BMJ Open

Reduction effect of oral pravastatin on the acute phase response to intravenous zoledronic acid: protocol for a real-world prospective, placebo-controlled trial

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To cite: Liu Q, Han G, Li R, *et al*. Reduction effect of oral pravastatin on the acute phase response to intravenous zoledronic acid: protocol for a real-world prospective, placebo-controlled trial. *BMJ Open* 2022;**12**:e060703. doi:10.1136/bmjopen-2021-060703

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2021-060703).

Received 30 December 2021
Accepted 27 June 2022

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ABSTRACT

Introduction Zoledronic acid (ZA) has been used as a first-line treatment in patients with osteoporosis (OP) who receive an annual injection of 5 mg. However, side effects of bone pain and fever, known as the acute phase response (APR), have often been observed after clinical usage. A meta-analysis reported that the incidence of APR was 49.4% among patients with OP who received ZA for the first time and that 30% of patients with these adverse effects refused treatment in the following year. As a clinically used hypolipidaemic drug, statins can inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase to block the pathway upstream of farnesyl pyrophosphate synthase. This process can decrease the accumulation of isopentenyl pyrophosphate to prevent γδT-cell activation and inflammatory factor production, blocking APR occurrence. The aim of this study is to determine the reduction effect of oral pravastatin on APR and investigate the possible mechanisms underlying the effect in vivo.

Methods and analysis This will be a single-centre, placebo-controlled trial. Female participants will be allocated at a 1:1 ratio to receive either oral pravastatin or a placebo at 1-hour predose and 24 and 48 hours post-administration of ZA. The primary outcome will be the incidence of APR within 72 hours after ZA infusion. The secondary outcomes will include the occurrence time and severity of APR and the frequency and amount of acetaminophen usage within 72 hours after ZA infusion. This study will determine the preventive effect of oral pravastatin on APR in Chinese patients with OP, supporting the clinical application of ZA to alleviate concerns regarding safety and increase patient compliance.

Ethics and dissemination This study protocol has been registered with ClinicalTrials.gov. This study protocol was reviewed and approved by the Peking University Third Hospital Medical Science Research Ethics Committee. The results will be published in scientific peer-reviewed journals.

Trial registration number NCT04719481.

Strengths and limitations of this study

- ⇒ This is the first clinical trial to demonstrate the effect of 80 mg pravastatin on blocking the occurrence of acute phase response.
- ⇒ Strict quality control will be required to ensure the integrity of the data as much as possible in terms of data collection, recording and storage.
- ⇒ The sample size of this study will be relatively small, with only female participants included.
- ⇒ The results obtained from this study may not be extrapolated to men with osteoporosis whose physiological factors differ from those of women.

INTRODUCTION

Background

Osteoporosis (OP) is a bone disease characterised by low-bone strength, diagnosed by measuring the combination of bone density and bone quality, that leads to an increased risk of fractures.[1] In China, approximately 36% of adults over 60 years old suffered from this disease in 2016, 49% of whom were women. In addition, based on the number of osteoporotic fractures in 2010 (2.33 million), it was estimated that approximately 5.99 million people in China would be affected by OP by 2050, with corresponding medical expenditures as high as ¥174.5 billion.[2] Thus, OP has become the predominant public health issue affecting the quality of life of the elderly population in China.

As one of the widely used drugs for OP treatment, bisphosphonates (BPs) selectively bind to the mineral bone surface and inhibit the bone resorption activity of osteoclasts. BPs are divided into nitrogen-containing bisphosphonates (N-BPs) and others according to whether the R^2 side chain contains a nitrogen moiety. With a higher capacity of binding to bone minerals, zoledronic acid (ZA) has been recommended at a dose of 5 mg per year for clinical OP treatment.[3]

