients [66]. Two knockout mouse studies [67,68] have strongly implicated ADAMTS-5 as the major aggrecanase responsible for the proteoglycan breakdown seen in arthritis. However, in humans, this remains to be demonstrated. Although these enzymes as well as the MMP-3 expression profile indicate that they are expressed in the early stage of the disease [69–71], the exact mechanisms by which they are activated are currently unknown, as are their exact roles in OA pathophysiology, whether they are in fact responsible for cartilage degradation in human OA, and at what stage of disease their role is most significant.

While several series of potent ADAMTS-4 and/or ADAMTS-5 inhibitors have been identified (see review [72]) there is, to our knowledge, only one study on an ADAMTS-4/-5 inhibitor (AGG-523) that has recently completed Phase I clinical trials for OA (Table 3). In a recent study in the rat model of meniscal tear-induced joint instability, treatment with oral AGG-523 significantly attenuated the level of aggrecanase-generated aggrecan fragments [73].

ADAM family members manifest some of the MMP signature sequence. As a result, many small molecule inhibitors of MMP activity also demonstrate significant efficacy against ADAMs and ADAMTS. This is due to the congruity between the MMP and adamalysin catalytic sites. Consequently, discrimination between these two families is a real challenge. However, the resolution of the crystal structures of these two aggrecanases [74] showed some structure differences that could meet selectivity for aggrecanases. Several inhibitor scaffolds possessing zinc-chelating groups were identified, and a thioxothiazolidinones compound appears to be selective for ADAMTS-5 versus ADAMTS-4, MMP-12 and MMP-13 [75,76].

Although the aggrecanase inhibitor compounds to date fall into three broad classes: 1) molecules containing the traditional zinc chelators such as hydroxamate, carboxylate or tartrate, 2) molecules containing non-traditional zinc chelators, and 3) molecules without an obvious zinc chelating group, the majority of the disclosed aggrecanase inhibitors possess moieties that bind to Zn^{+2} (carboxylic acids, tartrates, hydantoins, etc.) and are lipophilic, acidic compounds with molecular mass > 400, properties that usually do not permit good systemic exposure. The major issue of aggrecanase inhibitors thus appears to be the difficulties in obtaining high potency compounds with low molecular mass and less acidity.

As a new strategy, recent works indicate that inhibiting the interaction of syndecan-4 with some of its target molecules will protect cartilage destruction in OA. Syndecans are heparan sulfate proteoglycans expressed on the surface of adherent cells that interact with growth factors, cytokines, proteinases, adhesion receptors and extracellular matrix components through their heparan sulfate chains. These proteoglycans modulate homeostatic processes and tissue injury [77]. Syndecan-4 is specifically induced in type X collagen-producing chondrocytes in human OA and controls the activation of ADAMTS-5 through direct interaction with the protease [78].

Another product, calcium pentosan polysulfate (CaPPS), a chemically sulfated xylanopyranose from beechwood, has also been suggested as a therapeutic treatment for OA. CaPPS was shown to interact with the non-catalytic spacer domain of ADAMTS-4 and the cysteine-rich domain of ADAMTS-5, blocking activity against aggrecan with IC_{50} of 10–40 nM and increase the affinity of TIMP-3 for ADAMTS-4 and ADAMTS-5 by more than 100-fold [79].

Blocking nitric oxide

Nitric oxide (NO) as well as its primary metabolites is toxic to cells and creates extracellular matrix damage in OA. NO is synthesized from L-arginine by the action of an enzyme, NO synthase (NOS). The enzyme responsible for the production by chondrocytes of high and sustained levels of NO in response to pathologic factors is the inducible NOS (iNOS) which is expressed after cell activation by cytokines or inflammatory factors.

The constitutive NO production was shown to be necessary for the regulation of numerous physiological processes including blood pressure, platelet adhesiveness, gastrointestinal motility, and neurotransmission. OA cartilage produces a larger amount of NO than normal.

NO produced in response to cytokine stimulation exerts a number of catabolic effects that promote the degradation of articular cartilage. It reduces proteoglycan synthesis, enhances MMP activity, decreases synthesis of the IL-1 receptor antagonist (IL-1Ra), contributes to the excess production of prostaglandin E_2 (PGE_2), inhibits the production of TGF-β by chondrocytes treated with IL-1, decreases matrix production in response to insulin-like growth factor 1 (IGF-1), inhibits chondrocyte proliferation, and induces apoptosis.

The discovery and characterization of the functions of the iNOS isoenzyme have provided the impetus for developing a potential new class of drug. The challenge has been to have a selective inhibitor target only the inducible form of NOS in order not to downregulate the constitutive isoform. In vivo investigations into the potential of a selective iNOS inhibitor in a surgically induced OA animal model [80,81] showed that the compound, L-N6-imminoethyl-L-lysine (L-NIL), under prophylactic conditions, reduces the progression of early lesions, and that the inhibition of NO production was associated with a reduction in MMP activity in the cartilage. Moreover, it was shown that L-NIL decreased in situ the level of chondrocyte apoptosis and, more particularly, reduced the level of caspase-3. In an animal model, treatment with the selective iNOS inhibitor was also associated with a reduction in the levels of the pro-inflammatory mediators IL-1β and PGE_2 and nitrite/nitrate in the OA synovial fluid, as well as a marked reduction in joint effusion volume. Collectively, these data suggest that selective iNOS inhibitors may not only be effective agents for treatment of the signs and symptoms of OA, but may also exert disease-modifying activity. Phase I and II studies with an iNOS inhibitor have been conducted, and a Phase III DMOAD trial in knee OA is currently underway (Table 3).

