Dual JAK and ROCK inhibition with CPL'116 in patients with rheumatoid arthritis with inadequate response to methotrexate: a randomised, double-blind, placebo-controlled, phase 2 trial

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Summary

Background Janus kinase (JAK) inhibitors are an effective treatment option in rheumatoid arthritis and other autoimmune diseases. However, the use of JAK inhibitors is associated with increased total cholesterol, LDL cholesterol, triglycerides, and creatinine kinase, reducing the net clinical benefit of using them. Adding Rho-associated protein kinase (ROCK) inhibition to JAK inhibition might provide cardioprotection as ROCK inhibitors have been shown to reduce vascular inflammation, improve endothelial function, and prevent cardiac remodelling in preclinical models. In this study we investigated CPL409116 (hereafter referred to as CPL'116), a novel dual JAK and ROCK inhibitor, in patients with rheumatoid arthritis with inadequate response to methotrexate, to assess dose-dependent effects on disease control, pharmacokinetics, and laboratory abnormalities among other safety events.

Methods This phase 2, randomised, double-blind, dose-ranging, placebo-controlled, parallel group trial enrolled patients aged 18–75 years at nine hospitals or clinics in Poland and Ukraine. Main inclusion criteria were a documented diagnosis of adult-onset, moderate-to-severe rheumatoid arthritis for at least 6 months before screening, and inadequate response to current methotrexate treatment (oral or injected 15–25 mg once weekly, or ≥10 mg once weekly if reduced due to side-effects or intolerance). Participants were randomly assigned via an interactive web response system (1:1:1:1, block size of four, stratified by age) to oral CPL'116 (60 mg, 120 mg, or 240 mg) or placebo twice daily for 12 weeks, with continuation of background methotrexate. The primary endpoint of the study was the change from baseline in Disease Activity Score based on 28 joints and C-reactive protein (DAS28-CRP) at week 12, analysed with a mixed-effects repeated measures model by a modified intention-to-treat approach. p values were nominal. Safety parameters including adverse events, vital functions, and laboratory results were closely monitored and reported for the safety analysis population, comprising all participants who received at least one dose of CPL'116 or placebo. People with lived experience were not involved in the study. The trial was registered with ClinicalTrials.gov, NCT05374785, and is completed.

Findings Between May 30, 2022, and Feb 7, 2024, 106 patients were randomly assigned (27 in the CPL'116 60 mg group, 25 in the 120 mg group, 26 in the 240 mg group, and 28 in the placebo group). Overall mean age was 54·4 years (SD 10·5); 79 (75%) of 106 participants were women and 27 (25%) were men, and all individuals self-reported as White. The least squares mean difference in the change from baseline in DAS28-CRP at 12 weeks with CPL'116 versus placebo was –0·15 (95% CI –0·81 to 0·52; p=0·67) for the 60 mg dose; –0·56 (–1·25 to 0·12; p=0·10) for the 120 mg dose; and –0·89 (–1·56 to –0·22; p=0·010) for the 240 mg dose. Thus the primary endpoint was met for the 240 mg dose only. Overall CPL'116 was well tolerated. Two participants had serious treatment-emergent adverse events (one in the 60 mg dose group [non-fatal non-ST-elevation myocardial infarction, deemed possibly related to CPL'116] and one in the 240 mg dose group [non-muscle invasive bladder cancer, deemed unrelated to CPL'116]); both events led to discontinuation of CPL'116. CPL'116 was also discontinued due to leukopenia which was deemed to be possibly related to CPL'116 in one participant who received the 240 mg dose. No severe adverse events or deaths were reported. No laboratory or haematology abnormalities were recorded at any CPL'116 dose.

Interpretation The findings of this study suggest a dose-dependent response with CPL'116, with significant efficacy at the high dose of 240 mg twice daily. The drug was generally well tolerated and was not associated with lipid abnormalities or creatinine kinase increase. These findings warrant further investigation in larger studies and different clinical settings.

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Research in context

Evidence before this study

We searched PubMed from database inception up to March 1, 2022, with the terms "rheumatoid arthritis", "rheumatoid arthritis methotrexate", "JAK inhibition in rheumatoid arthritis ", "ROCK inhibition in autoimmune disease", "JAK inhibitors safety", "rheumatoid arthritis treatment refractory to methotrexate" "novel therapies in rheumatoid arthritis", "ROCK inhibitors in autoimmune diseases", and "ROCK inhibition cardioprotection", to identify articles published in any language regarding rheumatoid arthritis and its treatment. The therapeutic landscape for rheumatoid arthritis includes several treatment options, including methotrexate and various Janus kinase (JAK) inhibitors. Although JAK inhibitors have shown efficacy in managing rheumatoid arthritis, they have been associated with notable side-effects, including increased risk of altered lipid profile and cardiovascular events that often lead to treatment discontinuation. Therefore, many patients are left without sufficient therapy and, as well as living with everyday pain, might develop rheumatoid arthritis-related conditions that further decrease their quality of life. Emerging research on Rho-associated protein kinase (ROCK) inhibitors has suggested not only a potential role in modulating immune responses and inflammatory pathways relevant to rheumatoid arthritis, but also cardioprotective effects; these agents have been shown to reduce vascular inflammation, improve endothelial function, and prevent cardiac remodelling in preclinical models. In our laboratory, we have synthesised a novel dual inhibitor molecule (CPL'116), with the ability to selectively block JAK and ROCK pathways. The first article published by our group, using in vitro and in vivo models, reported the properties of this molecule and indicated its plausible beneficial role in treating autoimmune disease. The results of our recent phase 1 clinical trial of CPL'116 in healthy volunteers showed a tolerable safety profile and good pharmacokinetic properties, providing the key rationale for this study.

Added value of this study

In this phase 2, randomised, placebo-controlled trial, we evaluated three doses of CPL'116 (60 mg, 120 mg, and 240 mg twice daily) in patients with rheumatoid arthritis and inadequate response to methotrexate. In the tested population of over 100 individuals, findings suggested a dose-dependent response with CPL'116, with a significant change from baseline in disease activity at week 12 with the high dose of 240 mg. Two serious adverse events occurred during treatment with CPL'116, only one of which was possibly related to CPL'116. There were no severe adverse events and few instances of adverse events leading to treatment discontinuation. Additionally, CPL'116 was not associated with blood abnormalities or disruptions in vital functions. Thus, CPL'116 was generally well tolerated and did not evoke the negative side-effects typical of JAK inhibitors. These results provide preliminary evidence for a novel therapy for autoimmune disease such as rheumatoid arthritis, but also suggest a potential strategy for diseases with inflammatory and fibrotic components.

Implications of all the available evidence

CPL'116 tested in this phase 2 clinical trial in patients with rheumatoid arthritis showed promising therapeutic benefit compared with placebo after 12 weeks of treatment. CPL'116 acts simultaneously via JAK and ROCK molecular pathways. A large body of evidence has indicated the importance of JAK/STAT signalling in autoimmune diseases. Our findings build on this evidence base and indicate that by adding ROCK inhibition to a JAK inhibitory molecule, there is the possibility to obtain a safer profile with maintained efficacy. Therefore, CPL'116 is a novel potential drug candidate for the treatment of autoimmune diseases while possibly providing additional cardioprotective effects.

Introduction

Rheumatoid arthritis is a chronic autoimmune disease that primarily affects the joints, resulting in progressive disability and potentially leading to premature death due to arthritis-associated complications. Rheumatoid arthritis is linked to increased cardiovascular risks, with affected individuals being 48% more likely to have a cardiovascular event and 50% more likely to die from cardiovascular disease than the general population.[1] This increased risk is mainly due to chronic inflammation, endothelial damage, and the rheumatoid arthritis lipid paradox, whereby low LDL cholesterol worsens health outcomes.[2,3] Traditional risk factors such as hypertension, diabetes, and obesity further emphasise the need for regular cardiovascular monitoring in patients with rheumatoid arthritis.

Chronic inflammation and vascular dysfunction in rheumatoid arthritis contribute to increased pulmonary arterial pressure, and approximately 22% of patients with rheumatoid arthritis develop pulmonary arterial hypertension with resultant heart and lung dysfunction.[4] Another complication of rheumatoid arthritis is interstitial lung disease. Studies show that anti-cyclic citrullinated peptide antibodies, which are highly specific to rheumatoid arthritis, are also found in the lung tissue of patients with rheumatoid arthritis,[5] highlighting that similar inflammatory processes driving rheumatoid arthritis might also contribute to the fibrotic changes seen in interstitial lung disease.

