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Osteoarthritis and Cartilage

The efficacy and safety of a fixed-dose combination of apocynin and paeonol, APPA, in symptomatic knee OA: A double-blind, randomized, placebo-controlled, clinical trial☆

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SUMMARY

Objective: Apocynin (AP) and paeonol (PA) are low molecular weight phenolic compounds with a broad array of anti-inflammatory and immunoregulatory effects. This study assessed of a fixed-dose combination of APPA in people with symptomatic knee osteoarthritis (OA).
Methods: A multi-center, randomized, placebo-controlled, double-blind phase 2a trial enrolled participants with radiographic knee OA (Kellgren-Lawrence, KL, grades 2–3) and pain ≥40/100 on WOMAC pain subscale, and evaluated the efficacy and safety of oral APPA over a 28-day period. APPA 800 mg or matching placebo was administered twice daily in a 1:1 ratio. Post-hoc analyses explored the response to APPA in sub-groups with more severe pain and structural severity.
Results: The two groups were comparable at baseline; 152 subjects were enrolled and 148 completed the trial. There was no statistically significant difference between groups with respect to the primary outcome, WOMAC pain (mean difference between groups was −0.89, 95% CI: −5.62, 3.84, p = 0.71), nor WOMAC function or WOMAC total. However, predefined subgroup analyses of subjects with symptoms compatible with nociplastic/neuropathic pain features showed a statistically significant effect of APPA compared to placebo. Adverse events (mainly gastrointestinal) were mild to moderate.
Conclusion: Treatment with APPA 800 mg twice daily for 28 days in subjects with symptomatic knee OA was not associated with significant symptom improvement compared to placebo. The treatment was well-tolerated and safe. While the study was not powered for such analysis, pre-planned subgroup analyses showed a significant effect of APPA in subjects with nociplastic pain/severe OA, indicating that further research in the effects of APPA in appropriate patients is warranted.
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Introduction

Osteoarthritis (OA) is the most common joint disease, involving pain and reduced mobility, with substantial impact on quality of life.[1] Disease progression can lead to joint 'failure' and the need for invasive surgical procedures such as joint arthroplasty. Currently no disease-modifying treatments are available to alter the course of disease progression, and available treatments are symptom-directed and either considered to be largely ineffective over longer periods[2,3] or moderately efficacious but associated with serious safety risks.[3,4] Thus, there is a great unmet need for new treatments to treat the symptoms and structural pathology of OA.

The influence of low-grade inflammation in OA has been highlighted as a major driver in the disease process,[5] and in contrast to previous failed attempts to target inflammatory cytokines in OA[6,7]

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recent results of a large trial have indicated that long-term treatment with a potent anti-inflammatory agent may be associated with reduced risk of joint arthroplasty.[8] Evidence indicates that many different pathways contribute to the development of OA[9] and that it is a disease with several different phenotypes.[10–12] The Nuclear-Factor Kappa-B (NF-κB) pathway is involved in multiple OA processes,[13] and mediates an array of inflammatory and tissue degrading processes.[14] The pathway itself is stimulated under inflammatory conditions, and results in increased release of extracellular matrix fragments activating additional inflammatory cascades,[15] and may therefore play an important role in maintaining a self-perpetuating destructive cycle driving disease development.[13]

The nuclear factor erythroid 2-related factor 2 (Nrf2) regulates the expression of an array of cytoprotective genes which include transcription of elements that reduce oxidative stress factors known to play pathological roles in rheumatic diseases including OA.[16,17] The NF-κB and Nrf2 pathways which are known to interact to maintain normal cellular homeostasis become deranged in OA and a number of other diseases.[18,19] Inhibition of NF-κB and upregulation of Nrf2 have both been identified as potential targets for treatment of OA.[20] It has also been shown that cell senescence, particularly of chondrocytes, may play an important role in the development of OA.[21,22]

Apocynin (AP) and paeonol (PA) are low molecular weight phenolic compounds and secondary metabolites of plant origin. A broad array of anti-inflammatory and immunoregulatory effects have been demonstrated for both AP and PA, suggesting they play important roles in the regulation of NF-κB, Nrf2 and other signaling pathways.[23] The combination of two synthetically produced isomers has the acronym APPA, a fixed combination product for oral use with a ratio of 2:7 (AP:PA). Previous studies have demonstrated that APPA reduces the expression of reactive oxygen species (ROS), matrix-metalloproteinases (MMP)-3 and MMP-13, and senescent chondrocytes.[24,25] Some recent data suggest that AP may inhibit neuronal senescence through interacting with the nucleotide-binding oligomerization domain (NOD)-like receptor protein 1 (NLRP1), which is responsible for causing proinflammatory molecules that promote neuroinflammation.[26]

We performed a Phase IIa, multi-center, randomized, double-blind, placebo-controlled study of APPA for the treatment of knee OA symptoms. The purpose of the study was to evaluate the efficacy, safety, and tolerability of one fixed-dose combination of APPA over a 28-day period in subjects with symptomatic and radiographic knee OA.

Methods

Study population and study design

The trial was a randomized, placebo-controlled, double-blind, parallel group trial. The trial was performed in three clinical trial sites located in Denmark.

The main inclusion criteria were: female or male participants between 40 and 85 years old who provided written informed consent prior to beginning of the study, with femorotibial knee OA according to the American College of Rheumatology (ACR) clinical and radiographic criteria[27]; OA Kellgren-Lawrence grade 2 or 3 on X-ray of the target knee[28] as graded by a central independent reading of the X-ray; a pain score of the target knee rated on a 11-point numerical rating scale (NRS) of at least 40 and not exceeding 90 (out of 100) in response to the Western Ontario and McMasters University Osteoarthritis Index (WOMAC)[29] after an adequate wash-out period of analgesic treatment at screening and at baseline; knee pain in the target knee; and inadequate response, or intolerance, to analgesics and/or non-steroidal anti-inflammatory drugs (NSAIDs). Principal exclusion criteria comprised: body mass index above 40 kg/m^2; intra-articular administration of corticosteroids within 3 months or hyaluronic acid within 6 months into the target knee or any other joint within 1 month of study entry; systemic corticoid treatment of more than 2 weeks during the last six months; major surgery of the target knee within the prior year; presence of any other clinically significant arthritis other than OA; women of childbearing potential with an insufficient method of contraception; malignancy within the last five years (with the exception of non-melanoma skin cancer); presence of significant radicular back pain; renal insufficiency or other significant medical illness or abnormal laboratory test result.

The trial was conducted in accordance with all applicable Good Clinical Practise guidelines and was approved by relevant health authorities and the Danish Ethics committee (approval number: S-20200097), and was registered in the EU Clinical Trials Register (EudraCT Number: 2020-000249-14) and clinicaltrials.gov (NCT04657926).

Randomization and investigational medicinal product

Subjects were randomized 1:1 by a central, computerized Interactive Web Responding System (IWRS) into one of two groups, oral APPA (with a ratio apocynin:paeonol of 2:7 containing 88.9 mg of apocynin (AP) and 311.1 mg of paeonol (PA)) or identical placebo capsules of 400 mg each, taken twice a day for 27 consecutive days. The ratio of apocynin and paeonol was selected based on data from animal experiments, where beneficial effects of APPA in this ratio had been observed.[30–32] The dose of APPA was selected based on observed tolerability in a previous phase 1 trial, and reasonable expectation of pharmacological activity based on pre-clinical experiments. Apocynin and paeonol for this study was manufactured synthetically and hence not derived from plant material.

The investigational medicinal product (IMP) was indistinguishable to the placebo in appearance of the container, the label, as well as the appearance of the capsules. The capsules were packaged in individual blisters to avoid any potential differences in odor of the IMP. An electronic IWRS was used to assign blinded study drug to each subject. Investigators, all site personnel, sponsor operational staff and clinical research organization staff were blinded throughout the trial period.

Paracetamol/acetaminophen was dispensed as rescue medication, with a cap of 4 g daily, and use of paracetamol/acetaminophen was accounted for by pill-counts at study visits. Prohibited medication during the trial included use of any NSAIDs, opioids, Cox-2 inhibitors or other analgesic medication (as well as medical treatment neuropathic pain), except for the rescue medication. All concomitant medication was recorded.