Cartilage repair

The roles of growth factors in OA cartilage repair have been extensively studied due to their ability to enhance matrix synthesis. The efficacy of growth factors in cartilage repair is related to the

recruitment of chondrogenic cells, stimulation of proliferation and enhancement of cartilage matrix synthesis. Although growth factor therapy could be an attractive method for stimulating the repair of damaged cartilage matrix, there is evidence that with aging and in OA, articular chondrocytes may become unresponsive to growth factor stimulation. In general, in vivo administration of these agents leads to variable results. In some cases, the new cartilage is formed mainly of fibrous tissue that does not have the biomechanical properties of hyaline cartilage. In addition, the half-life of growth factors is too short to sustain therapeutic effects, and carrier systems are needed to achieve a controlled release into the joint.

Presently, there are two such drugs in clinical trials, osteogenic protein 1 (OP-1) also known as bone morphogenetic protein 7 (BMP-7), and fibroblast growth factor 18 (FGF-18) (Table 3). OP-1, unlike other members of the BMP family, exhibits very prominent anti-catabolic properties involving the IL-1β-induced catabolic effects, in addition to its strong pro-anabolic activity such as increasing synthesis of the key matrix proteins aggrecan and collagen. Animal studies demonstrated that OP-1 (BMP-7) has the ability to repair cartilage in vivo in various models of articular cartilage degradation. Phase I and II clinical trials are currently underway using a single intra-articular injection of BMP-7.

The FGF family of factors is known to play a central role in skeletal growth and development. In animals, FGF-18, contrary to some other members of this family (e.g. bFGF), showed significant anabolic effects on chondrocytes: it stimulated the growth of cartilage and proteoglycan synthesis. In a rat model of injury-induced OA, FGF-18 dose-dependently increased cartilage thickness in the tibial plateau [82]. There is currently one completed and one ongoing Phase I study on a human recombinant FGF-18 administered intra-articularly in patients with primary knee OA who are candidates for total knee replacement, and a Phase II trial in patients with acute knee cartilage injury.

Targeting synovial membrane inflammation

In OA, the role of synovial inflammation in the pathophysiology of OA is now widely accepted. Synovitis has been considered secondary to the cartilage changes yet findings indicate that synovial inflammation could be a component of the early events leading to the clinical stage of OA. Synovial inflammation leads to the production and release of pro-inflammatory cytokines and several other inflammatory mediators. Some of these factors, including the pro-inflammatory cytokines, diffuse through the synovial fluid into the cartilage, where they activate chondrocyte production of the catabolic factors through auto- and paracrine mechanisms.

Inhibiting IL-1β

Interleukin-1β plays a pivotal role in articular tissue destruction and is considered one of the main cytokines responsible for the processing of enzyme systems [42]. IL-1β stimulates its own production, increases the synthesis of enzymes, particularly MMPs, inhibits the synthesis of the major physiological inhibitors of these enzymes, and also inhibits the synthesis of matrix constituents such as collagen and aggrecan, thus making it a prime target for therapeutic approaches. The use of IL-1β inhibitors in experimental models of inflammatory arthritis and OA has provided a strong support for the role of IL-1β in the pathogenesis of these diseases. A better understanding of the regulation of mechanisms responsible for the increased synthesis of IL-1β in OA tissues has led to the development of promising new therapeutic strategies.

IL-1β is synthesized as inactive precursors, and must be activated by an enzyme before being released extracellularly in active/mature form. A protease belonging to the cysteine dependent protease family named IL-1β converting enzyme (ICE or caspase-1) has been identified, which can specifically generate the mature form of IL-1β [83]. ICE was detected in both human synovial membrane and in cartilage, with a marked and significant increase in expression and synthesis in OA tissues [84]. Based on these findings, inhibition of IL-1β activity by inhibiting ICE is an attractive therapeutic target. In vitro studies on OA cartilage and synovium explants showed that a specific ICE inhibitor can completely abrogate the formation of active IL-1β [84]. Likewise, an in vivo study in mice demonstrated that ICE inhibition effectively reduced the progression of type II collagen-induced arthritis (CIA) [85] and in induced and spontaneous mouse models of OA, joint damage was reduced [86]. A clinical trial in rheumatoid arthritis patients with an ICE inhibitor, pralnacasan, was stopped due to what is believed to be toxicity.

Cell signaling by IL-1β occurs through its binding to specific membrane receptors. Two types of receptors have been identified and named type I and type II IL-1R. Type I IL-1R is responsible for the signal transduction in articular tissue cells [87,88]. Modulating IL-1 activity is likely to be a promising strategy to reduce the progression of structural changes in OA. The therapeutic strategies of antagonizing IL-1β by either receptor blockade or molecular quenching appear to be of value.

One such factor is the IL-1Ra, a competitive inhibitor of IL-1β at the receptor level (Table 4). Preclinical studies using animal models showed that local administration or increased local production of IL-1Ra inhibits the development of structural changes associated with the disease [89]. To date, studies in humans using a human recombinant IL-1Ra have been conducted only in regard to relieving symptoms. Although an open-label study demonstrated the clinical effectiveness of intraarticular injections of IL-1Ra in patients with painful knee OA [90], a double-blind investigation conducted by the same investigators showed that a single intra-articular injection of IL-1Ra was not effective for relieving the symptoms of knee OA at 12 weeks [91]. However, significant short-term pain relief, as assessed by the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain subscore was shown at 4 days with the highest concentration compared with placebo. A number of reasons were suggested for the ineffectiveness of IL-1Ra on OA symptoms [91,92]. In a recent study, three patients with erosive hand OA who received daily subcutaneous injections of IL-1Ra for three months showed significant

Table 4
Trials on anti-inflammatory compounds.

