The "elusive DMOAD": Aggrecanase inhibition from laboratory to clinic

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ABSTRACT

From the time of their discovery in 1999, the aggrecanases, and ADAMTS-5 in particular, have been heavily investigated as targets for disease-modifying osteoarthritis drug (DMOAD) development. Here, we provide a brief narrative review of the discovery efforts to target these enzymes, and how this led to the current ongoing programmes that hold promise for the future. We discuss a comparison of inhibition of collagen breakdown versus inhibition of aggrecan breakdown. We then summarise existing programmes that target ADAMTS-5, including small molecule inhibitors, monoclonal neutralising antibodies and nanobodies, and gene editing technologies. We also briefly discuss the potential analgesic effects this strategy may offer in addition to its joint-protective effects.

Introduction

Successful disease modification in OA remains an elusive goal, in spite of significant progress in our understanding of osteoarthritis (OA) pathogenesis and sophistication of methods to assess disease state and progression. As discussed in detail in the article by Oo and Hunter in this Supplement, a disease-modifying osteoarthritis drug (DMOAD) is a drug that modifies the underlying OA pathophysiology, thereby inhibiting structural damage to prevent or reduce long-term disability and offer potential symptomatic relief (1). Currently, there are no FDA or EMA approved DMOADs (2), but aggressive ongoing efforts by many organisations and teams in academia and industry are offering the hope that DMOADs are on the horizon. As depicted in Fig. 1 [and discussed in (1)], the current DMOAD pipeline is densely populated with active clinical trials.

It was not until the 1980s that the concept took hold that OA is not simply a mechanical "wear and tear" disease (3), but rather a condition in which well-defined biochemically mediated pathways bring about articular cartilage damage (4). These changing concepts brought the expectation that, one day, scientists would be able to develop inhibitors that prevent, slow down, halt, or even reverse cartilage damage. Since then, the developing concept that OA is a failure of the joint as an organ, where different tissues and the crosstalk between them, including articular cartilage, subchondral bone, and the synovium, drives the progression of the disease and associated symptoms (5). This has led to the identification of multiple targets in different joint tissues that contribute to disease. Drugs that are currently under investigation for their potential DMOAD effects include both anabolic (*i.e.* promoting cartilage repair) and anti-catabolic strategies, with some drugs intended for systemic administration while others, such as the anabolic FGF-18 (Sprifermin, EMDSerono) and the wnt-pathway inhibitor, SM04690 (Lorecivivint, Samumed), are being developed for intra-articular administration.

Elsewhere in this Supplement, Oo and Hunter discuss DMOADs that are in phase 2/3 (1). Exciting novel strategies are currently in earlier phases (phase 1/2), including a senolytic agent for intra-articular administration, UBX0101 (Unity Biotechnology), which aims to eliminate senescent chondrocytes from articular cartilage. Senescent cells (SnCs), which have lost proliferative potential, accumulate in all tissues with age and promote the ageing process through the secretion of the "Senescence Associated Secretory Phenotype" (SASP), a host of inflammatory cytokines, chemokines, and proteases that profoundly alter the tissue microenvironment (6, 7). Senescent chondrocytes are found in cartilage isolated