Efficacy and safety assessments

The primary study objective was to evaluate the change in pain in the target knee, as measured by the change from baseline to Day 28 in the WOMAC pain sub-scale evaluated on a 11-point NRS scale. In addition to the WOMAC pain, other efficacy assessments included: WOMAC function and stiffness sub-scales, the Weekly Average of Daily Pain (WADP), Patient Global Assessment (PGA), and Intermittent and Constant OA Pain (ICOAP).[33] In addition, PainDETECT scores for evaluation of neuropathic pain features[34,35] were obtained at baseline. All collected patient-reported outcomes except PainDETECT, which was collected on paper, were obtained electronically. Average daily pain was evaluated daily on an electronic pain diary, by a single question rating pain intensity score (from 0 to 10 numeric pain scale, with ‘no pain’ = 0 and ‘worse imaginable pain’ = 10) during the past 24 h. A Twenty Meter Walk Test was performed

as a measure of the average gait speed during two consecutive walks of each 20 m at a normal walking pace.[36]

As exploratory endpoints, biochemical markers of cartilage and collagens relevant for OA were analyzed, including N-terminal epitope of aggrecanase-mediated aggrecan degradation (ARGS), C-terminal fragments of crosslinked type I collagen (CTX-I), C-terminal fragments of crosslinked type II collagen (urine), degradation of type II collagen matrix (C2M), and a neo-epitope of matrix metalloproteinase (MMP)-1 and MMP-13 mediated degradation of type II collagen (Nordic Bioscience A/S, Herlev, Denmark).

The safety endpoints were the nature, frequency, and severity of treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs), 12-lead ECG parameters, vital signs, and body weight.

Statistical analysis

Sample size. The minimal difference between treatment groups in the change from baseline in WOMAC pain which could be detected with the planned sample size was calculated. It was estimated that a sample size of 67 evaluable subjects per treatment group, would provide a minimally detectable difference of 9.7 points between the active and the placebo groups on the WOMAC pain sub-scale (normalized to 0–100), by assuming an estimate of the common standard deviation of 20,[6,37–39] with 80% power and a two-sided 5% alpha. On the assumption that 10% of subjects discontinued the trial, the targeted enrollment in the trial was 75 patients per treatment group.

Analysis populations. The intention-to-treat (ITT) analysis set included all subjects randomly allocated to a treatment, based on the intention to treat "as randomized" principle (i.e., the planned regimen rather than the actual treatment given in case of any difference). The modified ITT (mITT) population included all subjects from the ITT analysis set who had a baseline *and* at least one post-treatment assessment of the primary endpoint, i.e., the WOMAC pain sub-scale available. The PP analysis set included all subjects from the mITT analysis set who had been treated according to the trial protocol. The PP analysis set was used to perform sensitivity analyses for the primary endpoint.

The mITT analysis set was used to perform all efficacy analyses and summaries. The ITT and the mITT populations were identical as illustrated in the Fig. 1. Subjects were analyzed according to the randomized treatment.

The Safety Analysis Population included all subjects who had been administered at least one dose of trial treatment. All subjects were analyzed according to the actual treatment received.

General statistical analysis considerations. Missing data on the WOMAC scale were managed as described in the WOMAC User's Guide, and similarly, the ICOAP User's Guide for the ICOAP.

The weekly mean of the average daily intensity was calculated by starting from 6 days prior to the baseline visit and until last on-treatment visit. If four or more diaries required to calculate the weekly mean were missing, then the weekly mean was set to missing. If there were less than four missing diaries, the weekly mean was calculated from the diaries available.

Efficacy analyses. The primary endpoint was the absolute change from baseline in WOMAC pain sub-score at week 4.

The treatment effect of all continuous variables were assessed using a restricted maximum likelihood based repeated measures mixed model on the dependent variable of absolute change from baseline. The analysis included the covariates of baseline value of the dependent variable, treatment, timepoint, sex, the subject characteristic of unilateral/bilateral knee OA at baseline and treatment-by-timepoint interaction. An AR(1) covariance structure was used to model the correlations between within-subject repeated measurements. The Kenward-Roger approximation was used to estimate denominator degrees of freedom and adjust standard errors.

All patient reported outcome scores including WOMAC and its subscales, are reported standardized to a 0–100 scale.

The primary efficacy analysis was performed on the mITT Analysis Set. Analysis of OMERACT-OARSI response was performed in accordance with Pham et al,[40] and compared between treatment groups using a logistic regression analysis.

Proportional changes from baseline in biomarker concentrations were analyzed using the repeated measures mixed model as described above.

A pre-defined subgroup analysis in subjects with a baseline PainDETECT score > 12, which would indicate the potential presence of nociplastic/neuropathic pain features, was performed. This subgroup analysis indicated a positive effect of APPA in participants with possible neuropathic pain features. Based on hypotheses associating higher prevalence of neuropathic pain features such as central sensitization with late-stage OA and features indicative of a higher symptomatic burden for a longer period of time such as the presence of pain at rest,[41,42] post-hoc analyses were undertaken to further assess the effects of APPA in subgroups of participants with higher disease severity.

Specifically, groups of participants with higher baseline pain, defined as target knee WOMAC pain > 50 at baseline (Group 1), a KL-grade of the non-target knee ≥2 (Group 2) or a combination of these two criteria (Group 3) were assessed.

Results

A total of 334 subjects were screened for the study of whom 152 were randomized between 23-SEP-2020 and 05-MAR-2021, and 149 (98%) completed the trial. The details of the study participant disposition are outlined in Fig. 1, and as shown the ITT- and mITT-populations were identical. The baseline characteristics are summarized in Table I.

Efficacy results

The results of the main efficacy outcomes are shown in Table II.

The primary endpoint of change in WOMAC pain from baseline to Day 28 was not met, as mean difference between APPA and placebo was −0.89 (95% CI: −5.62, 3.84, p = 0.71, Fig. 2A). Similarly, no significant differences were found on other secondary endpoints Fig. 2B–D, and in Table II.

Fig. 3A shows that analysis of participants with PainDETECT > 12 at Baseline (APPA, N = 20; Placebo, N = 25) where a statistically significant mean difference of 11.20 (95% CI: −20.29, −2.11, p = 0.0165) which favored APPA was found.

The proportion of subjects using at least one dose the dispensed rescue medication (paracetamol 500 mg tablets) during the trial was 61.6% and 63.6%, for APPA and placebo groups, respectively. The average dose of paracetamol per study day was 402 mg (95% CI: 258 to 545 mg) and 351 mg (95% CI: 212 to 489 mg) for APPA and placebo. The observed difference in the average use of rescue medication was not statistically significantly different between the study groups (p = 0.62).

Post-hoc subgroup analyses

Results of the post-hoc subgroup analyses are shown in Fig. 3B–D.

In the group with WOMAC pain > 50 at baseline (*Group 1*; n = 95) the observed mean difference: −2.61, 95% CI: −8.98 to 3.76, p = 0.42 (Fig. 3B), and in Group 2 consisting of participants with a KL-grade of the non-target knee ≥2 (n = 105) a mean difference of −4.01, 95% CI:

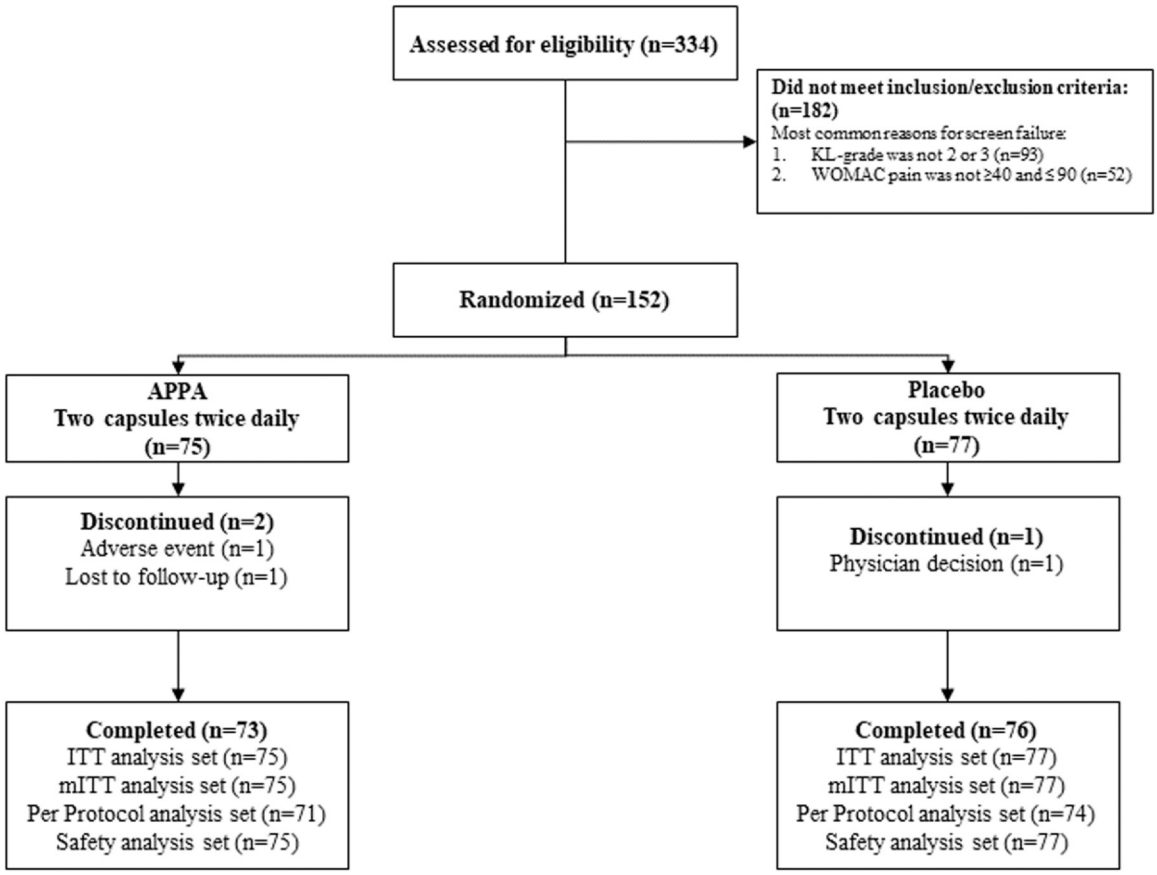


Fig. 1 Osteoarthritis and Cartilage

Participant disposition. ITT, intention-to-treat; mITT, modified intention-to-treat; APPA, apocynin+paeonol.

−9.35 to 1.33, p = 0.14 (Fig. 3C), or a combination of these two criteria (*Group 3*; n = 64) in Fig. 3D with a mean difference −8.32, 95% CI: −15.48 to −1.16, p = 0.02). The results of the main study analyses including the pre-planned and subgroup analyses are illustrated in Fig. 4.

Biomarker results

As shown in Table III, there were no statistically significant differences in the change from baseline between the two groups with respect to any of the biochemical markers studied for the entire study population. Thus, for ARGS, CTX-I, CTX-II, C2M, and T2CM the percent change from baseline was similar between groups during the study).

Safety results

At least one TEAE was reported by 36.0% and 41.6% of study participants receiving APPA or placebo, respectively. In general, APPA was found to be well tolerated (Table IV) and no differences in frequencies of reported AEs were noted, apart from a higher proportion of trial subjects reporting mild to moderate gastrointestinal discomfort reported with APPA compared to placebo (12% vs. 6.5%), most frequently reporting transient diarrhea (4% of subjects receiving APPA vs. 0% placebo). All but one reported AEs were mild to moderate, and self-limiting. In total, three participants discontinued the study; two in the APPA group, and one receiving placebo. One AE, which was deemed related to study treatment by the investigator (termed "diarrhea") in a participant receiving APPA led to discontinuation from the trial. During the trial one serious adverse event (SAE) ("prostate cancer") was reported, in a participant receiving placebo. No clinically relevant changes were found on clinical biochemistry or hematology parameters, urine dipstick, vital signs nor ECG parameters, including QTc-intervals.

The sensitivity analysis of the Per Protocol population found no difference to the mITT population and is therefore not considered relevant for further discussion.

Discussion

The current report describes the first multicenter, double-blind, randomized placebo-controlled trial of a fixed-dose combination of AP and PA in patients with symptomatic knee OA. In this clinical study, we found that APPA administered orally at 800 mg daily for 28 consecutive days did not result in statistically significant changes in any of the clinical outcomes compared with placebo in the ITT population. The treatment was found to be safe and well tolerated.

With regards to the mechanism of action, studies have been focusing on the two molecules (AP and PA) and on their combination. AP is a potent natural nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor, seems to be mainly through an effect on reactive oxidative species (ROS), such as hydrogen peroxide and

ITT population	APPA (n = 75)	Placebo (n = 77)
Mean age, yrs (SD)	62.5 (8.1)	60.7 (8.6)
Male sex, n (%)	45 (60.0)	32 (41.6)
BMI, kg/m^2 (SD)	30.8 (4.3)	29.9 (4.7)
KL grade, target knee, n (%)		
2	37 (49.3)	38 (49.4)
3	38 (50.7)	39 (50.6)
Mean OA symptom duration, months (SD)	83.4 (69.2)	106.8 (92.1)
Prior use, %		
Paracetamol/acetaminophen	76.0	83.1
Any NSAID or COXib	34.6	49.4
Opioids	2.6	0.0
Baseline WOMAC pain, target knee (0–100) (SD)	54.4 (10.1)	56.2 (10.4)
Baseline WOMAC pain, non-target knee (0–100) (SD)	27.3 (22.1)	29.4 (21.7)
Baseline WOMAC Function (0–100) (SD)	48.8 (13.7)	49.6 (14.6)
Baseline ICOAP total score (0–100) (SD)	54.0 (13.0)	49.9 (14.0)
20-meter walk test, mean gait speed, m/s (SD)	1.25 (0.16)	1.26 (0.17)
PainDETECT neuropathic pain (−1 to 38) (SD)	9.4 (5.5)	9.5 (5.8)
Extent of OA, n (%)		
Bilateral	47 (62.7)	47 (61.0)
Unilateral	28 (37.3)	30 (39.0)

BMI, body mass index; KL, Kellgren Lawrence; ICOAP, intermittent and constant OA pain; OA, osteoarthritis; NSAID, non-steroidal anti-inflammatory drug; COXib, cyclooxygenase-2 inhibitor; SD, standard deviation; WOMAC, Western Ontario McMaster University Osteoarthritis Index; yrs, years.

Table I Osteoarthritis and Cartilage

Baseline characteristics.

superoxide, thus modulating the oxidative stress-mediated pathway that has been shown to play a pivotal role in chronic pain.[43–45] In addition, experimental data have shown AP to inhibit NF-κB activation and upregulate Nrf2 gene expression with consequent downstream effects.[46] As for PA, it exerts its effect via inhibiting NF-κB activation with modulation of the inflammatory responses, through inhibition of proinflammatory cytokines TNF-α, IL-1β, IL-6, metalloproteinases and of MCP-1 expression.[47]

With respect to the combination of the two entities, AP and PA, APPA has been shown to have effects on several of the pathways that have been implicated in the pathogenesis of OA. Studies have demonstrated that although APPA does not interfere with neutrophil host defense against infections, it inhibits neutrophil degranulation and cytokine-driven signaling pathways (e.g., autocrine signaling and NF-κB activation), processes that are known to be associated with inflammatory diseases and to up-regulate gene expression of Nrf2, a key factor in response to oxidative stress. Furthermore, APPA is as effective in vitro as TNFα biologics in preventing endogenous TNFα-induced cytokine and chemokine expression.[23] Experiments using chondrocytes have shown that APPA downregulates gene expression of IL-8, TNF-α, MMP-13 and MMP-3.[48] whereas with cartilage explants APPA inhibited aggrecan degradation[49] and increased intermedial proteoglycan whilst reducing release of glycosaminoglycans.[48] Taken together these results provide evidence that APPA may be chondroprotective. Evidence from the rat meniscal tear model showed that APPA protected the animals from cartilage loss, unlike the individual components[31] and experience from dogs with naturally occurring disease support the potential for APPA to be effective treatment for OA in humans,[30–32] a conclusion that is supported by the findings of a case series of subjects with OA treated with APPA,[50] The reasons for the absence of a detectable clinical