Target	Example	Mode of action	Highest phase	Target joint/disease	NIH registration
IL-1	IL-1Ra (Anakinra)	Inhibition of IL-1 activity	II	Knee OA	NCT00110916
	Canakinumab (ACZ885)	IL-1 antibody	II	Knee OA	NCT01160822
	AMG 108	IL-1 receptor antibody	II	Knee OA	NCT00110942
	Diacerein	Inhibition of IL-1β production an proteases	IV	Hand and knee OA	NCT00685542, NCT00440661
TNF-α	Adalimumab	TNF-α antibody	III	Hand OA	NCT00597623
	Adalimumab	TNF-α antibody	II	Erosive hand OA	NCT00296894
	Infliximab	TNF-α antibody	IV	Knee OA	NCT01144143
	DLX 105	A single-chain (scFv) antibody fragment against TNF-α	I/IIa	Knee OA	NCT00819572

Table based on the NIH registry for clinical trials (www.clinicaltrials.gov) and MEDLINE; IL-1, interleukin 1; OA, osteoarthritis; TNF-α, tumor necrosis factor alpha.

improvement in pain and global disability [93]. The recombinant human IL-1Ra, anakinra, in combination with methotrexate, has been approved for the treatment of rheumatoid arthritis. It is thus suggested that new studies using different strategies for the administration of the drug to maintain a sustained therapeutic level over time should be performed before exclusion from future OA treatment.

A second line of inhibition of pro-inflammatory cytokines is the binding of the cytokine to free receptors. Such molecules are shed receptors named soluble receptors. For IL-1, two types have been found; type I and II IL-1 soluble receptors (IL-1sR). The shed receptors may function as receptor antagonists because the ligand binding region is preserved; they are capable of competing with the membrane associated receptors of the target cells. It is suggested that type II IL-1R serves as the main precursor for shed soluble receptors. Soluble receptors play an important physiological role in neutralizing cytokines. The sIL-1R binds to the free IL-1, preventing the cytokine from binding to its specific receptor, hence preventing its activity. However, IL-1Ra also binds to the sIL-1R, and the binding affinity of sIL-1R to both IL-1 isoforms and to IL-1Ra differs. Type II sIL-1R binds to IL-1β more readily than to IL-1Ra; in contrast, type I sIL-1R binds to IL-1Ra with high affinity. Therefore, the strategy using type II sIL-1R alone or in combination with IL-1Ra would seem more promising. However, soluble receptors have short plasma half-lives, and repeated doses, as in the case of IL-1Ra, would be required to neutralize the effects of the cytokine. This limitation can be circumvented by conjugating soluble receptors with a human proteolytic fragment of immunoglobulin G (IgG), which can extend the half-lives of these molecules. Another alternative could be to polymerize the soluble receptor, which can reduce its antigenicity and prolong the half-life.

Data from IL-1 signaling showed that after IL-1 binds to type I IL-1R, the IL-1 receptor accessory protein (IL-1RAcP) is recruited to form a high affinity receptor complex, which initiates the intracellular signaling cascade. In the CIA model, treatment with sIL-1RAcP showed a marked effect when given prophylactically [94]. The characteristics of this molecule make it an interesting inhibitor of IL-1 activity as it competes with membrane-bound IL-1RAcP for receptor complex formation with type I IL-1R. Moreover, sIL-1RAcP is an IL-1-specific target cell-discriminating inhibitor, as it can only induce a functional inhibition in the presence of IL-1 bound to type I IL-1R. sIL-1RAcP can also interact with the type II sIL-1R resulting in the formation of the soluble IL-1 scavenger receptor. It has been reported that sIL-1RAcP associates with ligand-bound type II sIL-1R, increasing the binding affinity of IL-1α and IL-1β to type II sIL-1R by approximately 100-fold, while leaving unaltered the low binding affinity of IL-1Ra to type II sIL-1R, thus enhancing inhibition of IL-1 when both IL-1Ra and sIL-1RAcP are present [95].

Relevant to an IL-1 neutralization strategy, a high-affinity "trap" was engineered by combining the extracellular domains of both the type I IL-1R and IL-1RAcP [96]. This IL-1Trap preferentially binds to IL-1β. However, a Phase II clinical trial [97] in rheumatoid arthritis with such a "trap," rilonacept, was discontinued due to limited benefit of the drug. Interestingly, this product is accepted for the treatment of some hereditary inflammatory disorders [97].

Another option would be the use of specific antibodies against IL-1 to neutralize its activity (Table 4). One such fully human monoclonal antibody, canakinumab (ACZ885), that neutralizes the bioactivity of human IL-1β was recently shown to completely suppress IL-1β-mediated joint inflammation and cartilage destruction in mouse arthritis models [98]. In 2009, the drug was approved by the FDA for the treatment of familial cold auto-inflammatory syndrome and Muckle–Wells syndrome, which are inflammatory diseases related to cryopyrin-associated periodic syndromes. The drug is currently being evaluated for its potential in the treatment of rheumatoid arthritis, systemic-onset juvenile idiopathic arthritis, chronic obstructive pulmonary disease, type 1 and 2 diabetes and ocular diseases. Reports from clinical trials suggest that canakinumab is well-tolerated in most patients, and no serious adverse effects have been reported. No study has yet been done with OA patients.