Despite several effective treatments for rheumatoid arthritis, not all patients have adequate responses. The increased risk of cardiovascular disease in patients with rheumatoid arthritis highlights the need for therapies that improve joint outcomes and address cardiovascular and pulmonary complications. In this context, CPL409116 (hereafter referred to as CPL'116) might be a promising therapeutic option. This drug candidate is a novel, dual inhibitor that exhibits enhanced selectivity for Janus

kinases (JAKs) and Rho-associated protein kinases (ROCKs). The molecule was selected from the Celon Pharma library after extensive in vitro and in vivo analyses, previously described by Dulak-Lis and colleagues.[6] Its activity as an ATP-competitive inhibitor of JAK and ROCK has been shown in cell-free kinase assays. Assessments with the KINOMEscan screening platform have further confirmed its greater selectivity and binding affinity for JAK1/3 and ROCK1/2 over a broad number of human kinases.[6] Although the exact mechanism of action of CPL'116 is still elusive, our previous studies have provided evidence that CPL'116 targets both pathways by decreasing phosphorylation of downstream proteins, including signal transducers and activators of transcription (for JAK) and myosin phosphatase targeting proteins and myosin light chain proteins (for ROCK) in vitro[6] and in healthy volunteers in a phase 1 clinical trial.[7]

JAK inhibitors have gained recognition as effective treatments for inflammatory diseases and demonstrated significant efficacy in managing rheumatoid arthritis. Despite proven efficacy, their use is associated with potential adverse effects including lipid profile abnormalities such as increased total cholesterol, LDL, and triglycerides[8,9] and elevated creatinine kinase[10] which, if symptomatic, can lead to treatment discontinuation.

The Ras homolog family member A (RhoA)/ROCK pathway has been implicated in the pathogenesis of cardiovascular diseases, whereby upregulated ROCK activity is associated with vascular dysfunction and detrimental remodelling. Subsequently, ROCK blockade has been found to ameliorate vascular remodelling and elevated blood pressure and to reduce vascular inflammation in preclinical models.[11,12] Additionally, ROCK inhibition has been shown to correct lipid profile abnormalities and restore endothelial function in vitro.[13] The relevance of ROCK activity in rheumatoid arthritis-associated interstitial lung disease is seen in pulmonary fibrosis, a disease with similar outcomes to interstitial lung disease. Increased ROCK activity has been observed in fibrotic lung tissue[14] and ROCK inhibition has been shown to prevent and even reverse fibrosis in animal models,[15] highlighting its potential as a therapeutic target in various fibrotic conditions.

CPL'116 has shown promising results stemming from its dual inhibition. In an in vivo model of systemic lupus erythematosus, we found that some of the observed anti-inflammatory effects of CPL'116 were mediated not only by JAK inhibition, but also by ROCK inhibition. We also observed renoprotective potential, likely linked with antifibrotic ROCK-dependent signalling.[6]

Based on these findings, we believe that the unique mechanism of action positions CPL'116 as a promising therapy for autoimmune diseases. By blocking JAK signalling while providing overall cardioprotective and antifibrotic effects of ROCK inhibition, CPL'116 is a compelling candidate for treating rheumatoid arthritis and its inter-related complications including cardiovascular risks and pulmonary fibrotic changes.

We previously conducted a phase 1 trial to evaluate the safety and pharmacokinetics of CPL'116.[7] 65 healthy White volunteers were administered CPL'116 (10–300 mg) in a single or multiple dose regime. The study revealed that the drug was well tolerated and did not evoke any severe adverse events, and plasma pharmacokinetics were predictable, showing a proportional increase in maximum plasma concentration and area under the plasma drug concentration–time curve. Based on the pharmacokinetic profile, twice daily administration was suggested for future testing. Encouraged by these positive outcomes, we conducted a randomised, phase 2 study of CPL'116 in patients with rheumatoid arthritis, to evaluate dose-dependent effects on disease control and pharmacokinetics, and its effect on laboratory abnormalities among other safety assessments.

Methods

Study design and participants

We did a phase 2, randomised, double-blind, dose-ranging, placebo-controlled, parallel group trial evaluating the efficacy, safety, and pharmacokinetics of CPL'116 in patients with moderate-to-severe rheumatoid arthritis, with an inadequate response to methotrexate. An overview of the study design is in the appendix (p 3). The study involved nine clinical sites (hospitals and clinics) in Poland and Ukraine, with all centres recruiting patients in parallel (appendix p 6).

See Online for appendix

The study population comprised adults aged 18–75 years with a documented diagnosis of adult-onset rheumatoid arthritis (diagnosed at age ≥16 years) at least 6 months before screening, as defined by the 2010 American College of Rheumatology (ACR) and European Alliance of Associations for Rheumatology (EULAR) classification criteria.[16] Individuals were required to have active disease at both screening and baseline, defined by having all three of: at least six tender/painful joints on the 68-joint count; at least six swollen joints on the 68-joint count; and Disease Activity Score based on 28 joints (DAS28) >3·2 (ie, at least moderate disease activity). Further eligibility criteria included class I, II, or III functional status according to the ACR 1991 revised criteria for global functional status in rheumatoid arthritis, and inadequate disease response on current methotrexate based on EULAR recommendations[17] and eligibility criteria (ie, active disease despite the use of methotrexate), determined by the investigator. The permitted dose of methotrexate was 15–25 mg once weekly, oral or injected (subcutaneous or intramuscular) for at least 12 weeks before screening, and with no change in dosage and route of administration for at least 8 weeks before day 1 (baseline); with a lower dose (≥10 mg once weekly) permitted on a case-by-case basis by the investigator if reduced due to side-effects or intolerance to methotrexate. Detailed inclusion and exclusion criteria

are listed in the appendix (pp 6–11). All participants provided written informed consent. Sex and ethnicity data were self-reported by participants. People with lived experience were not involved in the study.

The study was done in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. The protocol and informed consent forms received institutional review board approval and independent ethics committee approval (from the Polish Bioethical Committee, approval number: 209/2022) before study initiation. The trial was registered with ClinicalTrials.gov, NCT05374785. The protocol is available via ClinicalTrials.gov.

For the **protocol** see https://cdn.clinicaltrials.gov/large-docs/85/NCT05374785/Prot_000.pdf

Randomisation and masking

After completing screening evaluations, eligible individuals were enrolled by study investigators and randomly assigned (1:1:1:1) to one of four treatment groups: CPL'116 at dose 60 mg, 120 mg, or 240 mg, or placebo. Randomisation codes were automatically generated for each participant by an interactive web response system integrated into the electronic case report form. Participants were randomly assigned by the order in which they were enrolled for treatment, in randomly permuted blocks of four and stratified by age (<57 *vs* ≥57 years). After randomisation, masked study treatments were dispensed according to the treatment regimen, with investigators, site personnel, and participants masked to the assignment. The study pharmacist at each site was unmasked and responsible for preparing the appropriate drugs, which were packed identically for all the treatment groups. The drug packages were given to the patient by an investigator to administer at home according to the administration schedule. At each administration, participants received a set of four identically appearing tablets. Depending on the treatment group, the set contained the appropriate number of CPL'116 tablets at a dose of 60 mg each or matching placebo tablets. All participants, investigators, care providers, and outcome assessors were masked to treatment allocation.

Procedures

The study included a screening period (up to 4 weeks), a 12-week double-blind treatment period, and a 4-week follow-up period (appendix p 3). All treatments were administered orally, twice a day. The investigational product tablets were administered at the study site on the morning of ambulatory visits. All other doses were self-administered by the participants at home and documented in a participant diary. During the treatment period, participants received methotrexate as continuation of background rheumatoid arthritis therapy in addition to study treatment. The methotrexate dosage at baseline (ranging from 15 to 25 mg/week) and route of administration remained the same during the study and follow-up. As for the trial eligibility criteria, a lower dose of methotrexate (≥10 mg/week) was permitted if reduced due to side effects or intolerance. Participants were required to follow a schedule of visits, during which they underwent predefined assessments, outlined in the appendix (pp 4–5).