Variable		APPA (n = 75)		Placebo (n = 77)			
	Scale	Participants, n*	LS mean change (95% CI)	Participants, n*	LS mean change (95% CI)	LS mean difference (95% CI)	p-value for difference
WOMAC pain sub-score	0–100	73	-16.40 (−19.74; −13.05)	76	-15.51 (−18.80; −12.21)	-0.89 (−5.62; 3.84)	0.7119
WOMAC total score	0–100	73	-13.89 (−17.20; −10.58)	76	-11.61 (−14.87; −8.35)	-2.28 (−6.96; 2.40)	0.3373
WOMAC function score	0–100	73	-13.19 (−16.69; - 9.69)	76	-10.19 (−13.64; −6.74)	-3.01 (−7.96, 1.94)	0.2325
WOMAC stiffness score	0–100	73	-13.81 (−17.78; - 9.84)	76	-13.50 (−17.41; −9.59)	-0.31 (−5.92; 5.31)	0.9143
ICOAP total OA pain score	0–100	73	-14.36 (−17.92; −10.79)	76	-11.70 (−15.21; −8.19)	-2.66 (−7.72; 2.40)	0.3012
ICOAP constant OA pain score	0–100	73	-15.10 (−18.81; −11.40)	76	-11.87 (−15.51; −8.22)	-3.24 (−8.47, 2.00)	0.2252
ICOAP intermittent OA pain score	0–100	73	-13.51 (−17.33; −9.70)	76	-11.91 (−15.66; −8.15)	-1.61 (−7.02; 3.80)	0.5589
Gait speed (m/sec)	N/A	73	0.0514 (0.0271; 0.0757)	75	0.0470 (0.0230; 0.0710)	0.0044 (−0.0299; 0.0388)	0.8000
Weekly mean of average daily pain intensity	0–10	63	-1.2118 (−1.5512; −0.8724)	74	-1.0397 (−1.3573; −0.7221)	-0.17 (−0.64; 0.30)	0.4696
Patient global assessment score	0–10	73	-1.40 (−1.80; −1.00)	76	-1.27 (−1.66; −0.88)	-0.13 (−0.70; 0.43)	0.6379
WOMAC pain sub-score (subgroup PainDETECT > 12)	0–100	20	-25.48 (−32.07; −18.89)	25	-14.28 (−20.15; −8.42)	-11.20 (−20.29; −2.11)	0.0165
OMERACT-OARSI response, % (95% CI)	%	73	53.4 (42.0; 64.5)	76	59.2 (47.9; 69.6)	**Odds ratio (95% CI)** 0.79 (41.3; 1.51)	0.4768

WOMAC, Western Ontario McMaster University Osteoarthritis Index; ICOAP, intermittent and constant osteoarthritis pain index.
*: Number of participants with Week 4 data

Table II Osteoarthritis and Cartilage

Mean changes from baseline to week 4 in main efficacy outcomes (mITT population).

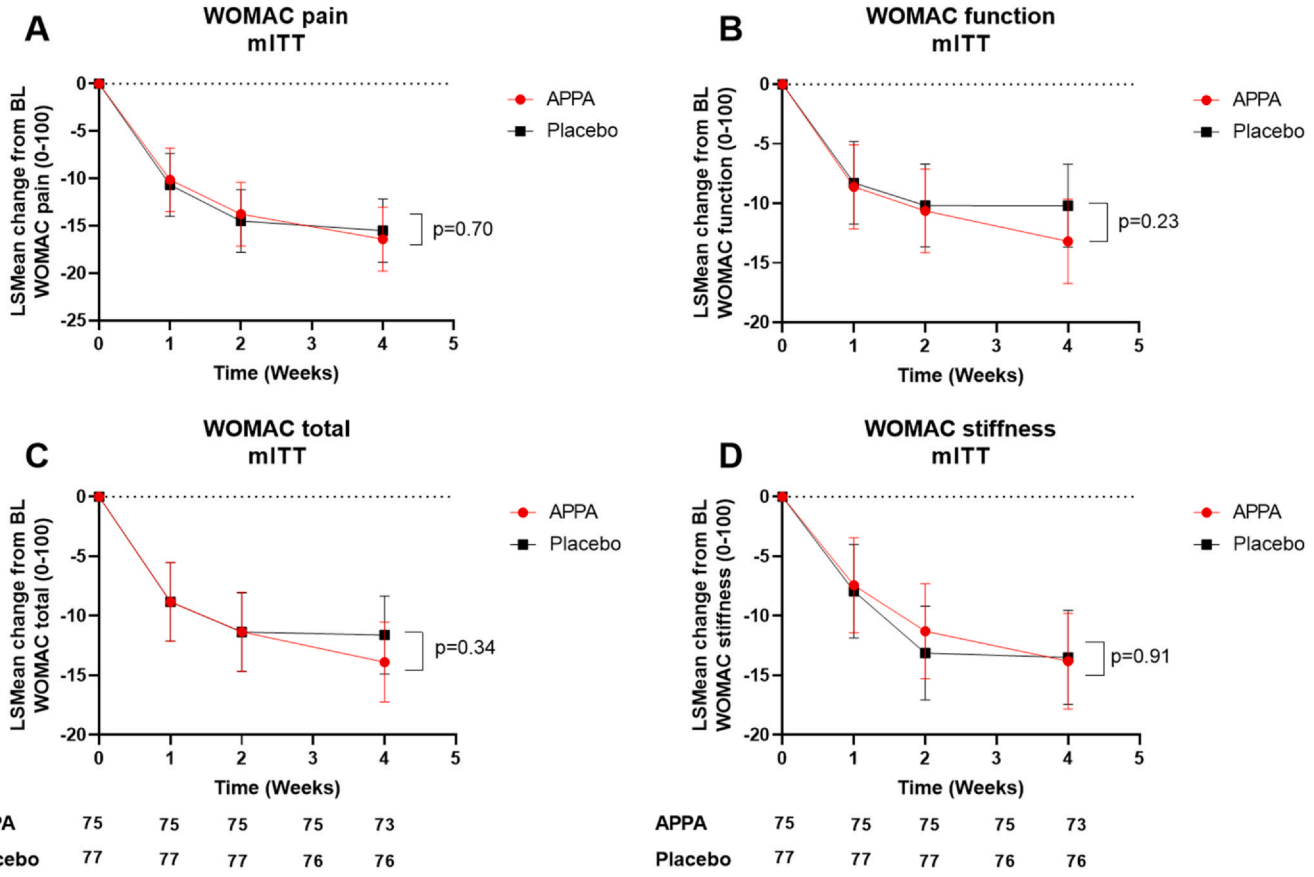


Fig. 2 Osteoarthritis and Cartilage

Change from baseline in WOMAC sub-scores. A, Pain in target knee. B, Function. C, Total D, Stiffness score. Data are LSmeans ± 95% confidence intervals.

effect of APPA in the overall population of this trial may be due to the risk of a suboptimal dose administered, or an insufficient trial duration, as discussed below. Considering the mechanism of action as described above, it is likely that any arthroprotective effects, whether directly related to cartilage or to other joint components or joint homeostasis as a whole, would require a longer treatment duration to manifest into a measurable clinical benefit in the broader, heterogeneous OA population.

The effect of APPA was studied in exploratory analyses for the subgroup of participants with a PainDETECT score > 12 indicating possible nociplastic and/or neuropathic features, as described below. We here found a significant effect of APPA compared to placebo (Fig. 3A). In the sub-groups evaluating the broader group of subjects with higher, primarily nociceptive OA pain (Group 1, Fig. 3B) or notable radiographic structural severity of the non-target knee (Group 2, Fig. 3C), the data suggests improved responses to APPA, albeit not statistically significant, while the response in the group combining these features was found to be higher (Group 3, Fig. 3D). This could be a reflection of APPA possibly being efficacious in patients with a more chronic and painful pathological state. This could include clinical situations where the more pronounced structural disease is associated with increased synovitis/joint inflammation[51] and synovitis being associated with higher pain severity pain, as described in the literature.[52,53] The fact that a significant effect of APPA was observed in subjects with more advanced symptomatic OA suggests that careful selection of the OA subjects is critical in terms of drug efficacy.