However, influenza-like symptoms, such as fever, headache, myalgia and other discomfort, caused by the infusion of BPs have been observed clinically. A phenomenon known as the acute phase response (APR) occurs between 24 and 72 hours after administration, especially with the use of ibandronic acid and ZA.[4–8] One prospective study of 411 patients showed that the total incidence rate after the infusion was 47.7%, and an incidence of 55.6% was observed after the first administration.[9] Reid *et al* discovered that there was a greater incidence among non-Japanese Asian patients than among those from Europe and the USA.[10] In addition, an analysis of 2709 patients in a multicentre clinical study in China showed that approximately 28.65% developed a fever within 7 days of the first infusion, and 54.36% had pain symptoms.[11] Thus, patients may worry about the safety of ZA for OP treatment, resulting in compliance reduction and an increased risk of osteoporotic fracture. It has been reported that nearly 30% of patients who suffered APR refused to receive the second infusion in the next year.[12]

Currently, the management of APR is usually focused on symptomatic treatment, such as using non-steroidal anti-inflammatory drugs (NSAIDs) to relieve fever, headache or muscle pain.[7 13] Wark *et al* showed that ibuprofen (500 mg, every 6 hours) or paracetamol (500 mg, every 6 hours) administered for three consecutive days after ZA infusion can reduce the proportion of patients with an elevated body temperature and APR scores.[9] However, symptoms were reduced in only approximately 30% of patients with APR.

According to current research, APR may be related to methyroxylic acid pathway blockade and the γδT-cell activation in circulation. N-BPs act as anti-bone absorption agents by inhibiting farnesyl pyrophosphate synthase (FPPS) activity to inhibit the prenylation of small G protein and reduce osteoclast function. FPPS is a key enzymes, and its inhibition may lead to the accumulation of its substrates isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate, the former of which can activate γδT cells.[14] Moreover, one clinical study concluded that increased levels of interferon (IFN)-γ after ZA infusion in patient plasma is the central actor in the inflammatory reaction that mediates cytokine cascade reactions involving interleukin (IL)-6 and tumour necrosis factor (TNF)-α.[15]

As stated previously, it is not ideal to simply alleviate the symptoms of APR with NSAIDs or other methods. The use of N-BPs would more likely be accepted if APR could be blocked from the source. Statins can inhibit the conversion of 3-hydroxy-3-methylglutaryl coenzyme A to mevalonate, thereby reducing the accumulation of IPP. Therefore, it is hoped that statins can be taken in advance to prevent patients from experiencing APR. In addition, results in vitro have confirmed the feasibility of this theory. Compared with a control group, patients treated with pravastatin (1 mM), simvastatin (100 nM) or fluvastatin (100 nm) 2 hours before exposure to N-BPs exhibited significantly decreased levels of γδT cells and TNF-α in human peripheral blood mononuclear cells (PBMCs).[14]

Therefore, the main purpose of this clinical trial (named ORZAAPR) is to determine the preventive effect of oral pravastatin on APR caused by ZA treatment in Chinese patients.

Objectives

Primary objectives

To determine the preventive effects of oral pravastatin on APR caused by ZA in the treatment of OP.

Secondary objectives

To investigate the mechanism of the effect of pravastatin on APR in vivo.

METHODS AND ANALYSIS

Study design

This will be a single-centre, prospective, placebo-controlled trial with a 1:1 allocation of Chinese