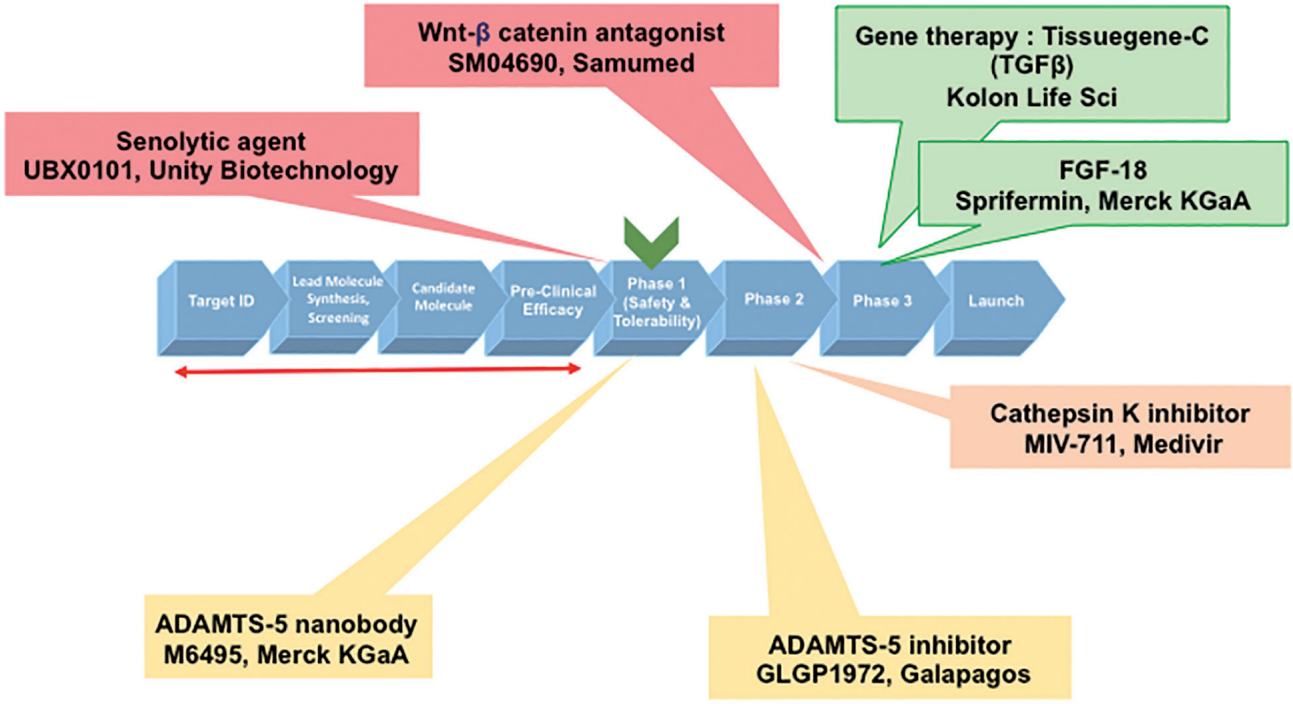

Fig. 1. Pipeline of potential DMOADs in Phase I.

from patients undergoing joint replacement (8, 9). In a surgical mouse model of OA, SnCs were found to accumulate in cartilage and synovium (10). UBX0101 is a potent senolytic small molecule inhibitor of the MDM2/p53 protein interaction, and disruption of this interaction triggers the elimination of senescent cells. In a surgical mouse model of OA, intra-articular treatment with UBX0101 attenuated joint damage and, importantly, the drug was able to reduce development of naturally occurring disease in ageing mice (10). A phase 1 study to evaluate safety, tolerability, and pharmacokinetics of a single intra-articular injection of UBX0101 in patients diagnosed with painful OA of the knee was recently completed. Results have yet to be posted on https://clinicaltrials.gov.

Targeting the cartilage matrix

The current pre-clinical and clinical pipeline comprises different strategies to block the activity of the collagenases and aggrecanases. In the current narrative review, we provide a brief overview of the discovery efforts to target these enzymes, and how this led to the current ongoing programmes that hold promise for the future.

Breakdown of articular cartilage is a hallmark of OA. Two key targetable pathways contribute to the enzymatic degradation of the cartilage extracellular matrix (ECM). Aggrecan and type II collagen make up the two major macromolecular components of articular cartilage and are essential for maintaining cartilage function and integrity, with aggrecan providing cartilage with its compressibility and collagen providing its elasticity [reviewed in (11)]. Degradation of these macromolecules is mediated by proteolytic cleavage, thus representing druggable mechanisms of intervention requiring the design of protease inhibitors with the proper pharmacokinetic properties. Aggrecan breakdown is mediated by the aggrecanases, predominantly ADAMTS-4 and ADAMTS-5, while collagen unwinding and degradation is mediated by the collagenases, predominantly MMP-1, 8, 13 and 14, although MMP-8 has not been reproducibly found in articular cartilage like other members (unpublished results).

Targeting collagen degradation

There are pros and cons to inhibiting either collagen *versus* aggrecan degradation, which need to be considered carefully. While it is accepted that preventing loss of collagen from the cartilage ECM will preserve its elastic properties and integrity, systemic inhibition of collagenase activity may pose specific problems. Type II collagen is a hydrophobic molecule and has no self-elimination mechanism from the ECM. As cells produce more collagen as part of its normal anabolic maintenance, collagen will accumulate in the matrix until it is proteolytically degraded and removed by collagenase activity. If this activity is blocked, collagen has the potential to build up in the ECM causing fibroplasia. Indeed, musculoskeletal syndrome (MSS, which is defined as painless loss of range of motion in large joints, particularly in the shoulders, joint stiffness and joint swelling, soft tissue pain, and fibrosis of palmar tendons) has been observed in clinical studies with broad-spectrum MMP inhibitors and is a pharmacological effect likely due to the non-selectivity of collagenase inhibitors (12, 13). Therefore, it is important that drug discovery scientists design drugs that target the collagenase(s) responsible for catabolism observed in OA, but not the collagenase activity responsible for matrix homeostasis in cartilage and other tis-

