Translational strategies in drug development for knee osteoarthritis

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Osteoarthritis (OA) is a common disease worldwide with large unmet medical needs. To bring innovative treatments to OA patients, we at Merck have implemented a comprehensive strategy for drug candidate evaluation. We have a clear framework for decision-making in our preclinical pipeline, to design our clinical proof-of-concept trials for OA patients. We have qualified our strategy to define and refine dose and dosing regimen, for treatments administered either systemically or intra-articularly (IA). We do this through preclinical *in vitro* and *in vivo* studies, and by back-translating results from clinical studies in OA patients.

Introduction

For successful and cost-effective drug discovery and development, and to ensure that the path to drug approval is de-risked, several factors need to be clearly defined preclinically, well before a drug candidate enters the clinic. Failure or success of Phase II clinical proof-of-concept (cPoC) trials, which are most often a combination of dose finding and proof-of-mechanism (PoM), is dependent on effective and, where feasible, predictive preclinical models, to increase the likelihood that primary efficacy endpoints are achieved. In addition to preclinical work, studies have shown that drug development programs that can rely on biomarkers correlating with efficacy have a higher chance of success [1,2]. In translational medicine at Merck, by building on the three pillars that have been rigorously prioritized: identifying the right biological target, identifying the right patient population and identifying the right therapeutic window for the drug candidate's mechanism of action (MoA) [3], we aim to leverage the key data that we have and ensure precise bench-to-bedside translation, and bedside-to-bench back-translation, for a well-informed and highly specific approach to our clinical dosing strategy in support of our OA drug development, as described in this paper.

One of the indications that has been the focus of our research and drug development over the years, and one of the most challenging diseases to treat, is osteoarthritis (OA), the most common joint disorder in the world [4,5]. Current estimates put the number affected by OA at 250 million worldwide [6]. In the over-60 age group, 10% of males and 18% of females have the disease, and the prevalence of OA is expected to increase with increased life expectancy [7]. The unmet medical need is high, because there are no disease-modifying drugs on the market for the millions of patients with OA. The FDA has acknowledged that OA can be a serious disease [8], and therapies that can modulate the underlying pathophysiology are required to change the course of the disease and prevent long-term disability. For example, symptomatic knee OA accounts for >90% of knee replacements or joint reconstruction surgeries [9]. OA in general is characterized by chronic joint pain, swelling and stiffness that lead to activity limitations, sleep interruption, fatigue, depression, anxiety and ultimately loss of independence and a reduced quality of life. Thus, there is an urgent need for more treatment options for patients. The management of symptoms alone is insufficient because the disease continues to progress with current treatments, indicating that the underlying disease pathology needs to be addressed to secure sustainable clinical benefit. We believe that only a positive

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GLOSSARY

ADAMTS A disintegrin and metalloprotease with thrombospondin motifs.
Aggrecan The cartilage-specific proteoglycan core protein (CSPCP) or chondroitin sulfate proteoglycan. Aggrecan is encoded by the ACAN gene which is a family member of the lecticans (chondroitin sulfate proteoglycan). The proteoglycan is an integral part of the extracellular matrix in cartilagenous tissue and it withstands compression in cartilage by binding hyaluronan and water. Aggrecan has a molecular weight of 2500 kDa and it consists of two structural domains (G1 and G2) at the N terminus and one domain (G3) at the C terminus, separated by an extended domain (CS) that is modified with glycosaminoglycans. Articular cartilage contains up to 10% proteoglycan, whereby most of that is aggrecan. Aggrecan plays a crucial part in cartilage homeostasis and its loss is an early sign of osteoarthritis.
Aggrecanase A proteolytic enzyme that acts on aggrecan in the cartilage, and a member of the ADAMTS family.
Allometric scaling A technique used to explain the observed relationships between organ size and body mass of mammals.
Anabolic Constructive metabolism; the synthesis in living organisms of more complex substances or molecules from smaller or simpler ones.
Biomarker Measurable indicator of a biological state or condition.
Catabolic Destructive metabolism; the breaking down in living organisms of more-complex molecules into smaller or simpler ones, with the release of energy.
Chondrocyte A cell that generates and maintains the matrix of mostly collagen and proteoglycans found in cartilage.
Collagen The most abundant protein in mammals and major component of connective tissues, including tendons, ligaments, skin and muscles.
Effusion An escape of fluid into a body cavity.
Epigenetics The study of heritable phenotype changes, not involving alterations in the DNA sequence.
Etiopathogenesis The cause and subsequent development of an abnormal condition or disease.
Explant culture A technique to culture cells from pieces of tissue, involving an extensively sterilized extraction process, so that the culture can be maintained to near in vivo conditions for 2−3 weeks.
Femorotibial joint Articulatio femorotibialis; the main spheroid of the stifle knee joint. It is formed by the thick condyles of the femur articulating in a rolling movement with the flattened condyles of the tibia. The femorotibial joint can be separated into two compartments: the medial and lateral compartment of femorotibial joint. The femorotibial joint is not, but forms together with, the femoropatellar joint (the articulation between patella and femur) in the knee joint.
Matrix metalloproteinase Enzymes that are responsible for the degradation of most extracellular matrix proteins during organogenesis, growth and normal tissue turnover.
Mesenchymal cell Stem cells that can differentiate into a variety of cell types, including osteoblasts (bone cells), chondrocytes, myocytes (muscle cells) and adipocytes (fat cells).
Metabolomics The study of chemical processes involving intermediates and products of metabolism.
Microcomputed tomography A 3D imaging technique utilizing X-rays to see inside an object, slice by slice.
Neo-epitope Antigen regions generated by modification of the original antigen.
Osteoblast A terminally differentiated product of mesenchymal stem cells. Osteoblasts comprise several types of matrix proteins composing the bone tissue and there are responsible for bone formation.
Proteomics The large-scale study of proteins.
Synovium Vascularized connective tissue that mediates exchange of nutrients between joint fluid and blood.
Synovial fluid The viscous fluid found in the cavities of synovial joints.

effect on structure will be able to provide improvement in long-lasting clinical outcomes [10].

Currently available drugs for OA patients, such as steroids and nonsteroidal anti-inflammatory drugs (NSAIDs), focus on symptomatic relief. However, the pain relief is short-lived and transient, and these therapies do not delay the long-term structural impact of OA on joints. Furthermore, there is a long-term negative impact associated with using pain relief medications owing to the risk of adverse events, including substance dependence (and potentially abuse and a decreased quality of life), increased morbidity and mortality in patients taking opioid analgesics, and upper gastrointestinal bleeding and cardiovascular events in patients receiving NSAIDs [11–14].

The Osteoarthritis Research Society International (OARSI), among other organizations, has recently provided guidelines for the treatment of OA: an updated, patient-focused guideline with treatment recommendations for patients with OA was recently published [15], which also provided a treatment algorithm to support decision-making for individualized treatment. Studies focusing on the voice of the patient clearly state that current marketed drugs do not serve them, and that they hope for products that will stop disease progression and restore joint homeostasis [16]. Current hypotheses for OA drug development focus on inhibiting structural deterioration. Although many potential therapeutics have been investigated, no pharmaceutical agent has been approved for clinical use as a disease-modifying treatment for osteoarthritis (DMOAD) [17–20]. The development of DMOADs is very challenging, partly because of variable rates of disease progression [21,22], variable treatment responses in patients [23] and, although progress is ongoing, a lack of validated biomarkers as OA drug development tools. Defining dosing strategy also remains a challenge [18]. In addition, the new draft guidance from the FDA on OA drug approvals [8] emphasizes key considerations that sponsors should follow regarding structural endpoints:

i The challenge with addressing a multifactorial, complex and heterogenic disease population and a discordance between structural changes and pain modulation in the all-comer population.
ii The unknown translatability of structural change to sustainable clinically meaningful benefit.
iii The unknown translatability of structural change resulting in abandoning or postponing the need for joint replacement surgeries as the ultimate proof of DMOAD efficacy.