Adverse event reporting began from the date the informed consent form was signed and was continued until the final follow-up visit. Adverse events were examined according to Medical Dictionary for Regulatory Activities (MedDRA; version 25.1) system organ class and preferred term. Treatment discontinuations and the incidence and severity of adverse events and serious adverse events were recorded. Per the International Conference on Harmonization E2A guideline, a serious adverse event was defined as any adverse event, occurring at any dose, that resulted in death, was life-threatening, required inpatient hospitalisation or prolongation of existing hospitalisation, resulted in persistent or significant disability or incapacity, or was a congenital anomaly or birth defect. Study investigators assessed all adverse events for severity based on the intensity of the incident. Any undesirable signs, symptoms, or medical conditions occurring, or any worsening of pre-existing conditions, between signing informed consent and the first administration of study treatment were considered as pre-treatment adverse events. Any such occurrences after the first administration of study treatment were considered treatment-emergent adverse events. Every treatment-emergent adverse event was evaluated to assess its relation to study treatment, and documented as unrelated, unlikely related, possibly related, probably related, and definitely related. Taking a conservative approach, any adverse event that occurred during treatment and documented as unlikely related, possibly related, probably related, or definitely related, was described as a treatment-related adverse event.

Due to the nature of the drug (dual JAK and ROCK inhibitor), some adverse events were more likely to occur. Targeted medical adverse events were defined as any events occurring on or after the day of the first study treatment that met predefined criteria (MedDRA high-level group term: embolism and thrombosis; system organ class: infections and infestations, cardiac disorders [and the cardiac adverse event was classified as serious], or neoplasms benign, malignant and unspecified [including cysts and polyps]; or preferred term: gastrointestinal perforation, hypotension, or blood pressure abnormal). During each ambulatory visit, blood parameters, including biochemistry, haematology, and lipid profile, were monitored in all participants.

Plasma CPL'116 concentration was measured in samples taken at 0–6 h on day 1 (baseline), week 1 (day 8), and week 8 (day 57; eight timepoints: 0 h [predose], 0·5 h, 1 h, 1·5 h, 2 h, 2·5 h, 3 h, 4 h, and 6 h)

and at 0–24 h at week 12 (day 85; 13 timepoints: 0 h [predose], 0·5 h, 1 h, 1·5 h, 2 h, 2·5 h, 3 h, 4 h, 6 h, 8 h, 10 h, 12 h, and 24 h). At week 4 (day 29), two samples were taken and analysed: 0 h [predose] and 2·5 h after administration.

Outcomes

The primary endpoint was change from baseline in DAS28-C reactive protein (CRP) at week 12 (day 85). Predefined secondary efficacy endpoints were the change from baseline in DAS28-CRP at weeks 4, 8, and 16 (days 29, 57, and 113, respectively); the proportion of participants with DAS28-CRP remission at weeks 4, 8, 12, and 16 (defined as score <2·6); rates of ACR20, ACR50, ACR70, and ACR90 response (defined as 20%, 50%, 70%, or 90% improvement, respectively, in tender/painful join count, swollen join count, and at least three of Patient Global Assessment of arthritis, Physician's Global Assessment of arthritis, Patient's Assessment of Arthritis Pain, Health Assessment Questionnaire Disability Index [HAQ-DI], or CRP) at weeks 4, 8, 12, and 16; change from baseline in tender/painful and swollen joint counts on the DAS28 at weeks 4, 8, 12, and 16; and change from baseline in Physician's Global Assessment of arthritis at weeks 4, 8, 12, and 16. Secondary patient-reported endpoints measured at weeks 4, 8, 12, and 16 were change from baseline in Patient Global Assessment of arthritis, Patient's Assessment of Arthritis Pain by visual analogue scale (1–10), and HAQ-DI. Patient-reported secondary endpoints measured at the end of treatment (week 12) and during follow-up (week 16) were 36-Item Short Form Survey (SF-36; eight domain) scores, and Functional Assessment of Chronic Illness Therapy-Fatigue total score.

As secondary CPL'116 pharmacokinetic endpoints, we assessed the partial area under the curve of plasma concentration versus time, from dosing time to 6 h after dosing ($AUC_{[0–6h]}$), maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}), elimination half-life ($T_{1/2}$; if possible), and elimination rate constant (K_{el}; if possible) for CPL'116 and its M3 metabolite at baseline and week 1 (day 8), week 8 (day 57), and week 12 (day 85). Secondary safety endpoints were the safety and tolerability of CPL'116 including all adverse events, serious adverse events, vital signs, laboratory tests, and 12-lead electrocardiogram (ECG) results. All endpoint analyses were prespecified in the final statistical analysis plan before unmasking.

Statistical analysis

Sample size calculation for the trial, with the continuous DAS28-CRP outcome measure defined as change from baseline, was determined based on simulation of ANCOVA. The baseline measure, treatment, and clinical sites were included as covariates in the analysis. A previous phase 2 trial of E6011, a humanised IgG2 monoclonal antibody against fractalkine, in patients with rheumatoid arthritis with inadequate response to methotrexate,[18] served as the basis for the analysis. The following value assumptions were adopted. A decrease in the DAS28-CRP would indicate an improvement in health status, assuming the baseline mean scores were the same for the drug and placebo groups and equal to 5·5 (with SD equal to 1·0). We assumed a week-12 mean score for each CPL'116 dose group of 3·7 (SD 1·1) and in the placebo group of 4·5 (1·1). With 21 participants per group, the study would have greater than 80% power to detect a difference of 0·8 points or greater in each dose group compared with placebo, given a 5% significance level. Assuming a 16% dropout rate, the study needed to enrol approximately 100 participants.

Assessment of protocol deviations and allocation of participants to analysis populations were done before unmasking of treatment assignments. Major protocol deviations that could affect the primary endpoint were prespecified and included any violations that had major impact on the DAS28-CRP assessment; absence of valid assessment of DAS28-CRP either at baseline or at week 12; and substantial deviations in dose of study medicine or administration schedule.

The primary analysis and secondary efficacy endpoints were assessed in a modified intention-to-treat population, comprising all randomly assigned patients who received at least one dose of study treatment (60 mg, 120 mg, 240 mg CPL'116, or placebo) and completed baseline assessments allowing for calculation of DAS28-CRP score. Pre-specified supplementary analysis of the primary endpoint was conducted on the per-protocol subset, in all randomly assigned participants who completed the treatment period (up to week 12) without any major protocol deviations. Analyses included data from all CPL'116 dose groups and the placebo group, with the primary analysis comparing each dose group with the placebo group at week 12. The null hypothesis was that there would be no difference in the primary endpoint between each CPL'116 dose group and the placebo group. The alternative hypothesis was that at least one dose group would show a significant difference compared to placebo, at a two-sided significance level of 5%. The primary endpoint was analysed with a mixed-effects repeated measures model (MRMM), from which least-squares (LS) means were calculated, with 95% CIs derived from a Student's *t* distribution. All calculations were performed in R (versions 4.1.3 or 4.3.2) using the emmeans package. The MRMM included factors for dose, timepoint, interaction between dose and timepoint, age, sex, and clinical site (appendix pp 72–73). Baseline DAS28-CRP was included as the continuous covariate. Participant ID (the unique identifier for each participant) was treated as a random effect, with an unstructured covariance structure applied to account for the correlation among repeated measurements. With regards to missing data, a last observation carried

forward approach was initially specified in the protocol; however, this was amended in the final statistical analysis plan, with missing data assumed to be missing at random, and the final analysis done with the observed data, without imputation. Values missing due to a participant dropping out of the study due to lack of efficacy or adverse events were addressed in a sensitivity analysis of the primary endpoint prespecified in the statistical analysis plan, using a last observation carried forward imputation approach.

To ensure that results were consistent for MRMM analyses, a prespecified sensitivity analysis of the primary endpoint was done to evaluate the treatment effect of pooled CPL'116 compared with placebo at multiple timepoints (weeks 4, 8, 12, and 16) in the modified intention-to-treat and per-protocol sets. Change from baseline in DAS28-CRP was analysed based on LS means calculated by the MRMM model used in the primary endpoint analysis, without adjustments for multiplicity.