Another product, AMG 108, is a fully human IgG_2 monoclonal antibody that binds type I IL-1R and non-selectively inhibits the activity of both forms of IL-1 (IL-1α and IL-1β). This compound recently underwent a Phase II clinical trial evaluating its safety and tolerability on knee OA patients; this study has been completed.

Inhibiting TNF-α

TNF-α was also shown to be part of the OA pathological process, similar to IL-1β. As does IL-1β, TNF-α also increases the synthesis of proteolytic enzymes, inhibits the synthesis of the major physiological inhibitors of these enzymes, and inhibits the synthesis of the articular tissue matrix macromolecules. Biological agents that inhibit the action of TNF-α have shown excellent clinical efficacy at preventing structural damages associated with rheumatoid arthritis. As convincing pro-inflammatory and cartilage destructive effects of this cytokine have also been described in OA tissues, exploring the effect of TNF-α in OA patients appears logical. To date, there have been very few clinical studies that have explored the effect of anti-TNF-α therapy in an OA clinical setting and most of them included a small number of patients and were of short duration. However, clinical trials are now ongoing in OA (Table 4).

For one of these products, adalimumab, a fully humanized TNF antibody, a case study [99] has been reported in which a 68-year-old male patient with bilateral knee OA experienced complete relief from nocturnal pain when treated with this product. Furthermore, MRI analysis of the patient's target knee demonstrated a decrease in synovial effusion and synovitis, while bone marrow edema was nearly abolished after six months of adalimumab therapy [99]. Another small open-label study in patients with erosive hand OA demonstrated that treatment with adalimumab for 3 months did not significantly improve the signs and symptoms and most patients did not achieve an ACR20. However, this study did show that individual patients experienced some benefit [100]. A Phase II trial conducted on erosive OA of the interphalangeal finger joints showed that patients with baseline palpable synovial effusion had a statistically significant reduction in the occurrence of erosive progression [101]. A Phase III study is underway in symptomatic hand OA.

With regard to infliximab, an open-label pilot trial in patients with erosive hand OA showed a significant reduction in pain in all treated patients which was associated with a reduction in anatomical lesion radiological score at 12-month follow-up [99,102]. More recently, in a multicenter, randomized clinical trial designed to compare the efficacy of four treatment strategies in recent-onset active rheumatoid arthritis patients, the investigators scored hand X-rays for OA and compared the effect of infliximab to other drugs: treatment with infliximab was associated with reduced incidence of secondary OA in the proximal interphalangeal joint [103].

Another treatment, ESBA 105 (DLX 105), a single-chain (scFv) antibody fragment against TNF-α, is now in a Phase I/IIa study in patients with severe knee OA (Table 4). In pre-clinical studies in vitro in cell culture and in vivo in animals, ESBA105 demonstrated TNF-α inhibitory activity similar to infliximab but, in contrast to infliximab, it can penetrate the cartilage as well as the synovial tissue and surrounding tissues [104].

Inhibiting IL-6

Another pro-inflammatory cytokine, IL-6, could be of therapeutic benefit to OA patients. The levels of IL-6 are elevated in a variety of inflammatory conditions and in bone resorption. The role of IL-6 in inflammation differs from that of TNF-α, as IL-6 does not cause symptoms of inflammation when infused at high doses, but does cause hepatic production of acute-phase proteins such as C-reactive

protein (CRP). Many cell types within the joint are capable of producing IL-6 including synovial fibroblasts and articular chondrocytes. It is suggested that synthesis of IL-1β and TNF-α could induce the production of IL-6, and consequently CRP. Increased levels of these factors may in turn contribute to the development of OA. This is supported by data showing that in OA i) there is a relationship between elevated plasma levels of CRP, elevated synovial fluid levels of IL-6 and the presence of chronic synovial inflammation as graded histologically [105], ii) IL-6 was found to be a significant predictor of radiographic knee OA [106], and iii) that higher levels of this cytokine are predictive of greater risk of cartilage loss in OA and poor response to treatment [107]. IL-6 can therefore be considered a most interesting potential new target for OA treatment.

Inhibiting pro-inflammatory cytokine-induced signaling pathways (Table 5)

Several post-receptor signaling pathways have been implicated in the synthesis of cytokines, and pathways specifically involved in pro-inflammatory cytokine intracellular signaling cascades provide additional molecular targets for pharmacological intervention. After cytokines bind to their specific receptors at the cell membrane, multiple phosphorylation-dependent signaling pathways that regulate catabolic or anti-anabolic gene expressions are induced. These pathways include the serine–threonine kinases of the mitogen-activated protein kinase (MAPK) family and nuclear factor kappa B (NF-κB) cascades. The MAPK superfamily is made up of at least three main and distinct signaling pathways: the extracellular signal-regulated protein kinases (ERKs), the c-Jun N-terminal kinases or stress-activated protein kinases (JNK/SAPK), and the p38 family.

An in vivo study in an OA experimental rabbit model showed the therapeutic effect of a specific ERK inhibitor, PD198306 [108]. In brief, there was a significant reduction in structural changes (cartilage destruction and osteophyte width) as well as a decrease in the severity of synovial inflammation.