The goal of this paper is to highlight our strategy for de-risking early clinical development in OA, fine-tuning dose levels and dosing regimens, as typically determined through modeling for subcutaneous (SC) and for intra-articular (IA) administration routes, and leveraging biomarker discovery to inform these decisions.

Understanding OA mechanisms and potential drug targets for DMOADs: structural modification in the cartilage via pro-anabolic and anticatabolic pathways

Because OA is in part defined by the loss of cartilage structure and function, specifically proteoglycan aggrecan and collagen type II, our approach to date has been to target the molecular mechanisms involved in cartilage formation and degradation. There are several anabolic and catabolic pathways that are dysregulated in OA cartilage, and potential drugs that are pro-anabolic or anticatabolic could re-balance the physiology of the joint by targeting biologically affected pathways (see Glossary of terms). One approach is to promote cartilage repair mediated by recombinant fibroblast growth factor (FGF), particularly FGF-18, which can induce type II collagen and proteoglycan formation [24,25]. Human FGF-18 is a 19.8 kDa secreted protein expressed by lung tissue, chondrocytes and osteoblasts [24,26,27]. FGF-18 increases chondrocyte proliferation, differentiation and cartilage deposition [24,28–31]. Signaling by FGF-18 through FGFR3 has been shown to promote mesenchymal cell differentiation (chondrogenesis) and production of cartilage matrix, leading to repair and reconstruction of articular cartilage [29]. Sprifermin is a truncated 170-amino-acid form of FGF-18 in which the signal sequence and the 11 C-terminal acids have been removed. It has been shown to stimulate proliferation and cartilage matrix accumulation in chondrocytes and cartilage cultures *in vitro* [32–34]. In a Phase II dose-ranging placebo-controlled clinical study (FORWARD) of patients with knee OA, statistically significant structural improvement was observed following intra-articular administration of sprifermin, after clinical safety data were published in earlier Phase I studies [25,35–37]. At Year 2, statistically significant, dose-dependent increases in change from baseline in cartilage thickness of the total femorotibial joint (primary endpoint) in patients receiving sprifermin was observed [38,39]. In a recent *post hoc* analysis of the same trial, a subgroup 'at-risk' for disease progression [baseline minimum medial or lateral JSW 1.5–3.5 mm, and baseline Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain score of 40–90] who had been administered sprifermin for only four cycles up to 18 months displayed structural improvement, as well as relevant and significant improvement in pain scores at Year 3 [40].

Potential therapeutics that target the catabolic pathways include matrix metalloproteinase (MMP) inhibitors and aggrecanase inhibitors. The clinical development of the former has thus far been impeded by musculoskeletal toxicity probabilities attributable to off-target activities. Aggrecanase inhibitors specifically target members of a disintegrin and metalloproteinase with thrombospondin type 1 motif (ADAMTS) family of enzymes, which are responsible for aggrecan cleavage during early cartilage remodeling. Having been demonstrated preclinically to protect tissues from aggrecan loss and cartilage degradation, small molecules for oral treatment and large molecules for systemic administration are under development. In particular, ADAMTS-5 cleaves proteoglycans like aggrecan and is crucially involved in remodeling the extracellular matrix in OA. Aggrecan can attract water to the cartilage and enable unique biomechanical properties. The role of ADAMTS-5 in cartilage degradation has been demonstrated preclinically *in vitro* and *in vivo*, and inhibition of ADAMTS-5 in *ex vivo* explant cell-based assays and in OA animal models showed a decrease in neo-epitope levels and decreased joint damage [41–46]. One neo-epitope biomarker of aggrecan degradation (374-ARGS) is a product of ADAMTS-5 enzymatic activity. Elevated levels of this biomarker in serum and synovial fluid (SF) have been described to be associated with different cartilage pathologies [47]. Based on these data, ADAMTS-5 is regarded as a key target to inhibit and prevent cartilage degradation and OA.

Translational medicine approaches for DMOAD development

One of the key challenges specific to potential OA drug preclinical development, particularly dosing strategies, relates to the fact that many of these treatments are designed for local injection, as opposed to oral or systemic administration. Following IA injection (for knee OA), any possible post-treatment modulation to systemic biomarker levels has been extraordinarily challenging to detect *in vivo*, in animal models and in patients. Historically, the difficulty in linking systemic (blood) soluble biomarker modulations to IA OA therapy in humans or animals has been because biomarkers produced locally in the knee joint might be diluted in the circulation to below the lower limit of quantitation of any available biomarker assay. Newer array platform technologies enabling highly sensitive single-molecule protein detection could help circumvent this issue in the future. Although it is difficult to conduct synovial fluid sampling in patients participating in knee OA clinical studies, for local measurements of potential pharmacodynamic (PD) biomarkers, there is an impediment to analogous collection in classical OA small-animal models, owing to joint size limitations. Inflammation and effusion increase the volume of liquids in the joints and therewith the probability of success when sampling. This results in an unwanted 'forced' selection of individual animals and investigating synovial biomarkers only in those that have an effusion phenotype. Therefore, in the preclinical phase there is a barrier to the translatability of our *in vivo* models with regard to local biomarker sampling. Nevertheless, we were able to implement a disciplined approach to developing drugs for OA, resulting in a portfolio that is diverse and inclusive of IA- and SC-administered drug candidates.

Global efforts to identify biomarkers in OA

Despite the challenges in identifying reliable efficacy biomarkers for OA, in the current era of precision medicine, the need for OA biomarkers continues to increase because it is considered to be essential for drug development [48]. In the past few years, several reports have been published describing OA biomarkers associated with the clinical outcomes of knee OA progression, collagen degradation, patient variability and levels of inflammation [48,49]. Other reports evaluated the combination of nonsoluble (e.g., imaging) with soluble biomarker readouts [50,51]. Given the complexity of the disease and the need to phenotype patients who develop the disease as a result of different risk factors, biomarkers

are needed to stratify patients for different therapeutic approaches based on their individual pathophysiology and OA endotype [52]. However, the challenge remains to identify biomarkers that are linked to efficacy in OA models and patients, which is hampered by a lack of understanding of the disease. For example, to the best of our knowledge, no soluble proximal or distal PD biomarker has been successfully shown to be directly transferable from preclinical experiments into the clinic and proven to reflect disease-modifying activity of a therapy in patients. This could be caused by the complexity of the disease and different OA endotypes, and because of sampling issues. We can assume high molecular variability in the composition of the synovial fluid in the respective joint and the reproducible quantification and correlation with disease stages of single biomarkers diluted in the periphery is a challenge [53]. However, local sampling requires an invasive biopsy of the joint. This is a risk for patients and is accompanied by the liability of an unwanted patient selection bias: the more effusion and inflammation the patient has at the time of sampling the better the chances are to successfully aspirate enough fluid for further investigations. This liability also accounts for the lack of translation between preclinical and clinical testing. In our experience, it is close to impossible to sample pure synovial fluid in experimental animals without lavage unless they have severe inflammation and effusion. As a result, the identification of soluble biomarkers to show the same disease-modifying activity locally preclinically and clinically remains unsolved.