Change in DAS28-CRP at weeks 4, 8, and 16 were assessed by the same MRMM approach as for the primary endpoint. Proportions of participants with DAS28-CRP remission (score <2·6) in each study group were presented together with 95% CIs, calculated with Wilson's model. A similar approach was applied for the ACR20, ACR50, ACR70, and ACR90 response rates. Differences in the proportions of participants in DAS28-CRP remission or with ACR responses between the CPL'116 groups and placebo group were presented together with 95% CIs, calculated with Wilson's method, and p values, calculated with a proportion test adjusted for multiple comparisons. Logistic regression models with baseline DAS28-CRP, treatment, and clinical site as independent variables were also prepared to explain the occurrence of DAS28-CRP remission. Change from baseline in the tender/painful and swollen joint counts and change from baseline in the Physician's Global Assessment of arthritis were analysed with MRMM models (with use of respective baseline values, dose, and clinical site as fixed effects and participant ID as a random effect). These analyses were done without adjustment for multiplicity.

Patient-reported outcomes were presented descriptively for each timepoint. Demographic and anthropometric variables were summarised by treatment for all participants in the full analysis set, comprising all randomly assigned participants, irrespective of receiving study treatment, and with any post-baseline data. In practice, the full analysis set was the same as the modified intention-to treat population, with no participants withdrawing consent prior to or missing the first treatment administration.

All safety analyses were conducted in the safety set, comprising all participants who received at least one dose of CPL'116 or placebo. Screening and baseline values for vital signs, physical examination, 12-lead ECG, chest x-ray, and rheumatoid arthritis baseline characteristics were summarised descriptively. Medical history data were collected for each participant during screening, including a description of the disease or procedure, MedDRA system organ class and preferred term for adverse events, start date, and stop date (or ongoing if applicable). All adverse events were listed and tabulated according to intensity and causality. The number and percentage of treatment-emergent adverse events were tabulated for each system organ class and preferred term.

A post-hoc analysis was done to evaluate changes from baseline at week 12 for selected laboratory parameters (total cholesterol, LDL, HDL, triglycerides, and creatinine kinase) using an MRMM model analogous to the model used for analysis of the primary endpoint. Changes from baseline data were summarised in graphical format.

Statistical analyses were done with R software (versions 4.1.3 or 4.3.2). The alpha level for each analysis was 0·05; all p values are the values obtained directly from the given tests without adjustment for multiple comparisons, and are nominal.

Role of the funding source

The funder of the study had a role in study design, data collection, data analysis, data interpretation, and writing of the report.

Results

Of 156 individuals assessed for eligibility, 106 were enrolled and randomly assigned between May 30, 2022, and Feb 7, 2024, with 27 participants assigned to the CPL'116 60 mg group, 25 to the CPL'116 120 mg group, 26 to the CPL'116 240 mg group, and 28 to the placebo group (figure 1). A summary of participants with major protocol deviations is provided in the appendix (p 11).

In the overall population, mean age was 54·4 years (SD 10·5), 79 (75%) of 106 participants were women and 27 (25%) were men, and all participants self-identified as White. Across treatment groups, baseline demographic and disease characteristics were generally balanced with some minor differences (table 1). Of the initial 106 randomly assigned participants, 99 (93%) completed the 12-week treatment period (placebo group: 27 [96%] of 28 participants; CPL'116 60 mg group: 24 [89%] of 27; CPL'116 120 mg group: 25 [100%] of 25; and CPL'116 240 mg group: 23 [88%] of 26; figure 1).

For the primary endpoint of change from baseline in DAS28-CRP at 12 weeks, the CPL'116 240 mg dose group showed a statistically significant difference compared with the placebo group (LS mean difference –0·89 [95% CI –1·56 to –0·22]; p=0·010). For the 120 mg dose group, DAS28-CRP at week 12 showed numerical improvement over placebo albeit the difference versus placebo was not statistically significant (LS mean difference –0·56 [–1·25 to 0·12]; p=0·10). The 60 mg dose group also showed no significant difference compared with the placebo group

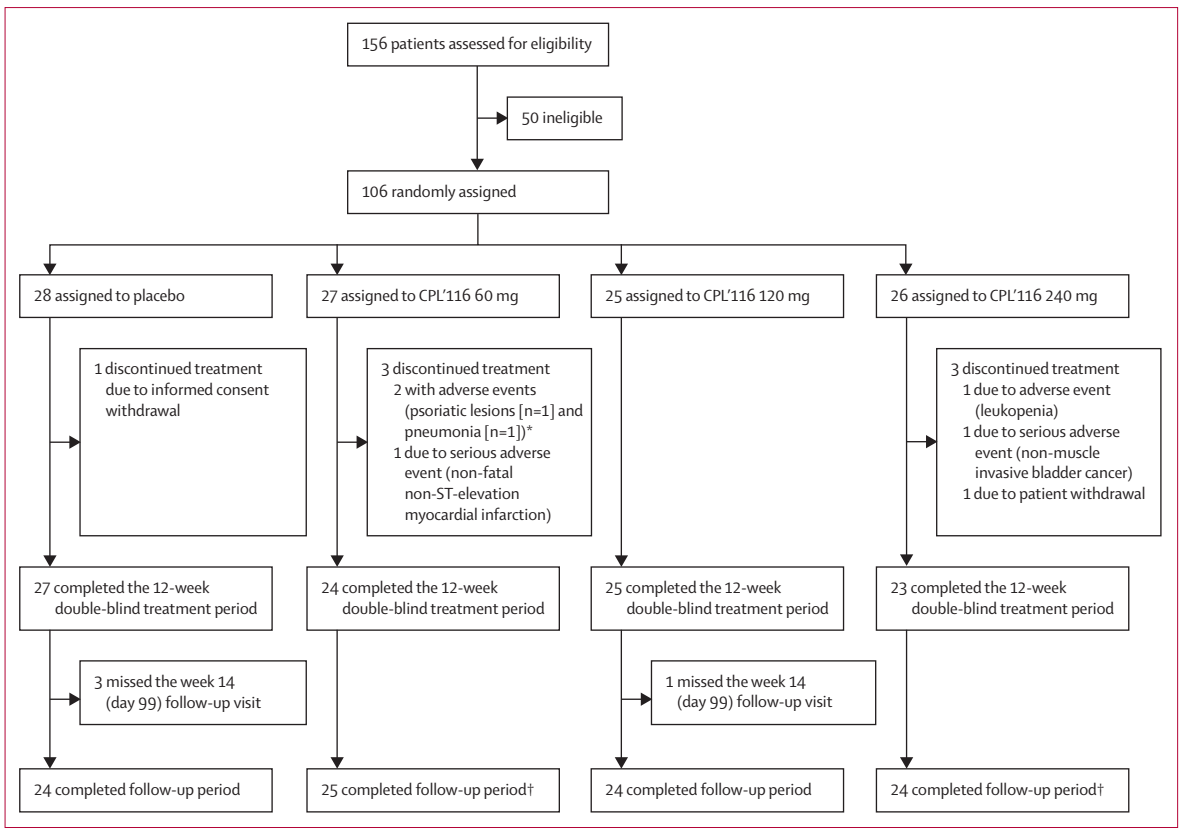

Figure 1: **Trial profile**

*Variation in reporting of reasons for discontinuation and adverse events meant that these adverse events were noted but not recorded as the final reason for discontinuation in the electronic case report form by the investigator, and thus these events were not defined as adverse events leading to treatment discontinuation; for the participant with pneumonia, the recorded reason for discontinuation was "dose interrupted", and for the participant with psoriatic lesions, the recorded reason was "other". †Including participants who discontinued treatment before the end of the treatment period who attended follow-up visits (week 14 [day 99] and week 16 [day 113]).

(LS mean difference –0·15 [–0·81 to 0·52], p=0·67; figure 2, appendix p 13). The results suggested dose-dependent improvement in DAS28-CRP (figure 2).

Detailed results for secondary endpoints are presented in the appendix (pp 12–66) and data for key endpoints are summarised herein. At weeks 4 and 8, DAS28-CRP score was significantly reduced at the highest dose of CPL'116 versus placebo. At week 16, the mean difference from placebo was no longer statistically significant (appendix pp 14–16). Proportions of participants in DAS28-CRP remission at any week of assessment were: five of 28 (18% [95% CI 7–38]) in the placebo group, six of 27 (22% [9–43]) in the CPL'116 60 mg dose group, nine of 25 (36% [19–57]) in the CPL'116 120 mg dose group, and 12 of 26 (46% [27–66]) in the CPL'116 240 mg dose group, representing no significant differences with placebo (all p>0·05; appendix pp 12–13). By contrast, the logistic model for the proportion of participants in DAS28-CRP remission at any week of assessment indicated a statistically significant difference with CPL'116 240 mg versus placebo with an odds ratio of 4·39 (95% CI 1·22–15·77]; p=0·023). ACR20, ACR50, ACR70, and ACR90 response rates at weeks 4, 8, 12, and 16 are presented in the appendix (pp 16–23). Numerically, ACR50 and ACR70 responses at the week 8, 12, and 16 timepoints were greater in the 240 mg dose group than in the placebo group but this was not statistically significant. However at week 8, there was a statistically significant difference for the 120 mg and 240 mg dose groups compared with placebo for ACR20.