The results of the pre-defined subgroup analysis in OA patients with probable nociplastic/neuropathic pain features are supported by pre-clinical data of AP and PA. Experimental data in rodents indicate that AP may inhibit the pathway involved in transmitting neuropathic pain,[54] and several reports indicating a beneficial effect of these two compounds evaluated individually in animal models of neuropathic pain have been published.[43,44] In a streptozotocin-induced diabetic neuropathy rat model, AP was found to dose-dependently increase the pain threshold of the animals, and appeared to do so by decelerating the oxidative-stress-mediated pathology in the sciatic nerve under study.[55] Similarly, in an identical model, AP was found to partially reverse allodynia, along with reversal of oxidative-stress markers in the spinal cord.[56] Similar results have been reported for PA in the same model[57].

Chronic pain in OA is thought to comprise elements of nociceptive pain and of neuropathic/nociplastic pain.[58] Central neuronal sensitization, caused by hyperactive and hyperexcitable neurons in the central nervous system,[59] plays a crucial role in amplifying pain hypersensitivity and is currently believed to be related to nociplastic pain.[60] There is evidence that central sensitization might be mediated via inflammation, and that the widespread augmentation of central pain processing is driven by circulating cytokines rather than directly by the nociceptors.[61] Further, data from the Multicenter

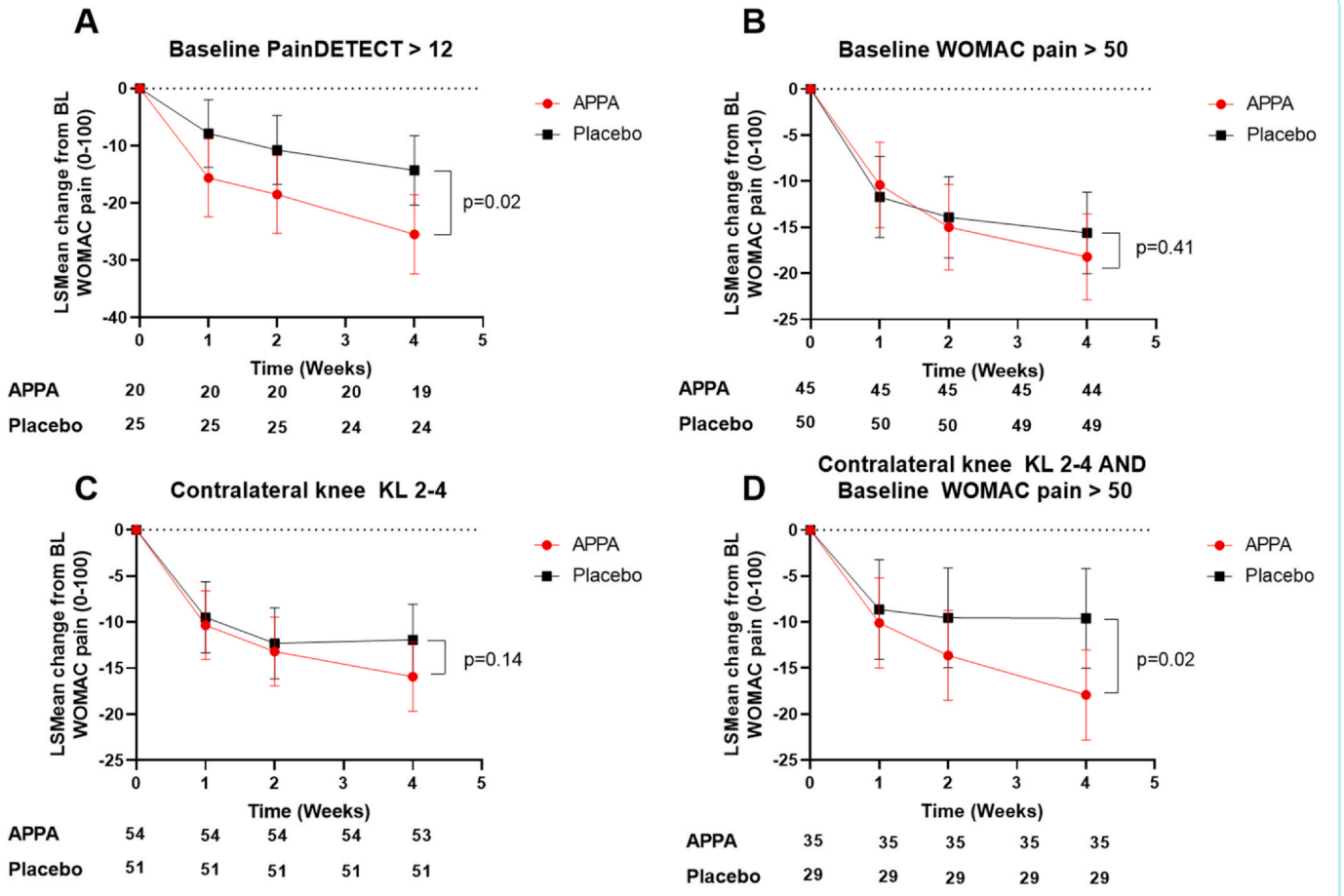


Fig. 3 Osteoarthritis and Cartilage

Change from baseline in WOMAC pain, exploratory sub-group analyses. A, Baseline PainDETECT > 12; B, Baseline WOMAC pain > 50 (Group 1); C, Baseline contralateral knee KL grade 2–4 (Group 2); D, Baseline contralateral knee KL grade 2–4 combined with Baseline WOMAC pain > 50 (Group 3). Data are LSmeans ± 95% confidence intervals.

Osteoarthritis Study (MOST) suggest that local inflammation in OA, synovitis and/or joint effusion, can lead to chronic pain by increasing the nociceptive input, is associated with pain sensitization.[62] In addition, localized pain (e.g., in the knee) seems to be a significant risk factor for development of widespread pain through central sensitization.[63] Taken together, this indicates that APPA may potentially affect pain sensitization through direct influences on the inflammatory processes.

Literature reports describe improvements in WOMAC pain ranging from 7–12 out of 100 as being perceptible and potentially clinically relevant.[64–66] Due to their modest sizes, the clinical relevance of the observed differences between APPA and placebo in the ITT-population are considered negligible. Caution must be taken in the assessment of clinical relevance of trial results describing changes on a group level, as the data describing thresholds of clinical relevance are intended to be evaluated in the context of an individual patient, and not applicable to a population mean change. However, the mean difference in WOMAC pain observed in the population with painDETECT > 12 of −11.2 out of 100 on WOMAC pain is likely to be considered clinically relevant.

Cell senescence has been reported to play an important role in the development of OA through the senescence-associated secretory phenotype, mitochondrial dysfunction, oxidative stress, and inflammation.[22] In support, senescent cells have been demonstrated to be drivers of inflammation implicated in OA.[67] Recently APPA has been reported to have senolytic and senomorphic effects in chondrocytes which could contribute to the effects seen with this combination of two plant metabolites in chondrocytes and cartilage.[48]

The limitations of the study include the lack of multiple APPA doses for evaluation of dose-response relationships, and the trial duration. It is possible that higher doses of APPA are required to reach clinical efficacy in a larger proportion of OA patients, but the current trial did not involve more than one dose group. As discussed above, the duration of this trial (28 days) was relatively short, and the observations on potential efficacy of APPA presented later in the trial, suggesting that a longer duration could have separated APPA from placebo better. Evidence from veterinarian use of APPA supports this. In a 4-week study in dogs with naturally occurring OA, in which APPA was compared to meloxicam (plus famotidine) and placebo, APPA showed increasing improvements in outcomes between weeks 2 and 4 whilst in the meloxicam group the improvement had plateaued or even decreased.[32] Additionally, recent evidence indicates that there are several pheno- and endo-types in OA[10–12] and it is possible that identifiable pheno/endotypes particularly susceptible to responding to APPA exist.

