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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] 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 extra-cellular 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 signalling 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 (BMI) 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 odour 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 organisation (CRO) staff were blinded throughout the trial period.

Paracetamol/acetaminophen was dispensed as rescue medication, with a cap of 4 grams 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 – 10 numeric pain scale, with ‘no pain’ = 0 and ‘worse imaginable pain’ = 10) during the past 24 hours. A Twenty Meter Walk Test was performed as a measure of the average gait speed during two consecutive walks of each 20 meters 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 enrolment 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 Figure 1. Subjects were analysed 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 analysed 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 (MMRM) 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 analysed 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 neuroplastic/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 **Figure 1**, and as shown the ITT- and mITT-populations were identical. The baseline characteristics are summarized in **Table 1**.

Efficacy results

The results of the main efficacy outcomes are shown in **Table 2.**

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, **Figure 2A**). Similarly, no significant differences were found on other secondary endpoints **Figures 2B-D**, and in **Table 2**.

Figure 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)

<u>Post-hoc subgroup analyses</u>

Results of the post-hoc subgroup analyses are shown in figures 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 (**Figure 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: -9.35 to 1.33, p=0.14 (**Figure 3C**), or a combination of these two criteria (*Group 3*; n=64) in **Figure 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 **Figure 4.**

Biomarker results

As shown in Table 3, 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 4**) 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 “diarrhoea”) 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 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 defence against infections, it inhibits neutrophil degranulation and cytokine-driven signalling pathways (e.g., autocrine signalling 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 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 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 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 neuroplastic pain/severe OA, indicating that further research in the effects of APPA in appropriate patients is warranted.

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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.

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CONFLICTS OF 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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FIGURE LEGENDS

Figure 1: Participant disposition.

ITT: Intention-to-treat. mITT: Modified Intention-to-treat. APPA: Apocynin+Paeonol.

Figure 2: 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.

Figure 3. 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.

Figure 4. Forest plot of subgroup analyses of WOMAC pain change

Data shown as difference between APPA and placebo at Week 4.

TABLE LEGENDS

Table 1: Baseline characteristics.

BMI: Body mass index. KL: Kellgren Lawrence. SD: Standard deviation. WOMAC: Western Ontario McMaster University Osteoarthritis Index. Yrs: Years.

Table 2: Mean Changes from Baseline to Week 4 in Main Efficacy Outcomes (mITT population)

WOMAC: Western Ontario McMaster University Osteoarthritis Index. ICOAP: Intermittent and Constant OsteoArthritis Pain Index

Table 3: Mean proportional Changes from Baseline to Week 4 in Biomarker Concentrations (%)

Table 4: 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).

TEAE, Treatment-emergent adverse event.