Compared with placebo, significant improvements in tender/painful joint count were observed with the 120 mg dose of CPL'116 (LS mean difference week 4: –2·24 [95% CI –4·41 to –0·08], p=0·042; week 8: –3·86 [–6·66 to–1·06], p=0·007; week 12: –3·27 [–5·94 to –0·61], p=0·017); and the 240 mg dose (week 8: –3·08 [–5·83 to –0·33], p=0·028; week 12: –2·79 [95% CI –5·41 to –0·18], p=0·037; appendix pp 23–25). Swollen joint count showed significant improvement versus placebo for the 120 mg dose (LS mean difference week 8: –2·23 [–3·98 to –0·49], p=0·013; week 12: –2·20 [–4·22 to –0·19], p=0·032); and for the 240 mg dose (week 8: –2·34 [–4·05 to –0·63], p=0·008; week 12: –2·21 [–4·19 to –0·23], p=0·029; appendix pp 25–27).

	Placebo (n=28)	CPL'116 60 mg (n=27)	CPL'116 120 mg (n=25)	CPL'116 240 mg (n=26)
Age, years	52·6 (12·0)	55·8 (9·1)	54·5 (11·1)	54·9 (9·9)
Sex				
Male	8 (29%)	8 (30%)	4 (16%)	7 (27%)
Female	20 (71%)	19 (70%)	21 (84%)	19 (73%)
Race				
White	28 (100%)	27 (100%)	25 (100%)	26 (100%)
Country				
Poland	18 (64%)	14 (52%)	13 (52%)	14 (54%)
Ukraine	10 (36%)	13 (48%)	12 (48%)	12 (46%)
Weight, kg	77·2 (18·6)	73·3 (16·1)	75·4 (12·9)	80·1 (18·8)
Height, cm	167·5 (8·6)	168·3 (7·6)	163·9 (6·9)	166·3 (8·3)
BMI, kg/m²	27·5 (6·2)	25·8 (5·2)	28·0 (4·2)	28·8 (5·8)
Duration of rheumatoid arthritis, years	8·8 (10·0)	6·4 (5·5)	8·2 (7·5)	7·7 (8·6)
Duration of methotrexate treatment, years	5·8 (6·4)	5·2 (4·9)	5·8 (6·3)	4·5 (4·9)
Dose of methotrexate during the trial, mg/week	19·6 (4·7)	18·6 (4·5)	19·4 (4·4)	18·6 (4·9)
Tender/painful joints (DAS28)	11·4 (4·0)	12·2 (4·7)	11·4 (5·0)	10·9 (4·5)
Swollen joints (DAS28)	8·6 (2·2)	9·7 (2·7)	8·8 (2·7)	8·7 (2·9)
CRP, mg/L	18·1 (9·7)	22·8 (21·7)	17·7 (9·4)	20·9 (18·9)
DAS28-CRP	5·7 (0·6)	5·8 (0·6)	5·6 (0·6)	5·6 (0·7)
Disease activity based on DAS28-CRP				
Low (<3·2)	0	0	0	0
Moderate (3·2 to ≤5·1)	8 (29%)	2 (7%)	4 (16%)	5 (19%)
High (>5·1)	20 (71%)	25 (93%)	21 (84%)	21 (81%)
Biomarkers*				
Rheumatoid factor abnormal low	0	0	1 (4%)	0
Rheumatoid factor normal	6 (21%)	4 (15%)	7 (28%)	6 (23%)
Rheumatoid factor abnormal high	22 (79%)	23 (85%)	17 (68%)	20 (77%)
Anti-cyclic citrullinated peptide normal	6 (21%)	5 (19%)	8 (32%)	6 (23%)
Anti-cyclic citrullinated peptide abnormal high	22 (79%)	22 (81%)	17 (68%)	20 (77%)
Patient Global Assessment of arthritis total score	62·6 (15·7)	64·7 (20·1)	63·4 (14·2)	64·6 (18·9)
Patient's Assessment of Arthritis Pain total score	61·5 (18·7)	63·5 (19·3)	61·0 (15·3)	60·7 (16·7)
Physician Global Assessment of arthritis total score	66·1 (16·7)	68·1 (11·7)	69·0 (10·9)	63·9 (13·4)
Previous medication				
DMARDs: xenobiotics	16 (57%)	15 (56%)	13 (52%)	8 (31%)
DMARDs: biologics	4 (14%)	2 (7%)	2 (8%)	2 (8%)
Glucocorticoids	6 (21%)	5 (19%)	5 (20%)	5 (19%)
Non-steroidal anti-inflammatory drugs	6 (21%)	7 (26%)	5 (20%)	5 (19%)

Data are n (%) or mean (SD). Data are presented for the full analysis set, which in practice was the same as the modified intention-to-treat set. DAS28=Disease Activity Score based on 28 joints. CRP=C reactive protein. DAS28-CRP=Disease Activity Score based on 28 joints-C reactive protein. DMARDs=disease-modifying anti-rheumatic drugs. *Biomarker thresholds depended on the laboratory.

Table 1: **Baseline characteristics and disease activity (full analysis set)**

Change from baseline in Physician's Global Assessment of arthritis was improved at weeks 8 and 12 for both the CPL'116 120 mg and 240 mg dose groups, reaching statistical significance only for the 120 mg dose (appendix pp 27–29). The Patient Global Assessment score showed a consistent reduction across study groups throughout the treatment period, reaching the lowest mean values at week 12. At this timepoint, the CPL'116 240 mg dose group showed the greatest mean improvement (–33·88 points [SD 26·92]), followed closely by the 120 mg dose group. By contrast, the placebo group had the smallest change, with a mean improvement of –24·74 points (27·94; appendix pp 29–30).

Dose-dependent trends were observed in the extent and rate of absorption of CPL'116 (C_{max} and $AUC_{[0–6h]}$) at all timepoints assessed (baseline and weeks 1, 8, and 12) with dose proportionality in the 60–240 mg range (basic pharmacokinetic parameters presented in the appendix [pp 56–57]). Based on C_{max} values and accumulation ratios over time, a steady state was reached by day 8, and limited

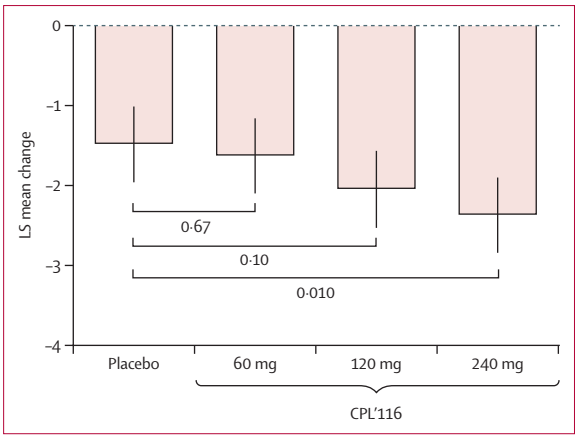

Figure 2: **Change from baseline in DAS28-CRP at week 12 by randomised treatment of CPL'116 (modified intention-to-treat set)**
Error bars show 95% CIs calculated from a Student's *t* distribution. p values are nominal. DAS28-CRP=Disease Activity Score based on 28 joints-C reactive protein. LS mean=least-squares mean.