Reports have suggested that higher pain reporting variability on an individual participant level has a negative impact on the ability to

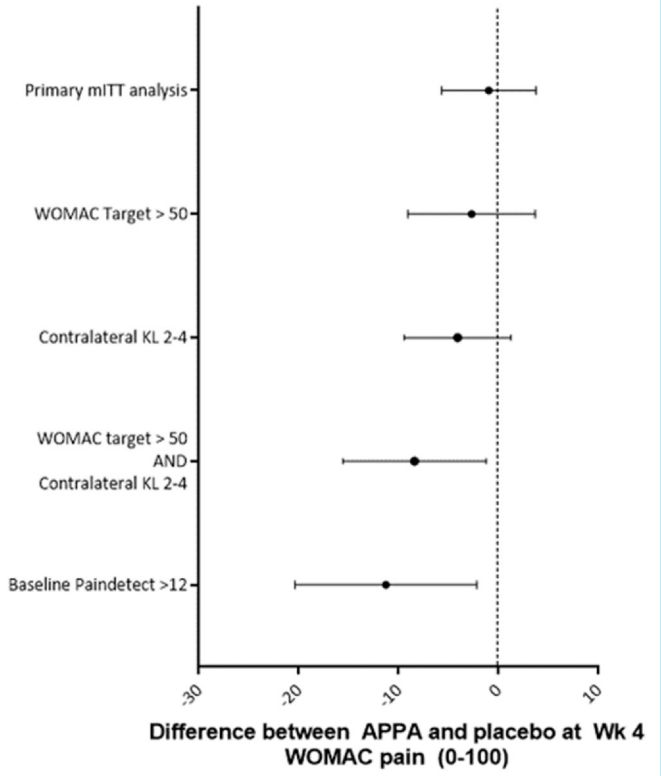


Fig. 4 Osteoarthritis and Cartilage

Forest plot of subgroup analyses of WOMAC pain change. Data shown as difference between APPA and placebo at Week 4.

detect meaningful differences between interventions in pain studies.[68–71] Some recent trials have utilized methods to screen participants for high pain variability, the resulting benefit of which, if any, is currently undescribed. The current trial did not include such methods, by which no exclusion of participants with higher pain variability was performed, which may have impacted the study power negatively, although this remains speculative.

The generalizability of the findings in the study is limited to populations with similar clinical and radiographic characteristics. Additionally, the study included only Caucasian participants, which may limit the applicability of the results to other racial groups.

In conclusion, treatment with APPA 800 mg twice daily for 28 days in patients with symptomatic knee OA was not associated with significantly improved outcomes compared to placebo. The treatment was well-tolerated and safe. While the study was not powered for such analysis, pre-planned subgroup analyses showed a significant effect of APPA in subjects with nociplastic pain/severe OA, indicating that further research in the effects of APPA in appropriate patients is warranted.

MedDRA system organ class preferred term	APPA (n = 75) n (%) E	Placebo (n = 77) n (%) E
All TEAEs	25 (33.3) 38	27 (35.1) 40
Gastrointestinal disorders	9 (12.0) 12	5 (6.5) 5
Diarrhea	3 (4.0) 4	0
Gastroesophageal reflux disease	4 (5.3) 4	0
General disorders and administration site condition	5 (6.7) 5	1 (1.3) 1
Feeling hot	3 (4.0) 3	0
Injury, poisoning and procedural complications	0	6 (7.8)
Contusion	0	3 (3.9) 3
Investigations	7 (9.3) 7	8 (10.4) 10
White blood cells urine	0	3 (3.9) 3
Nervous system disorders	4 (5.3) 5	5 (6.5) 5
Headache	3 (4.0) 4	2 (2.6) 2

TEAE, treatment-emergent adverse event.

Table IV Osteoarthritis and Cartilage

Treatment-emergent adverse events by system organ class and preferred term with a frequency of at least 3% in either treatment group, based on MedDRA Preferred Term (Safety population).

Declaration of Competing Interest

PGC has received consulting fees from AbbVie, AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Galapagos, Genascence, GlaxoSmithKline, Janssen, Levicept, Novartis, Pfizer, Stryker and UCB. RM has no conflict of interest to declare. AR and NL are full-time employees and shareholders of AKL Therapeutics Ltd. ARB and JRA are employees and shareholder in NBCD A/S, and at the time of trial conduct and manuscript preparation, IB was a full-time employee of NBCD A/S. PA is a full-time employee of Sanos Clinic, Vejle, Denmark, and HR a full-time employee of Sanos Clinic Gandrup, Denmark.

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Variable		APPA (n = 75)		Placebo (n = 77)			
	Unit	Participants, n	LS mean change (95% CI)	Participants, n	LS mean change (95% CI)	LS mean difference (95% CI)	p-value for difference
ARGS	pmol/L	73	0.51 (−1.02; 2.05)	76	0.68 (−0.83; 2.19)	-0.16 (−2.34; 2.01)	0.88
CTX-I	ng/mL	73	4.56 (0.09; 9.43)	76	4.86 (0.45; 9.26)	-0.29 (−6.6; 6.05)	0.92
CTX-II	ng/mmol	73	4.48 (−2.58; 11.53)	76	0.75 (−6.12; 7.63)	3.73 (−6.19; 13.65)	0.46
C2M	ng/mL	73	3.22 (0.32; 6.13)	76	1.37 (−1.48; 4.22)	1.85 (−2.24; 5.94)	0.37)
T2CM	ng/mL	73	-0.33 (−4.03; 3.39)	76	0.11 (−3.53; 3.76)	-0.44 (−5.67; 4.80)	0.87

Table III Osteoarthritis and Cartilage

Mean proportional changes from baseline to Week 4 in biomarker outcomes (%) (mITT population).

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Author contributions

The study conception and design was by ARB, AR, NL, JRA, and IB. The trial was conducted and supervised by ARB, IB, AR, PA and, HR. The first manuscript was drafted by PA and ARB. All authors participated in critical revision of the article for important intellectual content, and approved the manuscript for submission. IB provided statistical expertise. PA and HR participated in the collection of study data. All authors participated in study data analysis and interpretation.