The situation is different when referring to a systemic metabolic pathology and OA [54]. Evidence is increasing that aspects referred to as metabolic syndrome, summarizing a cluster of metabolic and cardiovascular complications, including obesity and visceral adiposity, insulin resistance, dyslipidemia, hyperglycemia and hypertension, are interlinked with OA and rheumatoid arthritis [55]. Besides differences in study protocols, patient heterogeneity and differences in OA phases are the likeliest reasons. The likelihood of identifying reliable systemic endotypes and PD biomarkers could increase, if we focus on the hypothesis of OA as a systemic instead of a local disease. In addition, a recent review detailing four distinct OA subtypes, including inflammatory, subchondral bone remodeling, metabolic syndrome and senescent age-related endotypes, suggested evaluation of the molecular biomarker pattern alongside other techniques such as imaging [56] to identify predictive biomarkers.

Given the obvious need to have translational biomarkers, particularly those that correlate to efficacy, extensive global initiatives are ongoing to address the need for soluble biomarkers, and imaging and genetic biomarkers associated with OA and OA treatment. Here, the aim is to leverage biomarkers identified through these initiatives to aid early disease diagnosis, to support drug development and to stratify OA patients appropriately. These highly collaborative, cross-industry and academia efforts include but are not limited to the following:

i FNIH/OARSI – the goal is to identify OA biomarkers with a greater prognostic ability to measure early structural and symptomatic OA progression. By enabling stratification and clinical trial enrichment of patients at the highest risk for OA progression, OA biomarkers are expected to facilitate smaller, shorter trials of new OA treatments. Currently, a retrospective analysis of placebo-treated patients from several randomized control trials is ongoing for imaging and soluble biomarkers. (https://fnih.org/what-we-do/biomarkers-consortium/programs/osteoarthritis-project).

ii IMI APPROACH – the goal here is to support the identification of different OA phenotypes, allowing formulation of recommendations for more-personalized treatments, by setting up a database of OA patients as well as a longitudinal cohort. This project will result in a cohort of >10 000 patients and healthy volunteers (https://www.approachproject.eu/about-approach).

iii OARSI/OMERACT – as part of an effort to highlight the importance of patient reported outcomes (PROs), this international collaboration of researchers, regulatory agencies, patients, healthcare professionals and others will build on recent publications [57,58] to determine and define clinically relevant biomarkers (https://www.oarsi.org/research/oa-biomarkers).

Novel protein and non-protein biomarker candidates will hopefully be discovered and developed through these and other ongoing efforts [59], to address the clinical and scientific need for applicable biomarkers in OA studies and OA therapeutic drug development. As these biomarkers emerge, more highly collaborative approaches that assess large numbers of samples across clinical trials will be needed for clinical validation.

Leveraging biomarkers for drug development in OA

Owing to the challenge of measuring meaningful and significant *in vivo* modulations of systemic soluble biomarkers after IA administration, we have turned to reliable *in vitro* human OA chondrocyte 3D cultures (as described by Gigout *et al.* [32]) and human OA tissue cultures to detect response to our drugs. These include a scaffold-free culture system, in which cells develop a cell–matrix construct over time, closely resembling cartilage. This system is ideal to test anabolic effects and the release of anabolic biomarkers. In addition, we employ cartilage and meniscus explant culture systems, either alone or in the presence of other types of articular tissue (the synovium, for example) as a co-culture to more-appropriately evaluate anticatabolic or anti-inflammatory approaches and associated biomarkers. This multifaceted *in vitro* system is a key component of a three-pronged preclinical framework for decision making in advancing potential OA therapeutics – *in vitro* assessments, *in vivo* efficacy models and back-translation using clinically effective drugs in the same pharmacology studies (Fig. 1). In this framework, and in parallel, we de-risk our clinical development programs for OA in the following ways:

i Identifying soluble PD biomarkers for cPoC trials using refined *in vitro* cell and tissue culture models. For this, soluble biomarkers are selected based on the known mode of action of the molecule and subsequently measured in the medium of the cell or tissue culture. To reflect the complexity of the joint and the crosstalk between tissues, tissue co-culture studies are also performed. The biomarkers that are modulated by the compound can be further evaluated in an *in vivo* setting.

ii We complement this with nonsoluble biomarker data gathered from *in vivo* OA models, using histology and microcomputed tomography (micro-CT) to assess structural changes, and incapacitance and gait analysis to assess pain [60]. These data are used to support human-equivalent dose

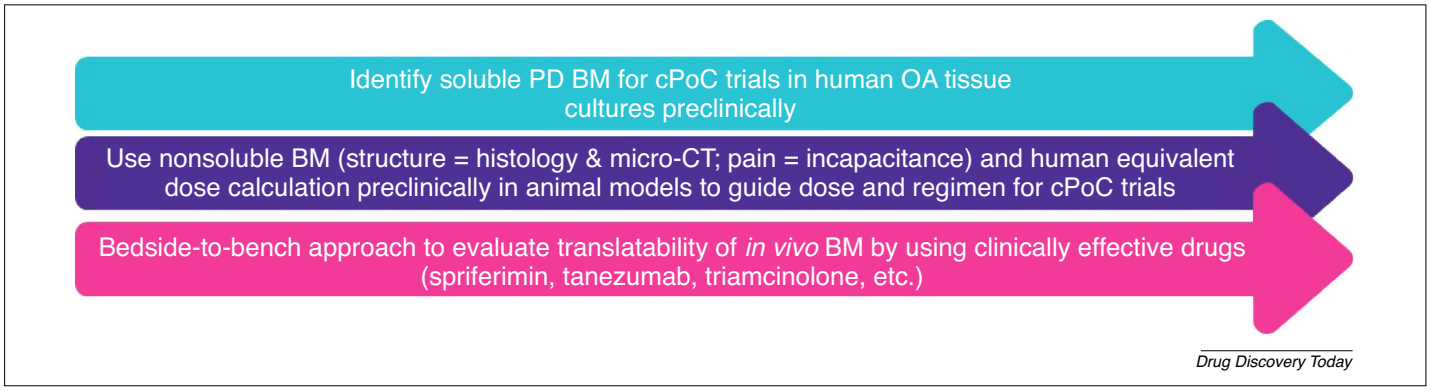


FIGURE 1

Tiered decision-making, for defining dose and dosing regimen to support clinical osteoarthritis drug development, using biomarker data. The data that we have generated internally for molecules in clinical trials (spriferimin, M6495) and molecules in early development (pro-anabolic and anticatabolic) exemplify and support this three-pronged approach. Abbreviations: BM, biomarkers; cPoC, clinical proof of concept; OA, osteoarthritis; PD, pharmacodynamic; micro-CT, micro computed tomography.

calculations (HED) to guide dose and dosing regimen for cPoC trials. To increase translation from animal models to humans and relevance of pain assessment in animal models, we employ sophisticated and innovative free-range housing conditions incorporating pain readouts. This is conducive to joint (over) loading and enabling novel observer-independent measures of pain, like voluntary walking on flat surfaces, stair climbing, jumping or contact-free incapacitance measurements [61].

iii Employing a bedside-to-bench approach to evaluate translation of the described non-soluble *in vivo* biomarkers by using clinically effective agents, such as spriferimin, as described above, or an anti-nerve-growth-factor monoclonal antibody (anti-NGF Mab) for preclinical PoC (pPoC), because these types of antibodies have been shown to be effective for treating OA pain [62]. When used in the same preclinical *in vivo* studies, these clinically validated compounds enable us to compare the effect size of drugs targeting new mechanisms to a clinical reference in the same animal model. With human equivalent doses of clinically effective spriferimin [39], we measured a significant increase in cartilage volume in animal models of OA via histology and an increase of joint space width and a significant impact on subchondral bone structures by microCT analysis [63,64]. Using clinically effective equivalent anti-NGF Mab doses we validated our pain readouts in rats and rabbits to be translational between animals and human patients [60,65].