	Placebo (n=28)	CPL'116 60 mg (n=27)	CPL'116 120 mg (n=25)	CPL'116 240 mg (n=26)
Total treatment-emergent adverse events	29	35	43	50
Infections and infestations				
Urinary tract infection	1 (3%)	1 (3%)	3 (7%)	2 (4%)
Upper respiratory tract infection	3 (10%)	0	1 (2%)	2 (4%)
Pharyngitis	0	0	0	3 (6%)
Nervous system disorders				
Headache	4 (14%)	2 (6%)	0	3 (6%)
Blood and lymphatic system disorders				
Leukopenia	0	3 (9%)	2 (5%)	3 (6%)
Lymphopenia	0	2 (6%)	0	1 (2%)
Gastrointestinal disorders				
Nausea	1 (3%)	6 (17%)	0	0
Diarrhoea	2 (7%)	0	1 (2%)	1 (2%)
Investigations				
Aspartate aminotransferase increased	1 (3%)	0	3 (7%)	2 (4%)
Alanine aminotransferase increased	0	1 (3%)	3 (7%)	3 (6%)
Lymphocyte count decreased	2 (7%)	0	0	1 (2%)
Renal and urinary disorders				
Leukocyturia	0	0	2 (5%)	3 (6%)
Urinary tract inflammation	0	2 (6%)	3 (7%)	2 (4%)

Data are presented as n (%) at the event level, where the denominator for percentages is the total number of reported treatment-emergent adverse events within each group. Multiple instances of the same event in the same patient were counted for each occurrence of the event. Further data on adverse events including treatment-related events are provided in the appendix (pp 58–66).

Table 2: **Most commonly reported treatment-emergent adverse events (≥5% incidence in any group; safety set)**

accumulation was observed regardless of dose. M3, the major active metabolite of CPL'116, exhibited a similar pharmacokinetic pattern to CPL'116 with a metabolite-to-parent ratio of 16–36% for C_{max} and 18–52% for $AUC_{(0–6h)}$.

In the safety set (n=106), treatment-emergent adverse event frequency was not markedly different in any group, however, a slight dose dependency was observed, with 29 events in the placebo group, 35 in the CPL'116 60 mg dose group, 43 in the CPL'116 120 mg dose group, and 50 in the CPL'116 240 mg dose group (appendix p 58). The most common treatment-emergent adverse events are presented in table 2. Two serious treatment-emergent adverse events were recorded during the study: non-fatal non-ST-elevation myocardial infarction (non-STEMI) was identified on day 13 in a participant with multiple cardiovascular risk factors in the CPL'116 60 mg dose group, and non-muscle invasive bladder cancer was identified on day 8 in a participant in the CPL'116 240 mg dose group. The incident of non-fatal non-STEMI was deemed possibly related to study drug, whereas the case of cancer occurrence was deemed unrelated. No severe treatment-emergent adverse events were recorded. Overall, 92 treatment-related adverse events were reported in 50 (47%) participants, with 16 events in 11 (39%) of 28 participants in the placebo group, 20 events in 11 (41%) of 27 participants in the CPL'116 60 mg dose group, 25 events in 13 (52%) of 25 participants in the CPL'116 120 mg dose group, and 31 events in 15 (58%) of 26 participants in the CPL'116 240 mg dose group (appendix pp 58–66). Treatment-related adverse events were most frequently reported under the system organ class of investigations (appendix pp 59–66). In total, 19 (18%) participants had a treatment-related adverse event in this class (five [18%] participants in the placebo group, one [4%] in the CPL'116 60 mg dose group, six [24%] in the CPL'116 120 mg dose group, and seven [27%] in the CPL'116 240 mg dose group). Within this class, treatment-related events of elevated alanine aminotransferase were reported in six (6%) participants overall (no participants in the placebo group, one [4%] in the CPL'116 60 mg dose group, two [8%] in the CPL'116 120 mg dose group, and three [12%] in the CPL'116 240 mg dose group). Treatment-related events of increased aspartate aminotransferase were reported in five (5%) participants overall (one [4%] participant in the placebo group, none in the CPL'116 60 mg dose group, two [8%] in the CPL'116 120 mg dose group, and two [8%] in the CPL'116 240 mg dose group). Treatment-emergent adverse events leading to discontinuation of study treatment occurred in one (4%) of 27 participants in the 60 mg dose group, two (8%) of 26 participants in the 240 mg dose group, and no patients in the placebo group or 120 mg dose group. These discontinuations included the two aforementioned serious adverse events in the CPL'116 60 mg and 240 mg dose groups. The additional discontinuation in the 240 mg dose group was due to leukopenia, which was deemed possibly related to the study drug. Infections and infestations, which we specified as targeted medical adverse events, were among the most common classes of adverse events across the study groups (table 2). The majority of

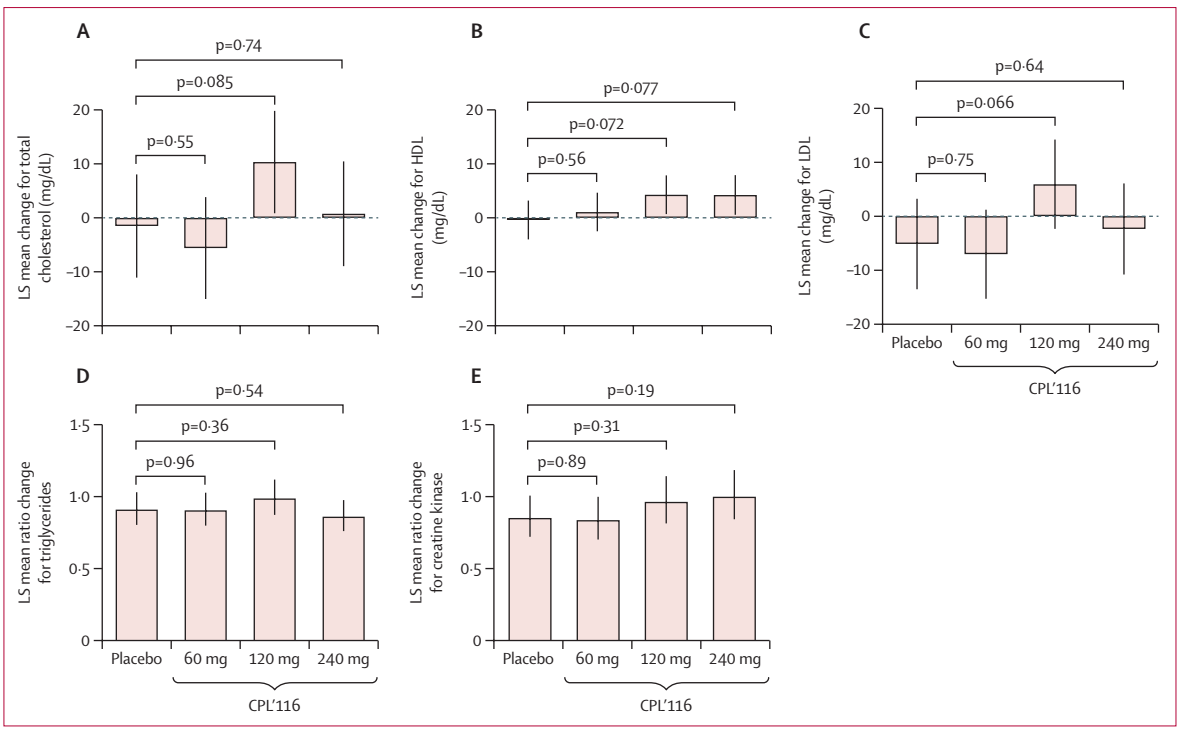

Figure 3: **Change from baseline in laboratory parameters at week 12 by randomised treatment of CPL'116 (safety set)**
In parts A, B, and C, LS mean change from baseline is presented. In parts D and E, change from baseline was calculated as the difference between the logarithm of the value at week 12 minus the logarithm of the value at baseline; thus data are presented as the ratio of values at week 12 versus baseline. Error bars show 95% CIs calculated from a Student' *t* distribution. p values are nominal. LS mean=least-squares mean.

infections and infestations were deemed unrelated to study treatment (appendix pp 60–62). The frequency of total targeted medical adverse events was overall similar between CPL'116-treated groups and the placebo group (appendix p 58). No deaths were reported during the study.

No changes in vital signs were recorded. Blood pressure was not modified and clinically significant aberrant ECG readouts were not identified for any dose of CPL'116 at any timepoint. No lasting changes in haematology parameters (platelets, haematocrit, and haemoglobin) were recorded at any dose. Similarly, biochemical parameters were not altered (appendix pp 43–55).

The sensitivity analyses confirmed robustness of the findings for the primary endpoint. The results were consistent between the modified intention-to-treat set (main analysis; figure 2) and per-protocol set (appendix pp 67–68). The analysis of the modified intention-to-treat population with imputed values also yielded similar outcomes to the primary analysis (appendix pp 68–69). In the analysis comparing pooled CPL'116 results to placebo, a greater decrease in DAS28-CRP in the active treatment group was observed at every timepoint in both the modified intention-to-treat and per-protocol sets, however, it reached significance only at week 8 in the modified intention-to-treat and per-protocol sets (appendix pp 69–72).