Role of the funding source

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References

1. Hunter DJ, Bierma-Zeinstra S. Osteoarthritis. Lancet 2019;393(10182):1745–59. https://doi.org/10.1016/S0140-6736(19)30417-9
2. Zhang W, Jones A, Doherty M. Does paracetamol (acetaminophen) reduce the pain of osteoarthritis?: A meta-analysis of randomised controlled trials. Ann Rheum Dis 2004;63(8):901–7. https://doi.org/10.1136/ard.2003.018531
3. Bannuru RR, Schmid CH, Kent DM, Vaysbrot EE, Wong JB, McAlindon TE. Comparative effectiveness of pharmacologic interventions for knee osteoarthritis: a systematic review and network meta-analysis. AnnInternMed 2015;162(1):46–54. https://doi.org/10.7326/M14-1231
4. Charlesworth J, Fitzpatrick J, Perera NKP, Orchard J. Osteoarthritis—a systematic review of long-term safety implications for osteoarthritis of the knee. BMC Musculoskelet Disord 2019;20(1). https://doi.org/10.1186/s12891-019-2525-0
5. Berenbaum F. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). Osteoarthr Cartil 2013;21(1522-9653 (Electronic)):16–21. https://doi.org/10.1016/j.joca.2012.11.012
6. Fleischmann RM, Bliddal H, Blanco FJ, *et al*. A phase II trial of Lutikizumab, an anti–interleukin-1α/β dual variable domain immunoglobulin, in knee osteoarthritis patients with synovitis. Arthritis Rheumatol 2019;71(7):1056–69. https://doi.org/10.1002/art.40840
7. Kloppenburg M, Peterfy C, Haugen IK, *et al*. Phase IIa, placebo-controlled, randomised study of lutikizumab, an anti-interleukin-1α and anti-interleukin-1β dual variable domain immunoglobulin, in patients with erosive hand osteoarthritis. Ann Rheum Dis 2019;78(3):413–20. https://doi.org/10.1136/annrheumdis-2018-213336
8. Schieker M, Conaghan PG, Mindeholm L, *et al*. Effects of interleukin-1β inhibition on incident hip and knee replacement: exploratory analyses from a randomized, double-blind, placebo-controlled trial. Ann Intern Med 2020;173(7):509–15. https://doi.org/10.7326/M20-0527
9. Gratal P, Lamuedra A, Medina JP, *et al*. Purinergic system signaling in metainflammation-associated osteoarthritis. Front Med 2020;7. https://doi.org/10.3389/fmed.2020.00506. (Published online).
10. Mobasheri A, Van Spil WE, Budd E, *et al*. 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 2019;31:80–9. https://doi.org/10.1097/BOR.0000000000000567. (Published online).
11. Mobasheri A, Saarakkala S, Finnilä M, Karsdal MA, Bay-Jensen AC, van Spil WE. Recent advances in understanding the phenotypes of osteoarthritis. F1000Research 2019;8. https://doi.org/10.12688/f1000research.20575.1. (Published online).
12. Deveza LA, Melo L, Yamato TP, Mills K, Ravi V, Hunter DJ. Knee osteoarthritis phenotypes and their relevance for outcomes: a systematic review. Osteoarthr Cartil 2017;25(12):1926–41. https://doi.org/10.1016/j.joca.2017.08.009. (Published online).
13. Rigoglou S, Papavassiliou AG. The NF-κB signalling pathway in osteoarthritis. Int J Biochem Cell Biol 2013;45(11):2580–4. https://doi.org/10.1016/j.biocel.2013.08.018
14. Choi MC, Jo J, Park J, Kang HK, Park YNF-. κb signaling pathways in osteoarthritic cartilage destruction. Cells 2019;8(7). https://doi.org/10.3390/cells8070734
15. Sharma N, Drobinski P, Kayed A, *et al*. Inflammation and joint destruction may be linked to the generation of cartilage metabolites of ADAMTS-5 through activation of toll-like receptors. Osteoarthr Cartil 2020. https://doi.org/10.1016/j.joca.2019.11.002. (Published online).
16. Bolduc JA, Collins JA, Loeser RF. Reactive oxygen species, aging and articular cartilage homeostasis. Free Radic Biol Med 2019;132:73–82. https://doi.org/10.1016/j.freeradbiomed.2018.08.038. (Published online).
17. Marchev AS, Dimitrova PA, Burns AJ, Kostov RV, Dinkova-Kostova AT, Georgiev MI. Oxidative stress and chronic inflammation in osteoarthritis: can NRF2 counteract these partners in crime? Ann N Y Acad Sci 2017;1401(1):114–35. https://doi.org/10.1111/nyas.13407. (Published online).
18. van der Horst D, Carter-Timofte ME, van Grevenynghe J, Laguette N, Dinkova-Kostova AT, Olagnier D. Regulation of innate immunity by Nrf2. Curr Opin Immunol 2022;78. https://doi.org/10.1016/j.coi.2022.102247. (Published online).
19. Wardyn JD, Ponsford AH, Sanderson CM. Dissecting molecular cross-talk between Nrf2 and NF-κB response pathways. Biochem Soc Trans 2015;43:621–6. https://doi.org/10.1042/BST20150014. (Published online).
20. Roman-Blas JA, Jimenez SA. NF-κB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis. Osteoarthr Cartil 2006;14(9):839–48. https://doi.org/10.1016/j.joca.2006.04.008. (Published online).
21. Collins JA, Diekman BO, Loeser RF. Targeting aging for disease modification in osteoarthritis. Curr Opin Rheumatol 2018;30(1):101–7. https://doi.org/10.1097/BOR.0000000000000456. (Published online).
22. Coryell PR, Diekman BO, Loeser RF. Mechanisms and therapeutic implications of cellular senescence in osteoarthritis. Nat Rev Rheumatol 2021;17(1):47–57. https://doi.org/10.1038/s41584-020-00533-7. (Published online).
23. Cross AL, Hawkes J, Wright HL, Moots RJ, Edwards SW. APPA (apocynin and paeonol) modulates pathological aspects of human neutrophil function, without supressing antimicrobial ability, and inhibits TNFα expression and signalling. Inflammopharmacology 2020;28(5):1223–35. https://doi.org/10.1007/s10787-020-00715-5. (Published online).
24. Fernandez-Moreno M, Larkins N, Reynolds A, Hermida-Gomez T, Blanco FJ. Anti-inflammatory effects of APPA -apocynin and paeonol- in human articular chondrocytes. Pharmaceuticals 2024;17(1):118. https://doi.org/10.1016/j.joca.2021.02.464
25. FERNANDEZ-MORENO M, Hermida-Gomez T, Larkins N, Reynolds A, Blanco-Garcia F. Effect of appa (Combination Of Apocynin And Paeonol) compound on cellular senescence using

human articular chondrocytes. Osteoarthr Cartil 2023;31:S401–2. https://doi.org/10.1016/j.joca.2023.01.466. (Published online).
26. Xu T, Sun L, Shen X, *et al*. NADPH oxidase 2-mediated NLRP1 inflammasome activation involves in neuronal senescence in hippocampal neurons in vitro. Int Immunopharmacol 2019;69:60–70. https://doi.org/10.1016/j.intimp.2019.01.025. (Published online).
27. Altman R, Asch E, Bloch D, *et al*. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 1986;29(0004-3591 (Print)):1039–49.
28. KELLGREN JH, LAWRENCE JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957;16(4):494–502. https://doi.org/10.1136/ard.16.4.494
29. Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. J Rheumatol 1988;15(0315-162X (Print)):1833–40.
30. Glasson S, Larkins N. APPA provides symptom relief in clinical canine osteoarthritis. Osteoarthr Cartil 2012;20:S287. https://doi.org/10.1016/j.joca.2012.02.494. (Published online).
31. Glasson S, Bendele A, Larkins N. Appa provides disease modification in preclinical osteoarthritis. Osteoarthr Cartil 2012;20:S72–3. https://doi.org/10.1016/j.joca.2012.02.054. (Published online).
32. Larkins N, King C. Effectiveness of apocynin-paeonol (APPA) for the management of osteoarthritis in dogs: comparisons with placebo and meloxicam in client-owned dogs. Matters 2017. https://doi.org/10.19185/matters.201608000001. (Published online).
33. Hawker GA, Davis AM, French MR, *et al*. Development and preliminary psychometric testing of a new OA pain measure—an OARSI/OMERACT initiative. Osteoarthr Cartil 2008;16(4):409–14. https://doi.org/10.1016/j.joca.2007.12.015. (Published online).
34. Sadosky A, Cappelleri J, Bienen EJ, Koduru V. Measurement properties of painDETECT by average pain severity. Clin Outcomes Res 2014. https://doi.org/10.2147/ceor.s68997. (Published online).
35. Freynhagen R, Tölle TR, Gockel U, Baron R. The painDETECT project—far more than a screening tool on neuropathic pain. Curr Med Res Opin 2016;32(6):1033–57. https://doi.org/10.1185/03007995.2016.1157460. (fPublished online).
36. Motyl JM, Driban JB, McAdams E, Price LL, McAlindon TE. Test-retest reliability and sensitivity of the 20-meter walk test among patients with knee osteoarthritis. BMC Musculoskelet Disord 2013;14(1):1. https://doi.org/10.1186/1471-2474-14-166
37. Yazici Y, McAlindon TE, Gibofsky A, *et al*. Lorecivivint, a novel intraarticular CDC-like kinase 2 and dual-specificity tyrosine phosphorylation-regulated kinase 1A inhibitor and wnt pathway modulator for the treatment of knee osteoarthritis: a phase II randomized trial. Arthritis Rheumatol 2020;72(10):1694–706. https://doi.org/10.1002/art.41315
38. Conaghan PG, Hunter DJ, Cohen SB, *et al*. Effects of a single intra-articular injection of a microsphere formulation of triamcinolone acetonide on knee osteoarthritis pain. J Bone Jt Surg 2018;100(8):666–77. https://doi.org/10.2106/jbjs.17.00154
39. Cai G, Laslett LL, Aitken D, *et al*. Zoledronic acid plus methylprednisolone versus zoledronic acid or placebo in symptomatic knee osteoarthritis: a randomized controlled trial. Ther Adv Musculoskelet Dis 2019;11:666–77. https://doi.org/10.1177/1759720×19880054
40. Pham T, van der Heijde D, Altman RD, *et al*. OMERACT-OARSI initiative: osteoarthritis research society international set of responder criteria for osteoarthritis clinical trials revisited. Osteoarthr Cartil 2004;12(5):389–99. https://doi.org/10.1016/j.joca.2004.02.001
41. Power JD, Perruccio AV, Gandhi R, *et al*. Neuropathic pain in end-stage hip and knee osteoarthritis: differential associations with patient-reported pain at rest and pain on activity. Osteoarthr Cartil 2018;26(3):363–9. https://doi.org/10.1016/j.joca.2018.01.002
42. Hensor EM, Dube B, Kingsbury SR, Tennant A, Conaghan PG. Toward a clinical definition of early osteoarthritis: onset of patient-reported knee pain begins on stairs. Data from the osteoarthritis initiative. Arthritis Care Res 2015;67(2151-4658 (Electronic):40–7.
43. Lu YF, Neugebauer V, Chen J, Li Z. Distinct contributions of reactive oxygen species in amygdala to bee venom-induced spontaneous pain-related behaviors. Neurosci Lett 2016;619:68–72. https://doi.org/10.1016/j.neulet.2016.03.015. (Published online).
44. Hsu HC, Chang WM, Wu JY, *et al*. Folate deficiency triggered apoptosis of synoviocytes: role of overproduction of reactive oxygen species generated via NADPH oxidase/mitochondrial complex II and Calcium Perturbation. PLoS One 2016;11(1). https://doi.org/10.1371/journal.pone.0146440. (Published online).
45. Hawkes ALCJ, Moots HLWRJ. APPA (apocynin and paeonol) modulates pathological aspects of human neutrophil function, without supressing antimicrobial ability, and inhibits TNFα expression and signalling. Inflammopharmacology 2020(0123456789):516–34. https://doi.org/10.1007/s10787-020-00715-5
46. Savla SR, Laddha AP, Kulkarni YA. Pharmacology of apocynin: a natural acetophenone. Drug Metab Rev 2021;53(4):542–62. https://doi.org/10.1080/03602532.2021.1895203
47. Qi J hong, Dong F xu, Wang X long. Exploring targets and signaling pathways of paeonol involved in relieving inflammation based on modern technology. Mol Divers 2022;26(3):1731–42. https://doi.org/10.1007/s11030-021-10301-8. (Published online).
48. Fernandez-Moreno M, Larkins N, Reynolds A, Hermida-Gomez T, Blanco FJ. Biological effect of APPA -apocynin and paeonol- in human articular chondrocytes. Osteoarthr Cartil 2021;29:S358. https://doi.org/10.1016/j.joca.2021.02.464. (Published online).
49. Thudium CS, Bay-Jensen AC, Gantzel T, Dziegiel MH, Larkins N, Reynolds A. Characterizing the effect of APPA on tissue turnover in cartilage and bone tissue cultures. Osteoarthr Cartil 2021;29, S152. https://doi.org/10.1016/j.joca.2021.02.213. (Published online).
50. Larkins N. AB0872 efficacy and safety of the combination of apocynin and paeonol (APPA) in patients with osteoarthritis: an uncontrolled patient case series. Ann Rheum Dis 2020;79(Suppl 1), 1742.1-1742. https://doi.org/10.1136/annrheumdis-2020-eular.1221
51. Guermazi A, Hayashi D, Roemer FW, *et al*. Synovitis in knee osteoarthritis assessed by contrast-enhanced magnetic resonance imaging (MRI) is associated with radiographic tibiofemoral osteoarthritis and MRI-detected widespread cartilage damage: The MOST study. J Rheumatol 2014;41(3):501–8. https://doi.org/10.3899/jrheum.130541
52. Dainese P, Wyngaert KV, De Mits S, Wittoek R, Van Ginckel A, Calders P. Association between knee inflammation and knee pain in patients with knee osteoarthritis: a systematic review. Osteoarthr Cartil 2022;30(4):516–34. https://doi.org/10.1016/j.joca.2021.12.003. (Published online).