Our proposed clinical dosing strategy

At Merck, we work within a framework for defining and refining dosing strategy based on route of administration (SC or IA). For both scenarios, we start with a deep understanding of the disease biology, using mechanistic preclinical models such as the rat anterior cruciate ligament transection (ACLT) or monoiodoacetate (MIA) models [29,46] and, in parallel, identify soluble biomarkers through human OA tissue culture [34].

Subcutaneous administration

As described in Fig. 2, for SC administration, we demonstrate pPoC by showing efficacy [structural and symptomatic (pain) benefit] at a similar dose, preferably in the same animal model study. For drug candidates synthesized for direct systemic administration, we rely on correspondingly significant and meaningful dose-dependent modulation of a PD (blood) biomarker. We then define a first-in-human (FIH) dose and dosing regimen by implementing translational pharmacokinetic (PK)/PD modeling for defining the human equivalent dose (HED), bearing in mind the maximum tolerated dose (MTD) and dosing regimen (although in all cases to date the safety margins are fairly high). Further refinement is possible as new data come in.

In the afore-mentioned case, we have been able to refine our HED with clinical data, including using data from our soluble PD biomarker analysis. Supporting the utility of our model, in the case of an SC-administered molecule M6495 (an anti-ADAMTS-5 nanobody), we accurately predicted the HED (Fig. 3) [66]. The data that we obtained for this molecule from Cynomolgus monkey PK and toxicity studies included soluble PD biomarker data after dense sampling (Fig. 3a, ARGS level modulation). After analyzing data from our Cynomolgus monkey PK/PD study and toxicology studies, a population PK/PD model was assessed to establish the relationship between PK and target engagement in Cynomolgus monkeys. We scaled the Cynomolgus monkey model using allometric scaling on model parameters according to best practices for biologics, then incorporated information on the PD biomarker in humans. Human dose was simulated, and we determined the dose range to be studied in the clinical trial (Fig. 3b). After our FIH trial, the clinical data confirmed the model predictions, and we were then able to refine the PK/PD model based on the FIH data, and subsequently results from our first safety study in patients.

Intra-articular administration

However, for IA injection of new biological entities, the framework we use for FIH dose and dosing regimen is different and much more complex. Based on our experience, the dose and dosing regimen cannot be established upon systemic exposure in IA-administered large molecules, owing to the ultra-low concentrations that are measurable. For new biological projects, our strategy is to first confirm that we have significant and meaningful dose-dependent efficacy in our preclinical models; then, considering

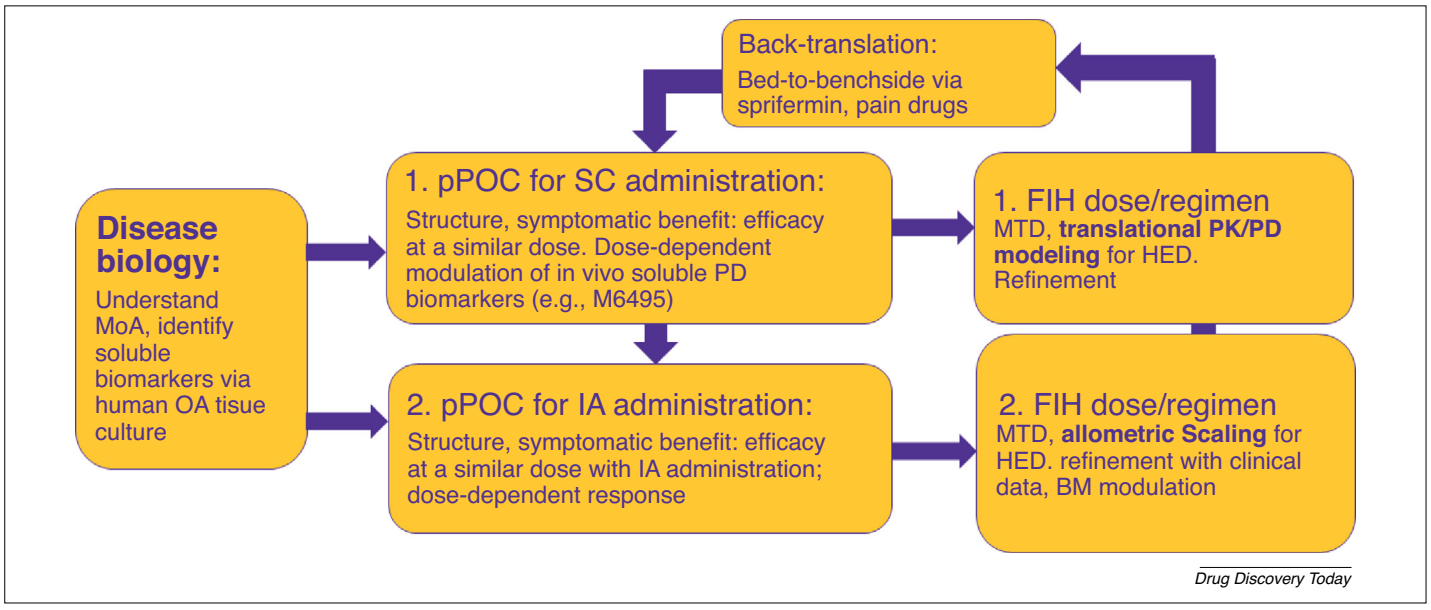


FIGURE 2

Refinement of the dosing strategy, depending on route of administration. Abbreviations: BM, biomarkers; SC, subcutaneous; HED, human-equivalent dose; IA, intra-articular; MoA, mechanism of action; MTD, maximum tolerated dose; PK, pharmacokinetic; PD, pharmacodynamic; pPoC, preclinical proof-of-concept; OA, osteoarthritis.

what we know to be the MTD (if any) from preclinical safety studies, we employ allometric scaling alone for HED.

This was the case for recombinant human fibroblast growth factor 18 (rhFGF18), sprifermin, administered IA, as demonstrated in the case study in Fig. 4. We showed, based on a mechanistic preclinical model and clinical data, that we could utilize allometric scaling through back-translating results from our FIH studies to our preclinical rat model. In preclinical studies, significant and sustained structural improvement in an OA rat model (internal results, Fig. 4a) was demonstrated through cartilage volume increases after a cumulative dose of 3 μg (1 μg in 3-weekly IA injections) per joint. Our preclinical studies *in vivo* provided meaningful and significant dose-dependent responses and the biological evidence to support an investigational new drug (IND) application package as expected in the FDA Draft Guidance [8]. Subsequently, in the clinic, we observed a dose-dependent cartilage thickness increase in OA patients (Fig. 4b), which further supported the MoA interpretation, through a clear pro-anabolic response with two cycles of 3-weekly 100 μg doses, administered six months apart (a cumulative dose of 600 μg per joint) [25]. This validated our allometric scaling based on the knee anatomy of the rat versus patients (Fig. 4c). Using knee cartilage surface area as an independent reference for rat doses, we calculated a theoretical HED at 588 μg per joint. Not only do we now have data that support our scaling factor but the results also qualified the rat model as a translational, mechanistic model supporting OA IA programs for the structural endpoint 'cartilage', thus providing us with a tool to scale animal data to human.

Clinical dosing strategy summary

The strategic framework for dose prediction utilizing preclinical structure data, then incorporating preclinical pain data at a similar dose, can determine HED across all OA programs. This approach is shown in Fig. 5. Given the right biological target, for structure data we can show the MoA via *in vitro* and *in vivo* pharmacology models, then confirm the therapeutic window and dose response via the pPoC study. For molecules that are administered systemically, we work to identify systemic soluble PD biomarkers supporting the PK/PD model and MoA, then conduct classical translational PK/PD modeling to determine the HED. For IA-administered molecules, we can identify soluble PD biomarkers for investigation in joint tissues through *in vitro* experiments initially, and then use allometric scaling for HED. In parallel, with preclinical models that show symptomatic benefit, we again confirm the target through mechanistic studies *in vivo* to show deep understanding of the disease biology, and then confirm the therapeutic window via pPoC, with similar dose response as with structural benefit, preferably in the same model. We can then define the FIH starting dose and, as more data from the sprifermin FORWARD study become available, we can refine the dose through back-translation, which could support disease and drug-related PD biomarker modulations.