sues. It is currently thought that MMP-1 and MMP-14 are homeostatic collagenases in cartilage and some other tissues, whereas MMP-13 appears to be responsible for the catabolism observed in OA (14-16). Designing selective MMP-13 inhibitors has proven difficult, however. The first and second generation inhibitors that were developed chelated the zinc atom in the catalytic pocket. Although zinc chelation provides high affinity, it also promotes promiscuity and lack of selectivity (17). More recently, several research groups have discovered what is known as S1' inhibitors that dock snuggly into the S1' loop of the collagenase without binding to the zinc atom, resulting in highly selective drugs (18). Time will tell if these can be brought forward into clinic trials.

Targeting aggrecan degradation

Unlike collagen, aggrecan has a self-clearance mechanism that is independent of proteolytic degradation. For example, when chondrocytes produce aggrecan as part of their anabolic maintenance, the aggrecan molecules that do not bind to hyaluronan via link protein are normally lost from the ECM through diffusion driven by charge repulsion. This charge-based repulsion mechanism is an effective clearance system for aggrecan, which relies only on the highly negatively charged properties of the molecules and is not dependent on proteolytic cleavage. Therefore, targeting the aggrecan degradation pathway through inhibition of the aggrecanases may be a safer alternative compared to inhibition of collagen degradation. In theory, systemic inhibition of the aggrecanases should only prevent loss of healthy cartilage aggrecan during disease, without the accumulation of newly synthesised aggrecan in cartilage and/or other tissues.

In 1999, ADAMTS-4 and ADAMTS-5 were cloned and characterised (19-21). Subsequent *in vitro* studies on bovine and human cartilage explants showed that these enzymes are the major proteases responsible for aggrecan degradation, and blocking their activity inhibited both aggrecan and collagen degradation in response to IL-1 (22-24). It was also found that *Adamts5* null mice are protected from joint damage in the antigen-induced arthritis model (25), as well as in a surgical model of OA, induced by destabilisation of the medial meniscus (DMM) (26). Aggrecanase-resistant aggrecan mutant mice are also protected from developing experimental OA after DMM surgery (27). Hence, from the time of their discovery, the aggrecanases, and ADAMTS-5 in particular, have been heavily investigated as targets for DMOAD development [discussed in (28)]. Currently, three categories of therapeutics targeting these enzymes have been explored: small molecule inhibitors, monoclonal neutralising antibodies, and gene editing technologies.

Small molecule inhibitors

Agg523 is an orally available small molecule selective inhibitor of ADAMT-4 and ADAMTS-5 developed by Pfizer (Wyeth) that successfully completed phase I safety trials in the USA. This was in 2008, and the programme was later discontinued. Currently, Galapagos N.V. has a programme centering on an orally available selective small molecule antagonist of ADAMTS-5, GLPG1972. It was reported to dose-dependently inhibit aggrecan turnover in human cartilage explants (IC50<1μM) and it has a protective effect when administered prophylactically in 2 animal models, an 8-week murine DMM model and a 3-week rat meniscectomy model (29, 30). In a recently completed phase 1 study, doses of 300 mg, 600 mg, and 1050 mg/day for two weeks were reported to be generally safe and well tolerated in healthy male subjects, and a favourable PK/PD profile was observed (31). Interestingly, at all 3 doses, the drug demonstrated the ability to reduce aggrecan ARGS neoepitope levels in plasma, indicative of suppressing ongoing cartilage degradation. Currently, the compound is being tested in phase 2. Collectively, all information that has been made publicly available regarding small molecule aggrecanase inhibitors suggests that systemic inhibition of these enzymes is well tolerated and safe, at least at the levels dosed.

Neutralising antibodies targeting ADAMTS-5

GSK developed a humanised ADAMTS-5-selective monoclonal antibody (GSK2394002), which was efficacious in the DMM model both in a prophylactic and a therapeutic protocol. Systemic treatment attenuated joint damage as well as mechanical allodynia, an indicator of pain (32, 33). Unfortunately, the programme was halted because toxicity studies showed that GSK2394002 resulted in modulation of cardiovascular functions that posed considerable challenges for clinical development (34). No further information on the mechanisms of this observed effect is available.