JNK inhibitors have demonstrated preventive effects on bone and cartilage destruction in rheumatoid arthritis [109,110]. However, little is known about the effect of these compounds in OA models. It has been reported that phenyl N-tert-butylnitrone, a spin-trap agent, downregulates the IL-1-induced MMP-13 expression via the inhibition of the JNK pathway in OA chondrocytes [111].

Although the p38 inhibitors showed anti-inflammatory effects in cartilage and in animal models [112,113], several clinical trials using p38 inhibitors in rheumatoid arthritis and other chronic inflammatory diseases were disappointing [114]. It is suggested that although p38 pathways regulate the expression of inflammation mediators, the lack of efficacy of p38 inhibitors in these trials may involve the disruption of one or more of the anti-inflammatory mechanisms operating in the articular tissues. Indeed, blockage of p38 could in turn also decrease the level of the anti-inflammatory cytokine IL-10, the expression of tristetrapolin which is involved in the degradation of some inflammatory mediators including TNF-α, IL-1α, cyclooxygenase 2 (COX-2) and IL-6, enhance JNK activity by reducing the expression of the MAPK phosphatase 1, and prevent the negative feedback control of TGF-β-activated kinase 1 (TAK1) activity on both JNK and NF-κB, thus enhancing the activities of these factors. Nonetheless, a p38α diaryl pyridinone inhibitor, PH-797804, is under clinical development for several inflammatory conditions and is presently in a Phase II clinical trial examining its effectiveness at relieving pain in subjects with flare-enriched knee OA.

Drugs targeting NF-κB activity/activation are also considered potential therapeutics for arthritis. NF-κB is a heterodimeric DNA binding protein that appears to be a major element in the regulation of pro-inflammatory cytokine production by activating a coordinated transactivation of their genes. NF-κB activation by the cytokines IL-1β and TNF-α has been found in many cells including OA chondrocytes and synovial fibroblasts. Growing evidence points to NF-κB signaling as not only playing a central role in the pro-inflammatory responses of cells, but also in the control of the chondrocyte differentiation process in which it could drive chondrocytes toward a more differentiated, hypertrophic-like state. In the rat CIA model, specifically blocking the activation of this factor suppressed the severity of joint destruction [115]. The role of NF-κB in OA was shown in in vitro studies using a specific NF-κB inhibitor or adenoviral gene transfer of IKBα (inhibitor of NF-κB) into OA synovial cells [116,117]. However, strategies targeted toward this factor should focus on highly specific drug modalities to prevent unwanted side effects on other proteins or signaling pathways. In the context of inhibiting NF-κB, an A3 adenosine receptor agonist is in a Phase II trial in knee OA patients. The A3 adenosine receptor belongs to the family of adenosine receptors, which are G-protein-coupled receptors that are involved in a variety of intracellular signaling pathways and physiological functions. The A3 adenosine receptor agonist was found to be a potent anti-inflammatory agent. Recently in a monosodium acetate rat model of OA, an A3 adenosine receptor agonist was shown to induce the apoptosis of inflammatory cells that had infiltrated the knee joint; however, it prevented the apoptosis of chondrocytes. Interestingly, the molecular mechanism appears to be a deregulation of the NF-κB signaling pathway resulting in down-regulation of TNF-α [118]

Alternative strategy: inhibition within the arachidonic acid pathway

The use of NSAIDs or COX-2 selective inhibitors has shown that PGE_2 inhibition alone does not seem to delay the natural course of progressive OA. COX is only one part of the arachidonic acid pathway and alternative targets in the arachidonic acid metabolic pathway have been identified.

For instance, the microsomal prostaglandin E synthase 1 (mPGES-1), an inducible enzyme acting downstream of COX, specifically converts PGH_2 to PGE_2 under basal as well as inflammatory conditions. Since the discovery of mPGES, several reports have suggested the possibility of mPGES-1 as a future therapeutic target for counteracting inflammation in inflammatory diseases. However, the major questions to be addressed are: Will the pharmacological inhibition of mPGES-1 mimic functional effects similar to its gene deletion [119,120], and will a therapy targeting mPGES-1 avoid the cardiovascular side effects associated with COX-2 inhibitors? These questions will remain unanswered until a potent (specific) inhibitor of mPGES-1 is tested in clinical studies.

Table 5
Trials on compounds targeting synovial signaling.

Target	Example	Mode of action	Highest phase	Target joint/disease	NIH registration
Adenosine 3 receptor	A3AR agonist (CF101)	Anti-inflammatory: decreases NF-κB leading to a decrease in TNF-α	II	Rheumatoid arthritis and psoriatic arthritis	NCT01034306, NCT00428974
MAP kinase	PH-797804	Diaryl pyridinone (p38α) inhibitor	II	Knee OA	NCT01102660
IKK	SAR-113945	IKK inhibitor	I	Knee OA	NCT01113333

Table based on the NIH registry for clinical trials (www.clinicaltrials.gov) and MEDLINE; IKK, inhibitor of kappa B-kinase; MAP, mitogen-activated protease; NF-κB, nuclear factor kappaB OA, osteoarthritis; TNF-α, tumor necrosis factor alpha.