Concluding remarks and future perspectives

We have described a clear approach for defining and refining the dose and dosing regimen for clinical PoC trials in OA, given our standardized requirements for decision-making across translational medicine, our confidence in our preclinical models for structural improvement and our defined preclinical strategy in translational studies across OA programs. Predictivity and translatability of preclinical models for pain remain a challenge. Biomarkers for OA and OA disease progression will continue to be investigated – *in vitro* through explant data and *in vivo* preclinically and in patients. ARGS is our target engagement biomarker for M6495 (SC administration), with clear evidence that it can support classical translational modeling, because clinical data to date have proven that preclinical data are predictive of PK in the clinic. We know that for

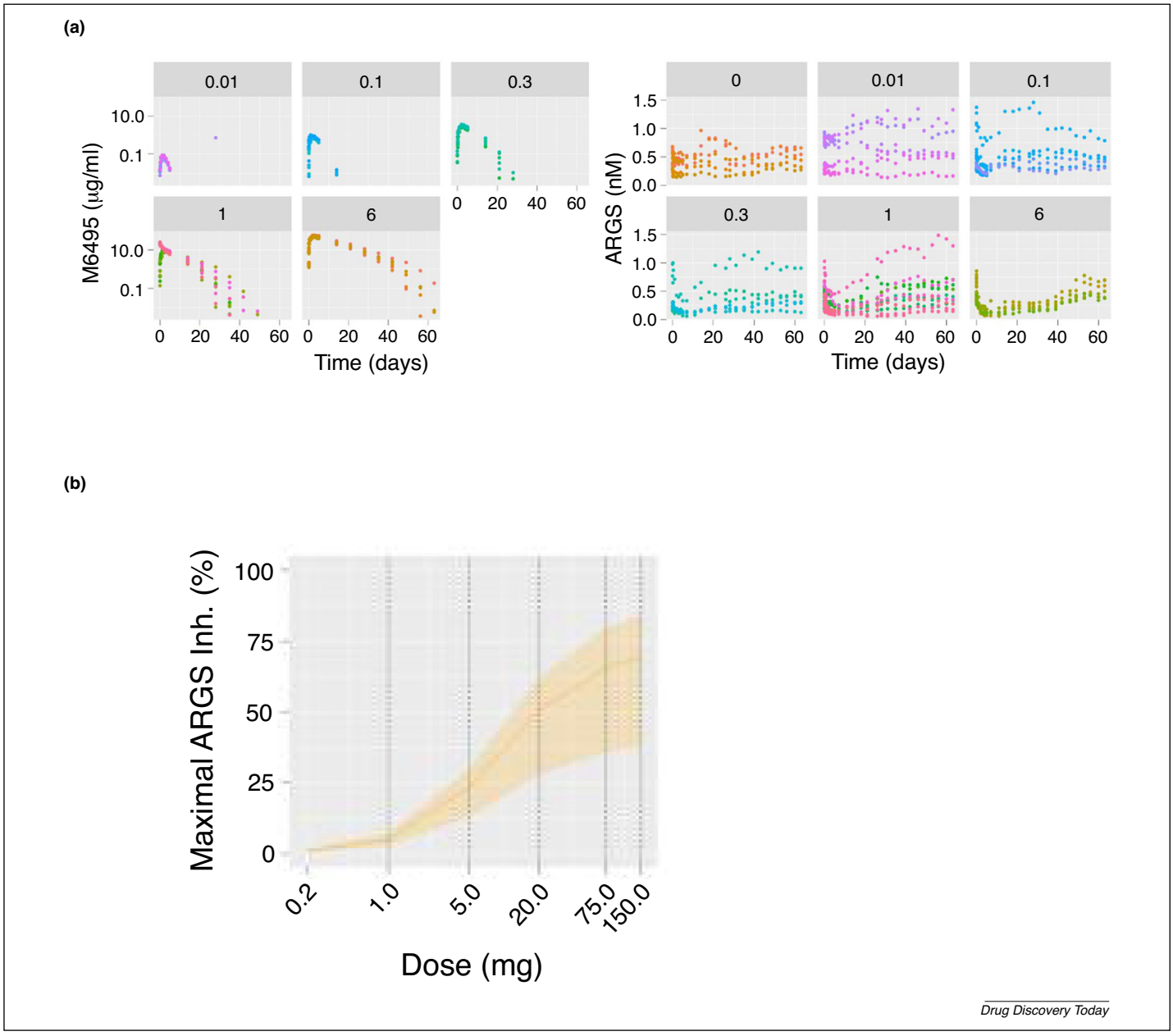
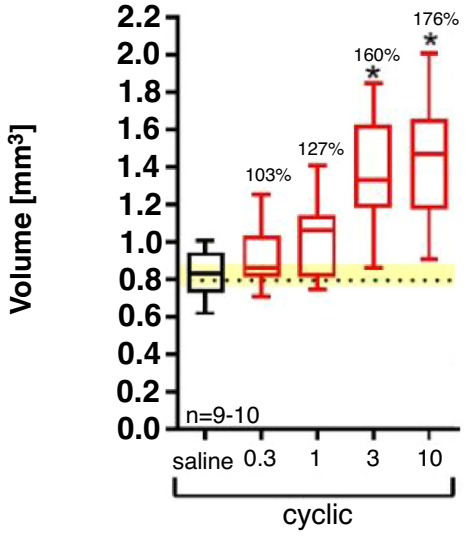


FIGURE 3

(a) Pharmacokinetics and pharmacodynamics of M6495 after a single dose (mg/kg) in Cynomolgus monkeys. Data collection for Cynomolgus pharmacokinetic/pharmacodynamic model showing M6495 exposure levels and modulation of ARGS for target engagement. Cynomolgus monkey data in this study, which included single-dose levels of M6495 at 0.01, 0.1, 0.3, 1 and 6 mg/kg subcutaneous, were used to support human-equivalent dose estimations. **(b)** Scaling of Cynomolgus monkey pharmacokinetic/pharmacodynamic model and refinement with clinical data – Project M6495. We employed allometric scaling on the Cynomolgus pharmacokinetic/pharmacodynamic model parameters according to best practices for biologics. Once we had information on the ARGS biomarker in humans, we were then able to incorporate this in the model and simulate the human-equivalent dose. Determination of dose range will continue to be studied in clinical trials. To date, this translational pharmacokinetic/pharmacodynamic model has accurately predicted the human-equivalent dose for Project M6495, as the clinical data collection has confirmed the model predictions. M6495 has predictable pharmacokinetics and exposure and ARGS modulation supported an accurate prediction of human pharmacokinetic/pharmacodynamics. Upcoming clinical proof of concept studies will gather data to assess correlation of ARGS modulation with clinical benefit.