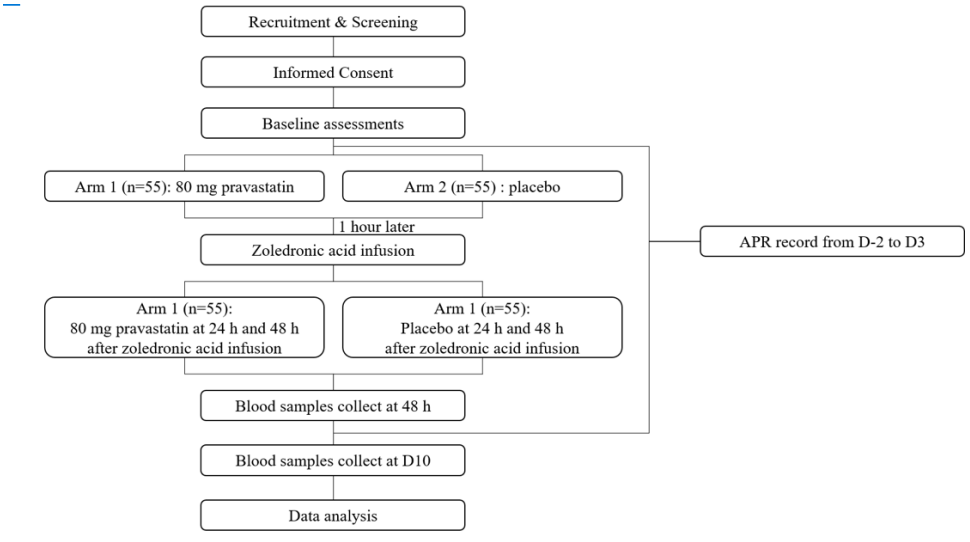

Figure 1 Flow chart of the study. APR, acute phase response.

	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation					
TIMEPOINT	D-20~D-17	D-17	D-2~D0	D0	D1	D2	D3	D14
Visit [V]	V1	V2	V3	V4	V5	V6	V7	V8
ENROLMENT:								
Eligibility screen	X							
Informed consent	X							
Medical history	X							
Demographics, physical exam	X							
BMD (DXA)	X							
Allocation		X						
INTERVENTIONS:								
5 mg zoledronic acid				X				
80 mg pravastatin sodium capsule				X	X	X		
placebo				X	X	X		
ASSESSMENTS:								
Body temperature	←	—	—	—	—	—	—	→
VAS score			←	—	—	—	→	
Complete blood count (CBC)	X			X		X		X
Clinical urine tests	X			X		X		X
Liver function tests	X			X		X		X
Renal function tests	X			X		X		X
25(OH)D3	X			X				
Creatine kinase	X			X		X		X
Serum calcium	X			X		X		
Blood sampling				X		X		X
ECG	X			X		X		X
Combination medication record	←	—	—	—	—	—	—	→
Adverse event				←	—	—	—	→
Acetaminophen usage record				←	—	—	→	
Issue participant daily card			X	X				
Recall participant daily card				X				X

Figure 2 Information on the schedule of screening, enrolment, interventions and assessments. BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry; VAS, Visual Analogue Scale.

postmenopausal women with OP (figure 1 shows the flowchart, and figure 2 shows the detailed schedule). The study protocol was written following the Standard Protocol Items: Recommendations for Interventional Trials guidelines.

Patient and public involvement

Patients or the public will not be involved in the design, conduct, reporting or dissemination plans of this study.

Recruitment

All eligible participants will be invited to participate in the trial. The primary recruitment approach will involve recruiting outpatients from our hospital via an electronic medical record search. Our research staff will identify interested participants, and the principal investigator or clinician will approach the interested participants, provide information on this study and ask them if they would like to participate. If the participants agree, they will provide written informed consent in the presence of a qualified clinician (online supplemental file 1). The clinician will then complete a screening process.

Eligibility criteria

Inclusion criteria

The participants will be eligible for this study if they meet all the following criteria:

1. Postmenopausal women of Chinese Han ethnicity.
2. Bone mineral density (BMD) of −2.5 SD or lower.
3. Willingness to participate in this study and sign the informed consent form (ICF).

Exclusion criteria

The participants will not be eligible for this study if they meet any of the following criteria:

1. Prior treatment with BPs (oral or intravenous).
2. Fever and/or any viral or bacterial infections within 30 days prior to enrolment.
3. Evidence of any cancer or a history of cancer.
4. Hypersensitivity to ZA, other BPs or ZA formulations (excipients).
5. Total serum calcium level <2.13 mmol/L (8.5 mg/dL), free serum calcium level <0.95 mmol/L (3.8 mg/dL) or untreated hypocalcaemia.