To better understand the safety profile of CPL'116, we did a post-hoc analysis of several key laboratory parameters. The analysis showed that total cholesterol, LDL, HDL, and triglycerides were not significantly altered at any dose compared with placebo. Creatinine kinase concentrations were stable over the course of the treatment at all doses and not different from the placebo group (figure 3).

Discussion

In this study, findings indicated dose-dependent efficacy of CPL'116 in patients with active rheumatoid arthritis and inadequate response to methotrexate. We found no evidence of efficacy with the 60 mg dose, the 120 mg dose had partial, albeit non-significant, effects, however the 240 mg dose had statistically significant efficacy for the primary endpoint and several secondary endpoints. The change from baseline in DAS28-CRP at week 12 with the 240 mg dose (LS mean difference from placebo –0·89 [95% CI –1·56 to –0·22]) is similar to other antirheumatic agents in patients with inadequate response to methotrexate on a background disease-modifying antirheumatic drug, as shown in a meta-analysis.[19] Interestingly, improvement from baseline to week 12 in the placebo group in DAS28-CRP (LS mean –1·50 [95% CI –1·97 to –1·02]; appendix p 13) was higher than the average DAS28 week 12

improvement with placebo (median –0·87 [95% CI –0·79 to 0·95]) reported in a previous meta-analysis that used a non-linear mixed effects modelling approach.[19] The placebo response measured by ACR20, ACR50, and ACR70 was also higher in this study compared with other randomised trials.[20] These high responses in the placebo group likely reduced the net clinical improvement with CPL'116 in this study.[20] The reason for the placebo group response remains unclear, but geographical bias in the study by recruiting patients only from less affluent countries (ie, eastern Europe) might have contributed by creating preferential incentives for inclusion to receive medical care.[21]

CPL'116 was well tolerated. There were few adverse events leading to treatment discontinuation. Non-STEMI was recorded early, in the second week of administration, in the lowest, inefficacious dose group. The event was deemed possibly related to the study drug, although it occurred in a participant with multiple cardiovascular risk factors. Non-muscle invasive bladder cancer recorded on day 8 was unlikely induced by the drug treatment given the long-term nature of this tumour, and was deemed unrelated to the study drug.

Post-hoc analysis showed no increases in lipid parameters at any dose of CPL'116 compared with placebo. These findings are in contrast to other studies testing JAK inhibitors, in which significant increases in total cholesterol, LDL, and HDL were observed.[8] This effect is not fully understood and might be partially explained by interleukin (IL)-6, which in the active inflammatory state increases LDL receptors on hepatocytes and enhances LDL catabolism. Supporting this mechanism is the fact that tocilizumab, an IL-6 receptor antibody, shows similar LDL and lipid-modulating effects as JAK inhibitors.[22]

The rheumatoid arthritis lipid paradox, a phenomenon of lower circulating lipids in active disease yet concurrent increased cardiovascular risk, has been under debate for more than a decade.[23] The increase in both LDL and HDL, thus maintaining a stable LDL to HDL ratio, after JAK inhibition in rheumatoid arthritis[23] might be misleading in atherosclerotic risk assessment. In rheumatoid arthritis, HDL composition differs from heathy volunteers with substantial loss of the anti-atherosclerotic HDL2 subfraction.[24,25] Under physiological conditions, HDL inhibits LDL oxidation and promotes cholesterol efflux from vascular foam cells.[23,26] It has protective anti-inflammatory and anti-atherosclerotic properties. Studies have shown that the anti-inflammatory effect of HDL is impaired during inflammation. As a result, HDL loses the ability to remove cholesterol from atherosclerotic plaques and protect LDL from oxidation, becoming pro-inflammatory HDL.[27,28] HDL changes from anti-inflammatory and anti-atherosclerotic to pro-inflammatory might partly explain the lipid paradox and the increased cardiovascular risk seen in rheumatoid arthritis.

It is unknown whether JAK inhibitors, which increase LDL and HDL, also proportionately increase the amounts of dysfunctional, atherosclerotic lipids (ie, oxidised LDL and pro-inflammatory and atherosclerotic HDL). This warrants urgent, detailed investigation and could provide insight into the link between JAK inhibitors, dyslipidaemia induction, and increased cardiovascular risk, as seen in previous studies comparing tofacitinib and adalimumab.[29]

In this study we did not observe changes in total cholesterol, LDL, or HDL with CPL'116. This beneficial outcome could be due to the unique dual mode of action of CPL'116, whereby an increase in lipid profile caused by JAK inhibition is balanced out by the protective effect of ROCK inhibition. Considering that ROCK activity is linked to endothelial dysfunction, which is closely associated with cardiovascular risk factors,[30] ROCK inhibition might help to maintain healthy function of the endothelium and lower LDL by enhancing clearance mechanisms. In support of this hypothesis, it has been reported that ROCK inhibition can disrupt LDL oxidation and reduce the effects of oxidised LDL on endothelial cells.[13] However, there has also been evidence of ROCK inhibition by statins not altering LDL concentration.[31] Although no direct evidence has linked ROCK activity with HDL reduction, ROCK inhibition has been associated with improved HDL functionality, likely due to its effects on the endothelium in reducing oxidative stress and enhancing anti-inflammatory properties in general.[32]

Another interesting outcome of this study is that the serum concentration of creatinine kinase was unchanged during CPL'116 treatment. Clinical studies have consistently reported that treatment with JAK inhibitors, but not IL-6 inhibitors, significantly increases creatinine kinase amounts, especially in the first few weeks of treatment.[10] CPL'116, despite its strong JAK inhibitory properties, did not show side-effects typical for these inhibitors. We believe that this result is a protective effect of its dual mode of action. There is a complex relationship between ROCK and creatinine kinase, however no tangible proof exists to support their direct interaction.[33] It is possible that inhibition of ROCK, which lowers blood pressure and improves vascular function,[11,12] potentially affects creatinine kinase concentration. One possibility is that ROCK activation promotes vasocontraction and increases blood pressure leading to increased ATP demand, which might be supplied via creatinine kinase activity.[33] Conversely, ROCK blockade decreases the demand for ATP thereby lowering creatinine kinase concentration.

This study had limitations that should be addressed. The trial was conducted only at clinical sites in Poland and Ukraine and all participants were White. This might limit the applicability of the results to patients in other regions and of other ethnic backgrounds, especially considering potential genetic, environmental, and

health-care system differences. The regions included in the study often have limited access to advanced medical treatments, especially with ongoing military conflict in Ukraine. Participating in the trial to receive medical care might have contributed to the placebo bias. Another limitation is that patients included in the study had insufficient response to methotrexate therapy, which narrows generalisability and excludes patients who are not treated with methotrexate or who respond well to it. Lastly, people with lived experience were not involved at any stage in preparation of the trial.

To our knowledge, we present the first evidence supporting the use of a dual JAK and ROCK inhibitor as a potential treatment option for patients with rheumatoid arthritis who have inadequate response to methotrexate. CPL'116 240 mg improved DAS28-CRP at week 12, with a high remission rate observed. Keeping in mind the small sample size, CPL'116 appeared to effectively target the JAK pathway, alleviating rheumatoid arthritis symptoms while avoiding the side-effects typically associated with JAK inhibitors. However, the observed favourable safety profile is yet to be assessed in phase 3 studies. Nevertheless, in this phase 2 trial, CPL'116 showed promising therapeutic potential for rheumatoid arthritis and possibly other autoimmune conditions.

Contributors

MW envisioned the study, sought funding, finalised the protocol, and oversaw the trial. MW, BK, PP, and DW conceptualised the trial and BK, PL, IVK, IV, and IK performed the clinical investigations. MK performed the pharmacokinetic analysis. MB and JK oversaw the data acquisition and statistical analysis. MW wrote the manuscript. MB and JK revised and edited the manuscript. All authors had full access to the source data in the study. BK, MB, and JK accessed and verified the data. All authors had final responsibility for the decision to submit for publication.

Declaration of interests

MW is a founder, Chief Executive Officer, and majority owner of and holds stocks in Celon Pharma. DW, PP, MK, MB, and JK are employees or were employees during the trial of Celon Pharma and received a salary. All other authors declare no competing interests.