Alternatively, arachidonic acid also gives origin to many lipid mediators, among which are the leukotrienes. Leukotrienes are well known for their major role in the development and persistence of the inflammatory process, and it is now clear that prostaglandins and leukotrienes have complementary effects. Leukotrienes are produced by the enzyme 5-lipoxygenase (5-LOX). Leukotriene A_4 (LTA_4) is the first to be synthesized and it is then processed into LTB_4 or LTC_4, then LTD_4 and LTE_4, which are potent chemotactic and inflammatory factors. On human OA synovial membrane, LTB_4 was shown to potently stimulate the release of pro-inflammatory cytokines such as IL-1β and TNF-α. Thus, the failure of NSAIDs to impact OA progression could be due to the fact that inhibiting only the COX pathways leads to a shunt to leukotriene production in these tissues. In view of the concept that prostaglandins and leukotrienes have complementary effects in the persistence of the inflammatory process, and chronic inhibition of COX may lead to a shunt of the arachidonic acid metabolism toward the leukotriene pathway, blocking both PGE_2 and LTB_4 production could have synergistic effects and achieve optimal anti-inflammatory activity. A dual COX/5-LOX inhibitor, named licofelone, was shown to exhibit anti-inflammatory, analgesic and antipyretic properties in animal models [121]. Data on the experimentally induced OA canine model revealed that this compound significantly reduced the severity of cartilage and subchondral bone alterations, as well as several disease catabolic pathways [122,123]. A Phase III study looking at the potential of this compound as a DMOAD showed that by using magnetic resonance imaging (MRI) and comparing licofelone to an NSAID (naproxen), the former significantly reduced global cartilage volume loss over time at 12 and 24 months, thus having a protective effect in knee OA patients [124]. Moreover, all clinical variables were improved with both licofelone and naproxen, with both having a good safety profile. Unfortunately, the development of licofelone was stopped for reasons not related to the safety of the drug.

Targeting subchondral bone remodeling

Emerging evidence suggests that changes in subchondral bone are closely involved in the disease progression. Data even indicate that the subchondral bone alterations may precede cartilage changes, and this tissue is suggested to be the site of the causally most significant pathophysiological events occurring in the cartilage. It is now believed that very early in the OA process, biological and morphological disturbances occur at the subchondral bone level that play a role in modulating articular cartilage metabolism. These disturbances in the subchondral bone appear, to a certain extent, to result from abnormal osteoblast metabolism [125,126]. However, the "myth" of harder subchondral bone explaining the sclerosis of this tissue must be put aside, as data indicate a generalized undermineralization of subchondral bone [127,128] with abnormal type I collagen quality [129]. Moreover, data also show a resorptive process in the subchondral bone; this was shown in vitro in humans [130], and in vivo in an OA animal model [131] and in humans [132,133]. Attempts to interfere with subchondral bone metabolism are thus of great interest.

A potential pharmacological approach is to target a reduction in abnormal subchondral bone metabolism activities, particularly in the early stages of disease. Two lines of intervention can be taken: preventing the resorption of this tissue and/or increasing the mineralization. A number of studies using OA animal models have explored the effects of drugs/agents that can modulate bone metabolism. To date, only a few have undergone human clinical trials (Table 6). In this context, anti-resorptive substances such as bisphosphonates, strontium ranelate, calcitonin, cathepsin K inhibitors, estrogen, and vitamin D would all seem well suited against the progression of OA.

Oral treatment with a bisphosphonate, risedronate, was shown in a Phase II clinical trial in knee OA patients to decrease CTX-II production [134]. However, in a Phase III trial, risedronate failed to show any disease modifying effect in such patients [135]. Explanations for the failure of such trials could be multiple. One reason may be that for treatment to be effective, the drug should be administered during the early stages of OA. Moreover, the imaging technology (X-ray) used in these investigations may not have been optimal for assessing a disease modifying effect. Indeed, data have shown that a few thousand OA patients would be required in order to demonstrate a statistically significant effect using X-ray technology [135]. Nonetheless, in a sub-analysis, it was demonstrated that in subjects with accelerated cartilage degradation at baseline, quantitative assessment of biochemical response, by urinary CTX-II, after six months of risedronate was associated with a significant reduction in radiological progression compared to subjects with no response [136]. In yet another sub-analysis, it was demonstrated that among those with significant radiographic progression, the 15 mg/day and the 50 mg/week doses of risedronate over 2 years retained trabecular structure and improved trabecular number, respectively, thereby preserving the structural integrity of the subchondral bone [137].

Strontium ranelate is a drug used in the treatment of postmenopausal osteoporosis [138,139]. Strontium ranelate is the only therapeutic approach that has been shown to reduce bone resorption at the same time as increasing bone formation, thereby improving bone architecture. This effect results from a decrease in the differentiation and resorptive activity of osteoclasts and increased osteoclast apoptosis [140–143]. The mode of action of the drug was demonstrated in a number of experimental studies on bone cells and pharmacological studies in animals and in clinical studies. Although the exact role of strontium ranelate in OA is still unknown, the effect of this drug on bone metabolism supports its potentially important role of promoting subchondral bone tissue homeostasis. Recently, clinical studies reported strontium ranelate to be of potential interest for OA patients. Indeed, this drug reduces the progression of radiographic features of spinal OA and back pain in women with osteoporosis and concomitant spinal OA [144]. Moreover, in a trial in postmenopausal women with different clinical levels of OA, it reduced the urinary level of the CTX-II biomarker [145]. A Phase III clinical study investigating the potential structure modifying effect of this drug in knee

Table 6
Trials on compounds targeting the subchondral bone.