HED calculation and allometric scaling we have a strong tool to determine dose and dosing regimen based on our preclinical data for IA administration, as confirmed by retrospective analysis with sprifermin on structural endpoints. We are also more confident that our preclinical models can predict quantitative benefit for IA administration and that the developed allometric scaling method can be used for potential future drug candidates that are administered IA. In addition, we have translational human disease tissue assays and animal models to investigate compound effects, to inform the MOA of potential targets and underline their value. We continue to aim to show structural and symptomatic benefit at a similar dose and dosing regimen, in the same animal if possible,

(a)



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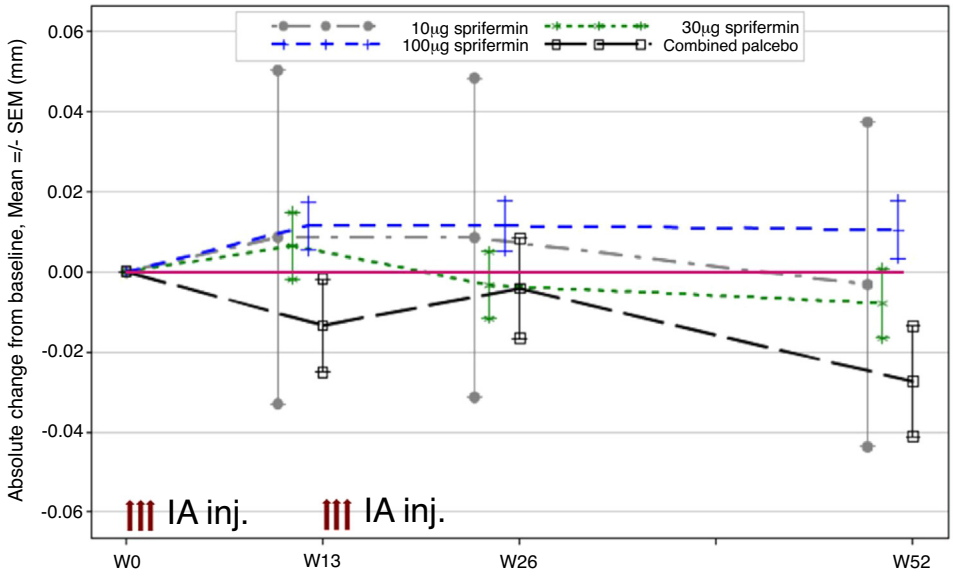

(c)

Species / Reference	Rat	Human	α
Efficacious dose (μg/joint)	**3**	**tbd**	N/A
Cartilage surface area (mm2)	6.2	1215	1

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FIGURE 4

Rat model data supported human-equivalent dose through a retrospective analysis based on sprifermin nonclinical and clinical data. **(a)** Rat ACLT model (3 weekly injections per cycle, with saline, 0.3, 1, 3, and 10 μg IA of sprifermin): early preclinical study *in vivo* serves as a mechanistic model, showing biological evidence of mechanism of action and dose-dependent response to sprifermin. This figure shows cartilage volume increasing dose-dependently in an experimental instability rat osteoarthritis model after weekly administration of sprifermin. With total doses of 3 μg, regardless of regimen, knee tibia plateau diameters were increased. **(b)** Dose-dependent cartilage thickness increase in patients with osteoarthritis. Clear pro-anabolic response. Phase Ib, Study 28980. 6X100 μg per joint (600 μg) [25]. **(c)** Data to support allometric scaling of dose to humans (based on knee anatomy [67]). Using independent scaling of rat doses and knee cartilage surface area as the reference we calculated the approximate dose that might have been applied in our first in human study (tbd = 588 μg/joint). The cumulative human dose in these initial patient studies relative to rat efficacious dose was within range (600 μg) and supported by the *in vivo* preclinical model. Thus, sprifermin first in human studies confirmed that allometric scaling could translate into human efficacious dose via a retrospective analysis. This provided confidence in our allometric scaling factor and qualified the rat ACLT model.

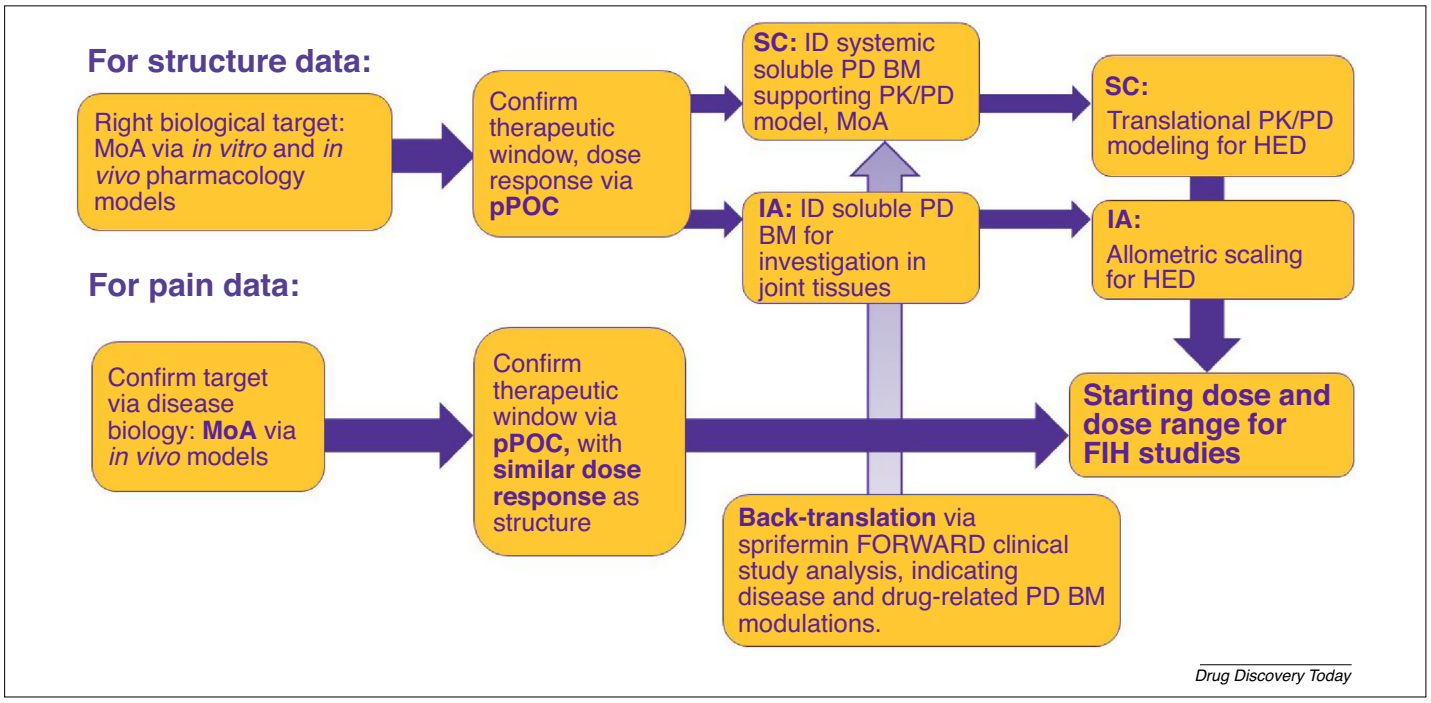

FIGURE 5

Strategic framework using structure and pain data, administered at a similar dose, to help determine the human-equivalent dose across all osteoarthritis programs. Abbreviations: BM, biomarkers; SC, subcutaneous; FIH, first in human; HED, human-equivalent dose; IA, intra-articular; MoA, mechanism of action; MTD, maximum tolerated dose; PK, pharmacokinetic; PD, pharmacodynamic; pPoC, preclinical proof-of-concept; Q1, quarter 1.

and in at least two species. As mentioned throughout, the challenge specific to OA drug development following local IA injection for knee OA is how to detect *in vivo* any post-treatment modulations using systemic biomarkers. As the industry continues to explore drugs for OA patients, we are aware that some support imaging biomarkers as being just as meaningful as soluble biomarkers to predict disease progression, owing to the lack of biomarker data in the OA community. Currently, therefore, we are confident that we have the appropriate tools for decision-making and for risk management of clinical development plans.