Data sharing

Deidentified summary data can be made available upon a reasonable, research-driven request to the corresponding author and after ethical approval, for professional individuals or organisations who are science oriented. The request will be judged on a case-by-case basis by a Celon Pharma representative. The platform to exchange documents will be discussed on an individual basis. Depending on the type of request, some data sharing may require a data access agreement. The protocol and statistical analysis plan are available online.

For the **protocol** see https://cdn.clinicaltrials.gov/large-docs/85/NCT05374785/Prot_000.pdf

For the **statistical analysis plan** see https://cdn.clinicaltrials.gov/large-docs/85/NCT05374785/SAP_001.pdf

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References

1 Avina-Zubieta JA, Thomas J, Sadatsafavi M, Lehman AJ, Lacaille D. Risk of incident cardiovascular events in patients with rheumatoid arthritis: a meta-analysis of observational studies. *Ann Rheum Dis* 2012; **71:** 1524–29.

2 Hansildaar R, Vedder D, Baniaamam M, Tausche AK, Gerritsen M, Nurmohamed MT. Cardiovascular risk in inflammatory arthritis: rheumatoid arthritis and gout. *Lancet Rheumatol* 2021; **3:** e58–70.

3 Rawla P. Cardiac and vascular complications in rheumatoid arthritis. *Reumatologia* 2019; **57:** 27–36.

4 Yang X, Mardekian J, Sanders KN, Mychaskiw MA, Thomas J 3rd. Prevalence of pulmonary arterial hypertension in patients with connective tissue diseases: a systematic review of the literature. *Clin Rheumatol* 2013; **32:** 1519–31.

5 Khan T, Jose RJ, Renzoni EA, Mouyis M. A closer look at the role of anti-CCP antibodies in the pathogenesis of rheumatoid arthritis-associated interstitial lung disease and bronchiectasis. *Rheumatol Ther* 2021; **8:** 1463–75.

6 Dulak-Lis M, Bujak A, Gala K, et al. A novel JAK/ROCK inhibitor, CPL409116, demonstrates potent efficacy in the mouse model of systemic lupus erythematosus. *J Pharmacol Sci* 2021; **145:** 340–48.

7 Rudzki PJ, Jarus-Dziedzic K, Włodarczyk D, et al. First-in-human study of CPL'116 —a dual JAK/ROCK inhibitor—in healthy subjects. *Front Pharmacol* 2025; **16:** 1583723.

8 Li N, Gou ZP, Du SQ, et al. Effect of JAK inhibitors on high- and low-density lipoprotein in patients with rheumatoid arthritis: a systematic review and network meta-analysis. *Clin Rheumatol* 2022; **41:** 677–88.

9 Koh JH, Lee BW, Kim WU. Changes in the cholesterol profile of patients with rheumatoid arthritis treated with biologics or Janus kinase inhibitors. *J Rheum Dis* 2023; **30:** 234–42.

10 Tada M, Okano T, Mamaoto K, et al. Comparison of creatine kinase elevation caused by Janus kinase inhibitors and interleukin-6 inhibitors in patients with rheumatoid arthritis: a propensity score-matched study. *Arch Rheumatol* 2024; **39:** 350–57.

11 Hartmann S, Ridley AJ, Lutz S. The function of Rho-associated kinases ROCK1 and ROCK2 in the pathogenesis of cardiovascular disease. *Front Pharmacol* 2015; **6:** 276.

12 Li C, Ma L, Wang Q, et al. Rho kinase inhibition ameliorates vascular remodeling and blood pressure elevations in a rat model of apatinib-induced hypertension. *J Hypertens* 2022; **40:** 675–84.

13 Zhang C, Adamos C, Oh M-J, et al. oxLDL induces endothelial cell proliferation via Rho/ROCK/Akt/$p27^{kip1}$ signaling: opposite effects of oxLDL and cholesterol loading. *Am J Physiol Cell Physiol* 2017; **313:** C340–51.

14 Shimizu Y, Dobashi K, Sano T, Yamada M. ROCK activation in lung of idiopathic pulmonary fibrosis with oxidative stress. *Int J Immunopathol Pharmacol* 2014; **27:** 37–44.

15 Nalkurthi C, Schroder WA, Melino M, et al. ROCK2 inhibition attenuates profibrogenic immune cell function to reverse thioacetamide-induced liver fibrosis. *JHEP Rep Innov Hepatol* 2021; **4:** 100386.

16 Aletaha D, Neogi T, Silman AJ, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010; **69:** 1580–88.

17 Smolen JS, Landewé RBM, Bijlsma JWJ, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. *Ann Rheum Dis* 2020; **79:** 685–99.

18 Tanaka Y, Takeuchi T, Yamanaka H, et al. Efficacy and safety of E6011, an anti-fractalkine monoclonal antibody, in patients with active rheumatoid arthritis with inadequate response to methotrexate: results of a randomized, double-blind, placebo-controlled phase II study. *Arthritis Rheumatol* 2021; **73:** 587–95.

19 Leil TA, Lu Y, Bouillon-Pichault M, Wong R, Nowak M. Model-based meta-analysis compares DAS28 rheumatoid arthritis treatment effects and suggests an expedited trial design for early clinical development. *Clin Pharmacol Ther* 2021; **109:** 517–27.

20 Bechman K, Yates M, Norton S, Cope AP, Galloway JB. Placebo response in rheumatoid arthritis clinical trials. *J Rheumatol* 2020; **47:** 28–34.

21 Kerschbaumer A, Smolen JS, Ferreira RJO, et al. Efficacy and safety of pharmacological treatment of psoriatic arthritis: a systematic literature research informing the 2023 update of the EULAR recommendations for the management of psoriatic arthritis. *Ann Rheum Dis* 2024; **83:** 760–74.

22 Robertson J, Porter D, Sattar N, et al. Interleukin-6 blockade raises LDL via reduced catabolism rather than via increased synthesis: a cytokine-specific mechanism for cholesterol changes in rheumatoid arthritis. *Ann Rheum Dis* 2017; **76:** 1949–52.

23 Venetsanopoulou AI, Pelechas E, Voulgari PV, Drosos AA. The lipid paradox in rheumatoid arthritis: the dark horse of the augmented cardiovascular risk. *Rheumatol Int* 2020; **40:** 1181–91.

24 Chung CP, Oeser A, Raggi P, et al. Lipoprotein subclasses determined by nuclear magnetic resonance spectroscopy and coronary atherosclerosis in patients with rheumatoid arthritis. *J Rheumatol* 2010; **37:** 1633–38.

25 Arts E, Fransen J, Lemmers H, et al. High-density lipoprotein cholesterol subfractions HDL2 and HDL3 are reduced in women with rheumatoid arthritis and may augment the cardiovascular risk of women with RA: a cross-sectional study. *Arthritis Res Ther* 2012; **14:** R116.

26 Liu SL, Sheng R, Jung JH, et al. Orthogonal lipid sensors identify transbilayer asymmetry of plasma membrane cholesterol. *Nat Chem Biol* 2017; **13:** 268–74.

27 Van Lenten BJ, Hama SY, de Beer FC, et al. Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cell cocultures. *J Clin Invest* 1995; **96:** 2758–67.

28 Ansell BJ, Navab M, Hama S, et al. Inflammatory/antiinflammatory properties of high-density lipoprotein distinguish patients from control subjects better than high-density lipoprotein cholesterol levels and are favorably affected by simvastatin treatment. *Circulation* 2003; **108:** 2751–56.

29 Ma XN, Shi MF, Wang SI, et al. Risk of dyslipidemia and major adverse cardiac events with tofacitinib versus adalimumab in rheumatoid arthritis: a real-world cohort study from 7580 patients. *Front Pharmacol* 2024; **15:** 1370661.

30 Noma K, Kihara Y, Higashi Y. Striking crosstalk of ROCK signaling with endothelial function. *J Cardiol* 2012; **60:** 1–6.

31 Nohria A, Prsic A, Liu PY, et al. Statins inhibit Rho kinase activity in patients with atherosclerosis. *Atherosclerosis* 2009; **205:** 517–21.

32 Liao JK. Does it matter whether or not a lipid-lowering agent inhibits Rho kinase? *Curr Atheroscler Rep* 2007; **9:** 384–88.

33 Brewster LM. Creatine kinase, energy reserve, and hypertension: from bench to bedside. *Ann Transl Med* 2018; **6:** 292.