53. Philpott HT, Birmingham TB, Pinto R, *et al*. Synovitis is associated with constant pain in knee osteoarthritis: a cross-sectional study of OMERACT knee ultrasound scores. J Rheumatol 2022;49(1):89–97. https://doi.org/10.3899/jrheum.210285. (Published online).
54. Nazıroğlu M. Activation of TRPM2 and TRPV1 channels in dorsal root ganglion by NADPH oxidase and protein kinase C molecular pathways: a patch clamp study. J Mol Neurosci 2017;61(3):425–35. https://doi.org/10.1007/s12031-017-0882-4. (Published online).
55. Olukman M, Önal A, Çelenk F, *et al*. Treatment with NADPH oxidase inhibitor apocynin alleviates diabetic neuropathic pain in rats. Neural Regen Res 2018;13(9):1657–64. https://doi.org/10.4103/1673-5374.232530. (Published online).
56. Zhao WC, Zhang B, Liao MJ, *et al*. Curcumin ameliorated diabetic neuropathy partially by inhibition of NADPH oxidase mediating oxidative stress in the spinal cord. Neurosci Lett 2014;560:81–5. https://doi.org/10.1016/j.neulet.2013.12.019. (Published online).
57. Adki KM, Kulkarni YA. Neuroprotective effect of paeonol in streptozotocin-induced diabetes in rats. Life Sci 2021;271. https://doi.org/10.1016/j.lfs.2021.119202. (Published online).
58. den Boer C, Dries L, Terluin B, *et al*. Central sensitization in chronic pain and medically unexplained symptom research: a systematic review of definitions, operationalizations and measurement instruments. J Psychosom Res 2019;117:32–40. https://doi.org/10.1016/j.jpsychores.2018.12.010. (Published online).
59. Matsuda M, Huh Y, Ji RR. Roles of inflammation, neurogenic inflammation, and neuroinflammation in pain. J Anesth 2019;33(1):131–9. https://doi.org/10.1007/s00540-018-2579-4. (Published online).
60. Bailly F, Cantagrel A, Bertin P, *et al*. Part of pain labelled neuropathic in rheumatic disease might be rather nociplastic. RMD Open 2020;6(2). https://doi.org/10.1136/rmdopen-2020-001326. (Published online).
61. Walsh DA. Synovitis and pain sensitization. Arthritis Rheumatol 2016;68(3):561–2. https://doi.org/10.1002/art.39487. (Published online).
62. Neogi T, Guermazi A, Roemer F, *et al*. Association of joint inflammation with pain sensitization in knee osteoarthritis: the multicenter osteoarthritis study. Arthritis Rheumatol 2016;68(3):654–61. https://doi.org/10.1002/art.39488. (Published online).
63. Carlesso LC, Segal NA, Curtis JR, *et al*. Knee pain and structural damage as risk factors for incident widespread pain: data from the multicenter osteoarthritis study. Arthritis Care Res 2017;69(6):826–832. https://doi.org/10.1002/acr.23086. (Published online).
64. Ehrich EW, Davies GM, Watson DJ, Bolognese JA, Seidenberg BC, Bellamy N. Minimal perceptible clinical improvement with the Western Ontario and McMaster Universities Osteoarthritis Index questionnaire and global assessments in patients with osteoarthritis. J Rheumatol 2000;27(11):2635–41.
65. Angst F, Benz T, Lehmann S, Aeschlimann A, Angst J. Multidimensional minimal clinically important differences in knee osteoarthritis after comprehensive rehabilitation: a prospective evaluation from the Bad Zurzach Osteoarthritis Study. RMD Open 2018;4(2). https://doi.org/10.1136/rmdopen-2018-000685
66. Devji T, Guyatt GH, Lytvyn L, *et al*. Application of minimal important differences in degenerative knee disease outcomes: a systematic review and case study to inform BMJ Rapid Recommendations. BMJ Open 2017;7(5):1–11. https://doi.org/10.1136/bmjopen-2016-015587
67. Loeser RF, Collins JA, Diekman BO. Ageing and the pathogenesis of osteoarthritis. Nat Rev Rheumatol 2016;12(7):412–20. https://doi.org/10.1038/nrrheum.2016.65. (Published online).
68. Harris RE, Williams DA, McLean SA, *et al*. Characterization and consequences of pain variability in individuals with fibromyalgia. Arthritis Rheum 2005;52(11):3670–4. https://doi.org/10.1002/art.21407
69. Farrar JT, Troxel AB, Haynes K, *et al*. Effect of variability in the 7-day baseline pain diary on the assay sensitivity of neuropathic pain randomized clinical trials: an ACTTION study. Pain 2014;155(8):1622–31. https://doi.org/10.1016/j.pain.2014.05.009
70. Gillving M, Demant D, Holbech JV, *et al*. Impact of variability in baseline pain on the placebo response in randomized, placebo-controlled, crossover trials in peripheral neuropathic pain. Pain 2022;163(3):483–8. https://doi.org/10.1097/j.pain.0000000000002374. (Published online).
71. Treister R, Honigman L, Lawal OD, Lanier RK, Katz NP. A deeper look at pain variability and its relationship with the placebo response: results from a randomized, double-blind, placebo-controlled clinical trial of naproxen in osteoarthritis of the knee. Pain 2019;160(7):1522–8. https://doi.org/10.1097/j.pain.0000000000001538