Category	Example	Mode of action	Highest phase	Target joint/disease	NIH registration
Bisphosphonates	Risedronate	Remodeling of the subchondral bone	III	Knee OA	–
Calcitonin	SMC021 (oral salmon calcitonin)	Inhibition of osteoclast activity	III	Knee OA	NCT00704847
			III	Knee OA	NCT00486434
Cathepsin K	Balicatib	Inhibition of cathepsin K activity	II	Knee OA	NCT00371670
Strontium ranelate	Protelos	Inhibition of bone resorption and increase bone formation	III	Knee OA	–
Vitamins	Cholecalciferol (vitamin D)	Increase intestinal calcium absorption, remodeling of subchondral bone	II	Knee OA	NCT00306774
			III	Knee OA	NCT01176344
			IV	Knee OA	NCT00599807

Table based on the NIH registry for clinical trials (www.clinicaltrials.gov) and MEDLINE. OA, osteoarthritis.

OA patients has been completed and the results should be available soon.

Calcitonin is one of a number of natural regulators of calcium and phosphate balance in the body. It has the ability to counteract the blood calcium by increasing the effects of parathyroid hormone. In states of calcium mobilization, calcitonin may attempt to protect against calcium mobilization from bones. Calcitonin possesses potent anti-resorptive properties, which are mediated by direct binding to its receptor on osteoclasts [146]. Although various sources of calcitonin exist, the salmon source is the most potent. While historically most of the effects of calcitonin have been studied in bone, over the past decade evidence that it can modulate both cartilage and bone metabolism has accumulated [147]. There is in vitro evidence that calcitonin directly attenuates type II collagen degradation by inhibiting MMPs in articular chondrocytes [148]. In a rat ovariectomy model of post-menopause, oral salmon calcitonin significantly mitigated articular damage in an OA meniscectomy model [149]. A small Phase II trial assessing the efficacy of oral salmon calcitonin in knee OA showed an improvement in function scores as well as a reduction in some biomarker levels [150]. However, in a recent Phase III trial in knee OA patients using X-rays, oral calcitonin was found not to have a protective effect on the progression of cartilage loss [151].

Inhibition of cathepsin K is an interesting candidate for OA as it could act on both cartilage and subchondral bone remodeling. Cathepsin K is a member of the papain cystein proteinase superfamily and can cleave both type II and I collagen. One clinical Phase II study in OA patients was stopped due to adverse events.

With regard to the estrogens, a recent clinical trial conducted for two years in postmenopausal women [152] investigated whether the administration of a synthetic steroid with estrogenic, androgenic, and progestogenic properties could have similar dual protective effects on both bone and cartilage turnover. Data from this trial suggest that bone resorption can be attenuated, yet without the positive effects on cartilage degradation being demonstrated.

Although vitamin D was also shown to be an important hormonal contributor to cartilage/chondrocyte homeostasis, and vitamin D receptors are present in chondrocytes [153], its role in OA is far less understood and still controversial. The mechanisms of action of vitamin D on cartilage remain unclear; however, it could be speculated that it has a direct effect via vitamin D specific receptors. An effect on the subchondral bone metabolism is also a possibility, as the vitamin D levels in serum were found to be associated with reduced subchondral bone area [154], a known risk factor for knee OA. However, while some clinical studies reported that low intake and low serum levels of vitamin D were associated with an increased risk for progression of knee OA and incidence of hip OA [155,156], others demonstrated no association between serum vitamin D levels and joint space loss or worsening cartilage score in knee OA [133,157]. In a study [154] in which knee OA structural changes were assessed both radiographically and by quantitative MRI, vitamin D serum levels were associated with decreased knee cartilage loss and insufficient vitamin D predicted knee cartilage loss over 2.9 years. A recent clinical trial addressing the effect of vitamin D on joint structure changes in knee OA patients evaluated by MRI reported that vitamin D supplementation at a dose sufficient to elevate serum levels above 30 ng/ml does not appear to have any symptom or structure modifying benefits for knee OA [158]. Moreover, there is an ongoing Phase IV clinical trial in knee OA patients who underwent unilateral total knee replacement to evaluate the effect of vitamin D on the pain and disability related to rehabilitation of the operated knee and to the expected high prevalence of OA in the contralateral knee.

Further prospects include osteoprotegerin (OPG) or an anti-receptor activator of nuclear factor-κB ligand (RANKL), as OPG and RANKL are highly involved in the control of bone biology. Indeed, RANKL, produced by the osteoblasts, stimulates osteoclastogenesis and osteoclast activity by binding to the cell surface receptor RANK on the osteoclasts, and OPG inhibits osteoclastogenesis by binding to RANKL. The pro-resorptive effect of RANKL on the osteoclastogenesis process could therefore be targeted via the use of either OPG or an anti-RANKL antibody. Such inhibition of RANKL has been proposed as a therapeutic approach in some osteolytic diseases [159,160]. However, in OA only relevant clinical trials will be able to provide such information.

The use of drugs/agents directed toward subchondral bone to treat knee OA and prevent the progression of the joint structural changes needs further exploration. There is a very good rationale behind the use of such agents since some are already being given to patients with osteolytic diseases and have been proven safe for long-term administration. The results of a number of previous studies have been disappointing. Possible explanations for such failure could be that the patients included in those studies were already in late stages of the disease upon study entry and that the technologies used to determine the DMOAD effect were not sensitive enough. As data show that subchondral bone resorption is involved early in the OA process, further trials, perhaps in patients with less advanced disease, and with the use of more reliable and sensitive imaging technology such as MRI and biomarkers, will likely answer this important question.