The preclinical models that we have to hand can now be used for mechanistic understanding, although we have yet to determine whether they are predictive of efficacy in OA patients. To improve clinical development, PD biomarkers (in synovial fluid, blood and urine) are useful to show target engagement and PoM but correlation with clinical benefit has yet to be demonstrated. For example, to date, researchers have not shown that ARGS modulation is indirectly or directly linked to pain benefit in preclinical models. In our preclinical investigations, we will continue to recognize the whole joint pathophysiology of OA and include data from other tissues, in addition to cartilage, through explant cultures. Also, through micro-CT, we investigate bony structure. We will focus on histology through therapeutic treatment in more-advanced OA animal models. We aim to investigate pain and structure in parallel as much as possible, and to consider aging models in addition to joint instability OA models. In the meantime, current technologies could measure differentiated serum PD biomarker profiles after IA treatment, potentially through clinical studies that investigate proteomics profiles or analyze soluble biomarkers before and after joint replacement in patients. However, the challenge remains in identifying reliable biomarkers that can be measured in blood and urine, and correspondingly robust, reproducible and sensitive assays that translate from preclinical matrices into patient sample analyses. Most important will be those biomarkers that are modulated in the early stages of OA that can potentially be used as sensitive diagnostic, prognostic and treatment decision tools. As we learn more about the patient pathophysiology of each OA phenotype, explore technological advances and work collaboratively across the industry, new and potentially impactful opportunities will arise to provide sustained and significant benefit for those patients suffering from OA.

Conflicts of interest

K.J.C., K.K-D and A.G. are employees of Merck; F.M., J.K., R.T. and J.D.M. are employees of EMD Serono (a business of Merck); H.G. is an employee of Servier.

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References

1 Heatherington, A.C. *et al.* (2018) How well are we applying quantitative methods to reverse translation to inform early clinical development? *Clin. Pharmacol. Ther.* 103, 174–176
2 Plenge, R.M. (2016) Disciplined approach to drug discovery and early development. *Sci. Transl. Med.* 8, 349ps315
3 Dolgos, H. *et al.* (2016) Translational Medicine Guide transforms drug development processes: the recent Merck experience. *Drug Discov. Today* 21, 517–526
4 Lawrence, R.C. *et al.* (2008) Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. *Arthritis Rheum.* 58, 26–35
5 Osteoarthritis Research Society International (OARSI) (2016) *Osteoarthritis: a serious disease.* Available at:2016 https://www.oarsi.org/sites/default/files/docs/2016/oarsi_white_paper_oa-serious-disease.pdf
6 Hunter, D.J. and Bierma-Zeinstra, S. (2019) Osteoarthritis. *Lancet* 393, 1745–1759
7 Glyn-Jones, S. *et al.* (2015) Osteoarthritis. *Lancet* 386, 376–387
8 U.S. Department of Health and Human Services *et al.* (2018) *Osteoarthritis: structural endpoints for the development of drugs, devices, and biological products for treatment, draft guidance for industry.* Available at:2018 https://www.fda.gov/media/71132/download
9 Culliford, D.J. *et al.* (2012) The lifetime risk of total hip and knee arthroplasty: results from the UK general practice research database. *Osteoarthritis Cartilage* 20, 519–524
10 Gregori, D. *et al.* (2018) Association of pharmacological treatments with long-term pain control in patients with knee osteoarthritis: a systematic review and meta-analysis. *JAMA* 320, 2564–2579
11 Hochberg, M.C. *et al.* (2012) American College of Rheumatology 2012 recommendations for the use of nonpharmacologic and pharmacologic therapies in osteoarthritis of the hand, hip, and knee. *Arthritis Care Res.* 64, 465–474
12 McAlindon, T.E. *et al.* (2014) OARSI guidelines for the non-surgical management of knee osteoarthritis. *Osteoarthritis Cartilage* 22, 363–388
13 Zhang, W. *et al.* (2019) Emerging trend in the pharmacotherapy of osteoarthritis. *Front. Endocrinol.* 10, 431
14 Fernandes, L. *et al.* (2013) EULAR recommendations for the non-pharmacological core management of hip and knee osteoarthritis. *Ann. Rheum. Dis.* 72, 1125–1135
15 Bannuru, R.R. *et al.* (2019) OARSI guidelines for the non-surgical management of knee, hip, and polyarticular osteoarthritis. *Osteoarthritis Cartilage* 27, 1578–1589
16 Arthritis Foundation (2017) In *The Voice of the Patient: Report of the 2017 Patient-Focused Drug Development Meeting.*
17 Alcaraz, M.J. *et al.* (2019) Emerging therapeutic agents in osteoarthritis. *Biochem. Pharmacol.* 165, 4–16
18 Karsdal, M.A. *et al.* (2016) Disease-modifying treatments for osteoarthritis (DMOADs) of the knee and hip: lessons learned from failures and opportunities for the future. *Osteoarthritis Cartilage* 24, 2013–2021
19 Oo, W.M. *et al.* (2018) Disease-modifying drugs in osteoarthritis: current understanding and future therapeutics. *Expert Opin. Emerg. Drugs* 23, 331–347
20 Tonge, D.P. *et al.* (2014) The hallmarks of osteoarthritis and the potential to develop personalised disease-modifying pharmacological therapeutics. *Osteoarthritis Cartilage* 22, 609–621
21 Leyland, K.M. *et al.* (2012) The natural history of radiographic knee osteoarthritis: a fourteen-year population-based cohort study. *Arthritis Rheum.* 64, 2243–2251
22 Soni, A. *et al.* (2013) Neuropathic features of joint pain: a community-based study. *Arthritis Rheum.* 65, 1942–1949
23 Arden, N.K. *et al.* (2008) A randomised controlled trial of tidal irrigation vs corticosteroid injection in knee osteoarthritis: the KIVIS Study. *Osteoarthritis Cartilage* 16, 733–739
24 Ellsworth, J.L. *et al.* (2002) Fibroblast growth factor-18 is a trophic factor for mature chondrocytes and their progenitors. *Osteoarthritis Cartilage* 10, 308–320
25 Lohmander, L.S. *et al.* (2014) Intraarticular sprifermin (recombinant human fibroblast growth factor 18) in knee osteoarthritis: a randomized, double-blind, placebo-controlled trial. *Arthritis Rheumatol.* 66, 1820–1831
26 Kapadia, R.M. *et al.* (2005) Glycogen synthase kinase 3 controls endochondral bone development: contribution of fibroblast growth factor 18. *Dev. Biol.* 285, 496–507
27 Ohbayashi, N. *et al.* (1998) Structure and expression of the mRNA encoding a novel fibroblast growth factor, FGF-18. *J. Biol. Chem.* 273, 18161–18164
28 Davidson, D. *et al.* (2005) Fibroblast growth factor (FGF) 18 signals through FGF receptor 3 to promote chondrogenesis. *J. Biol. Chem.* 280, 20509–20515
29 Moore, E.E. *et al.* (2005) Fibroblast growth factor-18 stimulates chondrogenesis and cartilage repair in a rat model of injury-induced osteoarthritis. *Osteoarthritis Cartilage* 13, 623–631
30 Valverde-Franco, G. *et al.* (2006) Defects in articular cartilage metabolism and early arthritis in fibroblast growth factor receptor 3 deficient mice. *Hum. Mol. Genet.* 15, 1783–1792
31 Valverde-Franco, G. *et al.* (2004) Defective bone mineralization and osteopenia in young adult FGFR3-/- mice. *Hum. Mol. Genet.* 13, 271–284
32 Gigout, A. *et al.* (2017) Sprifermin (rhFGF18) enables proliferation of chondrocytes producing a hyaline cartilage matrix. *Osteoarthritis Cartilage* 25, 1858–1867
33 Gigout, A. *et al.* (2012) Recombinant human fibroblast growth factor-18 (rhFG18) promotes bovine articular chondrocyte proliferation and cartilage matrix production *in vitro*. *Osteoarthritis and Cartilage* 20, S135
34 Reker, D. *et al.* (2015) Extracellular matrix changes in response to sprifermin studied in ex vivo cultures of articular cartilage. *Osteoarthritis Cartilage* 23, A142–143
35 Dahlberg, L.E. *et al.* (2016) A first-in-human, double-blind, randomised, placebo-controlled, dose ascending study of intra-articular rhFGF18 (sprifermin) in patients with advanced knee osteoarthritis. *Clin. Exp. Rheumatol.* 34, 445–450
36 Eckstein, F. *et al.* (2015) Brief report: intraarticular sprifermin not only increases cartilage thickness, but also reduces cartilage loss: location-independent *post hoc* analysis using magnetic resonance imaging. *Arthritis Rheumatol.* 67, 2916–2922
37 Roemer, F.W. *et al.* (2016) Structural effects of sprifermin in knee osteoarthritis: a *post-hoc* analysis on cartilage and non-cartilaginous tissue alterations in a randomized controlled trial. *BMC Musculoskelet. Disord.* 17, 267
38 Hochberg, M. *et al.* (2018) Efficacy and safety of intra-articular sprifermin in symptomatic radiographic knee osteoarthritis: pre-specified analysis of 3-year data from a 5-year randomised, placebo-controlled, Phase II study. *Ann. Rheum. Dis.* 77 (suppl. 2), 80
39 Hochberg, M.C. *et al.* (2019) Effect of intra-articular sprifermin vs placebo on femorotibial joint cartilage thickness in patients with osteoarthritis: The FORWARD Randomized Clinical Trial. *JAMA* 322, 1360–1370
40 Gühring, H. *et al.* (2019) Cartilage thickness modification with sprifermin in knee osteoarthritis patients translates into symptomatic improvement over placebo in patients at risk of further structural and symptomatic progression: post-HOC analysis of the Phase II forward trial. *Annal. Rheum. Dis.* 78, 70–71
41 Chockalingam, P.S. *et al.* (2011) Elevated aggrecanase activity in a rat model of joint injury is attenuated by an aggrecanase specific inhibitor. *Osteoarthritis Cartilage* 19, 315–323
42 Glasson, S.S. *et al.* (2005) Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature* 434, 644–648
43 Larkin, J. *et al.* (2015) Translational development of an ADAMTS-5 antibody for osteoarthritis disease modification. *Osteoarthritis Cartilage* 23, 1254–1266
44 Malfait, A.M. *et al.* (2010) ADAMTS-5 deficient mice do not develop mechanical allodynia associated with osteoarthritis following medial meniscal destabilization. *Osteoarthritis Cartilage* 18, 572–580
45 Miller, R.E. *et al.* (2015) An aggrecan 32-MER fragment generated in osteoarthritis directly excites nociceptive neurons. *Osteoarthritis Cartilage* 23, A41–42
46 Swearingen, C.A. *et al.* (2010) A short-term pharmacodynamic model for monitoring aggrecanase activity: injection of monosodium iodoacetate (MIA) in rats and assessment of aggrecan neoepitope release in synovial fluid using novel ELISAs. *Osteoarthritis Cartilage* 18, 1159–1166
47 Nguyen, L.T. *et al.* (2017) Review of prospects of biological fluid biomarkers in osteoarthritis. *Int. J. Mol. Sci.* 18, E601
48 Bay-Jensen, A.C. *et al.* (2018) Biochemical marker discovery, testing and evaluation for facilitating OA drug discovery and development. *Drug Discov. Today* 23, 349–358
49 Watt, F.E. (2018) Osteoarthritis biomarkers: year in review. *Osteoarthritis Cartilage* 26, 312–318
50 Bay-Jensen, A.C. *et al.* (2008) Biochemical markers of type II collagen breakdown and synthesis are positioned at specific sites in human osteoarthritic knee cartilage. *Osteoarthritis Cartilage* 16, 615–623
51 Dam, E.B. *et al.* (2009) Identification of progressors in osteoarthritis by combining biochemical and MRI-based markers. *Arthritis Res. Ther.* 11, R115
52 Dell'Isola, A. *et al.* (2016) Identification of clinical phenotypes in knee osteoarthritis: a systematic review of the literature. *BMC Musculoskelet. Disord.* 17, 425
53 Mobasheri, A. *et al.* (2017) The role of metabolism in the pathogenesis of osteoarthritis. *Nat. Rev. Rheumatol* 13, 302–311
54 Abella, V. *et al.* (2017) Leptin in the interplay of inflammation, metabolism and immune system disorders. *Nat. Rev. Rheumatol.* 13, 100–109
55 Francisco, V. *et al.* (2019) Adipokines: linking metabolic syndrome, the immune system, and arthritic diseases. *Biochem. Pharmacol.* 165, 196–206
56 Mobasheri, A. *et al.* (2019) Molecular taxonomy of osteoarthritis for patient stratification, disease management and drug development: biochemical markers associated with emerging clinical phenotypes and molecular endotypes. *Curr. Opin. Rheumatol.* 31, 80–89