Another anti-ADAMTS-5 antibody was developed by Rottapharm, CRB0017, which targets the ancillary domain of ADAMTS-5. It was reported to slow down disease progression in a mouse model of spontaneous OA (STR/Ort) upon intra-articular administration (35). No further information is available at this time.

An ADAMTS-5-inhibiting bifunctional Nanobody, M6495, is being developed by EMDSerono. Nanobodies are a novel class of proprietary therapeutic proteins based on single-domain antibody fragments. M6495 is a bifunctional Nanobody (Ablynx) of 28.1 kDa that binds ADAMTS-5, but not ADAMTS-1, -4, or -15, and inhibits its enzymatic activity. In this molecule, the target arm binding ADAMTS-5 is conjugated to a half-life extension arm for serum albumin. It has been reported that M6495 dose-dependently inhibited aggrecan turnover in human cartilage explants (36). In an 8-week murine DMM model, it slowed progression of joint damage when administered prophylactically (37). Phase 1 clinical trials with this nanobody, administered subcutaneously, were recently completed and results have not yet been posted.

Gene editing technologies

At the Guangzhou Institutes of Biomedicine & Health, a drug candidate was developed, B001-5, which is a mixture of two chemically modified small interfering RNAs targeting ADAMTS-5 and ADAM-17 that have a

long retention time in joints following intra-articular injection. The oligonucleotides were also conjugated to small molecule cholesterol analogs for enhanced chondrocyte and synovial fibroblast penetration. The IND application with the Chinese FDA for approval to conduct phase I safety trials will be completed in 2020 (38).

Will ADAMTS-5 inhibition provide symptomatic benefit?

The disconnect between the extent of joint damage and severity of pain has often been cited as a big hurdle for successful development of DMOADs [see also (1)]. After all, will patients care that their cartilage is no longer being degraded if they still feel pain? Recent reviews have discussed how clinical research is increasingly revealing specific structural changes that are correlated to pain and sensitisation in patients with OA (for a good review on this subject please see ref. 39).

In the case of ADAMTS-5 blockade, it should be noted that preclinical evidence suggests that blocking the activity of ADAMTS-5 will be accompanied by an analgesic effect. In *Adamts5* null mice, attenuated joint damage after DMM surgery was associated with protection from mechanical allodynia, an indicator of pain and sensitisation of the sensory nervous system (40). In agreement with this finding, it was reported that inhibiting ADAMTS-5 with a neutralising antibody blocked mechanical allodynia after DMM surgery, both in a prophylactic and in a therapeutic treatment protocol (32, 33). Likewise, the M6495 Nanobody improved gait performance in a surgical rat model in a dose-dependent manner (37).

It is worth highlighting that ADAMTS-4/5-mediated degradation of aggrecan may have a direct effect on nociceptors and thus directly cause pain. Indeed, we recently reported that a 32 amino-acid fragment that is generated by the orchestrated enzymatic cleavage of the aggrecan core protein by ADAMTS-5 and MMPs ("32-mer", ^{342}F–E^{373}) (41) can activate cultured nociceptors, specifically through Toll-like receptor (TLR)-2 (42). Furthermore, intra-articular injection of this 32-mer peptide (but not a scrambled peptide) elicited knee hyperalgesia in wild-type but not *Tlr2* null mice. Remarkably, "Chloe mice", a transgenic line in which the MMP cleavage site (N^{341}↓^{342}F) in the aggrecan interglobular domain is mutated, thus preventing production of the 32-amino acid fragment (27), were protected from knee hyperalgesia after DMM surgery despite exhibiting more severe cartilage damage (42). Thus, this single TLR-2 ligand, which is a product of aggrecanase-mediated aggrecan cleavage, may play a central role in driving knee pain but not joint damage in murine OA. It will be of interest to test if circulating levels of this 32-mer fragment can be developed as a marker of OA pain.

Summary

In conclusion, despite the substantial unmet medical need in OA, the development of DMOADs has proven elusive. Despite decades of research and development efforts by many research groups, no drug has made it into the hands of patients. Reasons for this include the complexity of OA disease and its multi-gene nature, less support for musculoskeletal disease research by funders and big pharma compared to other therapeutic areas, fewer researchers working in the OA field as compared to other more "trendy" areas of research, as well as other factors (for example, the erroneous perception that OA is not a severe disease, as discussed in this Supplement) (43). However, with candidate drugs slowly making their way into clinical trials, there is hope that a "true" DMOAD will finally make its way to patients in the foreseeable future. As we have discussed here, inhibition of ADAMTS-5 remains an attractive strategy that may offer pain relief in addition to structural protection, and the activity of which can be monitored quite easily.

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