DMOAD clinical research

Based on experience from the conduct of DMOAD trials in the last decades, there is no doubt that such studies present major challenges. Firstly, protocols for these trials were highly variable from one study to another. For example, inclusion/exclusion criteria including disease severity, demographics, risk factors, and structural changes differed quite significantly among the studies, having an obvious impact on the results and making it very difficult to analyze and compare the results of a particular drug. Efforts should be made to better standardize these criteria to ensure a homogeneous and representative population. This would also allow better standardization and balancing of the risks factors associated with the disease progression, which is essential in such studies.

The issue about the “extent of effect” that a DMOAD should have on disease symptoms to be “acceptable” is also problematic. To start with, the questionnaires and evaluation scales recommended and used in these studies have been “imported” from short-term trials dealing with the efficacy of OA treatment on symptoms. One might question the reliability of using these pain questionnaires for long-term DMOAD studies. It is also of note that a drug targeting a single tissue such as the cartilage will not act on the clinical symptoms of OA since, as mentioned above, OA pain could originate in the various tissues. Indeed, data from genetically modified animal models are inconclusive as to whether the inhibition of cartilage breakdown alone will result in improvement in OA symptoms [161].

Another important challenge in the development of DMOADs is finding an ideal study outcome measure. Ideally, a relatively “hard target” such as the reduction in or need for total joint replacement is most interesting. However, as a practical matter, since the incidence of total joint replacement during clinical trials is fairly low, particularly in knee OA trials, the concept of a “responder criteria” combining the impact of DMOAD treatment of disease symptoms and progression of structural changes, has recently been put forward [162], and should be looked at more carefully.

Many trials have been hindered by a combination of slow and unpredictable disease progression and relatively insensitive detection tools. These include the imaging technology and the very limited experience with chemical biomarkers in DMOAD trials. This often led to trial failure because the subjects, at least in the control groups, did not progress during the study period, thus no treatment effect could be measured. It is suggested to perform larger and longer clinical trials; however, in the past this has stopped drug development. There is a general consensus that a study duration of two years is realistic.

Another solution is to select individuals likely to progress more rapidly, who tend to be those with more severe disease, limb malalignment, and meniscal extrusion. However, in patients with more severe OA, the disease will be less likely to alter with an intervention. Moreover, if the drug has a significant effect at a later stage of the disease, it may not be appropriate at an early stage.

A query that remains largely unanswered is whether the etiopathogenesis of this disease is homogeneous in nature, implying the very same form from one joint to another, from weight-bearing to non-weight-bearing joints, from peripheral to central joints and between individuals. This question is most relevant in the context of the development of DMOADs. The fact that a considerable amount of information available on OA is derived from studies performed on weight-bearing joints may possibly create a bias in therapeutic developments. Thus, one might question whether studying one target joint is adequate and representative of a drug's effect. If the effect of treatment is systemic, should more than one joint be studied? These questions are highly relevant and need to be addressed.

The imaging method used for the assessment of a DMOAD effect has traditionally been X-ray, which is still recommended today. Should this technology still be the gold standard given the progress made in the field of OA imaging in the last decade? Probably not, given the limitations imposed by the use of X-rays [163,164]. Advanced technology such as MRI can provide more comprehensive and reliable information on the progression of the disease and the effects of DMOAD treatment [165]. The recent improvements in image analysis have led to the reliable quantitative measurement of cartilage volume and thickness in both normal and disease conditions such as in OA, and the changes in this tissue over time [166–168]. Another advantage of MRI compared to conventional imaging technologies is its ability to globally assess all the major joint structures, including the cartilage, meniscus, bone marrow, synovial membrane, and synovial effusion. This is important, as cartilage volume loss can be dependent on other joint tissue damage. The recently described new applications of MRI allowing specific quantification of articular tissue lesions, not only at the onset of OA but also of the disease progression over time, should enable the discrimination of subgroups and lead to a better understanding of the key players and risk factors in the initiation and progression of the disease [169,170]. To date, only manual or semi-automated quantitative MRI assessment methods have shown enough stability to produce cohort-scaled results. However, in recent years our group has developed a second generation tool of fully automated joint structure segmentation and quantification of volume assessment solutions which demonstrated robust stability and reproducibility of MRI readings for OA patients. These were developed for knee OA bone contours and osteophytes [171] as well as for the quantitative volume assessment of cartilage [172] and synovial fluid [173]. MRI has been approved and used for a long time as a reference tool in many fields of research including cancer and central nervous system trials. The time has come for the field of OA research to follow suit as this technology can assist in the development of DMOAD studies and reduce the number of patients to be included in Phase II and III trials.

Conclusion

A number of major hurdles are responsible for the almost idle effort to develop new DMOADs and these can be explained quite simply by three major factors that are intertwined: the slowly progressive loss of cartilage, the multifactorial nature of the disease, and the cyclical course with periods of active disease followed by remission. The unsatisfactory efficacy of currently available treatment options has led the current development efforts to focus mainly on pain drugs. This effort is short-sighted, as agents that relieve pain rarely have structure modification properties. Now is the time to concentrate efforts on developing DMOADs. The recognition of early changes in the subchondral bone and the importance of the cross-talk between the subchondral bone and cartilage during the disease process present a promising new therapeutic approach. Moreover, the concept of multi-drug regimens may eventually provide a more successful approach to the treatment of this debilitating disease.

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