57 Smith, T.O. *et al.* (2019) Uptake of the OMERACT-OARSI Hip and Knee Osteoarthritis Core Outcome Set: review of randomized controlled trials from 1997 to 2017. *J. Rheumatol.* 46, 976–980
58 Smith, T.O. *et al.* (2019) The OMERACT-OARSI Core Domain Set for measurement in clinical trials of hip and/or knee osteoarthritis. *J. Rheumatol.* 46, 981–989
59 Carlson, A.K. *et al.* (2019) Characterization of synovial fluid metabolomic phenotypes of cartilage morphological changes associated with osteoarthritis. *Osteoarthritis Cartilage* 27, 1174–1184
60 Brenneis, C. *et al.* (2017) Automated tracking of motion and body weight for objective monitoring of rats in colony housing. *J. Am. Assoc. Lab. Anim. Sci.* 56, 18–31
61 Chang, Y.-M. *et al.* (2016) Penalized linear discriminant analysis reveals divergent gait patterns in three surgically-induced OA models. *Osteoarthritis Cartilage* 24, S107–108
62 Tive, L. *et al.* (2019) Pooled analysis of tanezumab efficacy and safety with subgroup analyses of Phase III clinical trials in patients with osteoarthritis pain of the knee or hip. *J. Pain Res.* 12, 975–995
63 Kleinschmidt, K. *et al.* (2013) Sprifermin increased joint space width and reduced subchondral bone sclerosis as translational primary outcome measures in a rat OA model. *Presented at ICRS 2013 11th World Congress of the International Cartilage Repair Society*, Izmir, Turkey
64 Menges, S. *et al.* (2014) μCT parameters as translational biomarkers for an experimental rat OA model. *Presented at 2014 annual Bruker μCT User Meeting*, Ostend, Belgium
65 Brenneis, C. *et al.* (2016) Spontaneous home cage activity is sensitive to anti-NGF treatment or can be linked to joint stiffness in different models of surgery-induced osteoarthritis. *Osteoarthritis Cartilage* 24, S498–499
66 Pereira, J. *et al.* (2018) Pharmacokinetic and pharmacodynamic modelling of the novel anti-adamts-5 nanobody M6495 using the neo-epitope args as a biomarker. *Arthritis Rheumatol.* 70, 1163–1164
67 Wehr, B. *et al.* (2010) Tibial cartilage surface area, thickness and volume in various animal species and in humans. *Osteoarthritis Cartilage* 15 (suppl. C), C54–55