6. Pregnant or breastfeeding women.
7. Creatinine clearance <35 mL/min.
8. Patients currently receiving aminoglycosides, diuretics or thalidomide within restrictions related to ZA usage.
9. Hypersensitivity to pravastatin or other excipients in the pravastatin formulation.
10. Patients with severe liver insufficiency, a history of severe liver insufficiency, active liver disease or continuously elevated transaminase, severe renal insufficiency or a history of severe renal insufficiency, as well as those currently receiving fibrates (eg, bezafibrate), immunosuppressive drugs (eg, cyclosporine) or niacin in accordance with restrictions on pravastatin usage.
11. Any physiological or medical condition deemed by the investigator to preclude the participant from participating in this trial.

Withdrawal criteria

Participants will be free to withdraw their informed consent and withdraw from the study. The investigator may withdraw the participant from the study under any of the following conditions:

1. Participants withdraw consent.
2. Serious changes in clinical or laboratory examination results during the study that would be detrimental to the subject's healthcare as observed by the investigator.
3. Participants who exhibit poor compliance, refuse to undergo all required tests and cannot adhere to the study plan.
4. Participants who are unable to attend the follow-up or drop out.
5. Any patient the investigator deems not clinically able to continue to participate in this study.

Written informed consent and assent will be obtained from participants after a full explanation of the study objective, method, benefits and risks by the investigator. Participants have the right to refuse to participate or to withdraw from the study at any time. The clinician will call, invite and make individual appointments for each potential participant. The investigator will inform the participants in a timely manner and reobtain an updated ICF if significant changes in the protocol or ICF have been made. Two copies of the ICF will be documented and signed: one for the researcher and the other for the participant.

Interventions

In clinical trials on whether statin usage inhibits APR, we found that the free drug concentration in circulation under routine clinical doses could not reach the effective concentration used during in vitro experiments to block γδT-cell activation. For pravastatin, the effective concentration of reducing γδT-cell activation and decreasing cytokine levels was 45 ng/mL based on in vitro experiments. In contrast, the total maximum in vivo concentration of pravastatin after a 40 mg dose was 69 ng/mL, with a plasma protein binding (PPB) rate of 50%. Thus, we believe that the free drug concentration of 80 mg pravastatin could have an effect in vivo. However, due to safety considerations, this study mainly focuses on abnormal liver function, rhabdomyolysis and secondary renal function abnormalities in patients. Several clinical trials have shown no significant difference in the occurrence of adverse events (AEs) between the experimental and the control groups when 80 mg pravastatin is continuously administered for at least 6 weeks. Even in subjects with chronic liver disease, there have been no significant increases in alanine transaminase (ALT) levels or other AEs after continuous administration for 2 weeks. In our study, with a 3-day dosing regimen (1 hour before ZA infusion and 24 and 48 hours after infusion), we believe that administration of 80 mg pravastatin will be safe.

Participants will be eligible if all the trial inclusion criteria are met and none of the exclusion criteria apply. Participants allocated at a 1:1 ratio to the two arms will be divided into an experimental group (ZA plus 80 mg pravastatin) or a control group (ZA plus placebo) according to the principle of shared decision-making.

Arm 1 (pravastatin 80 mg): Participants in this group will take pravastatin capsules three times, 1 hour before ZA infusion and 24 and 48 hours after the infusion.

Arm 2 (placebo): Oral administration of placebo capsules at the same time as in Arm 1. In total, three doses will be administered.

Before conducting the study, the investigator should strictly follow the requirements of the protocol and formulate a detailed monitoring plan. In addition to ensuring that participants are fully informed, they need to understand the study process. Thus, the investigator will inform the subjects about the precautions and possible AEs. Participants will complete a self-documentation form and return unused products. The researcher will review the documentation and the number of remaining products. In addition, participants will be contacted regularly via text messages or phone calls to improve adherence.

Any medication used during the trial will be recorded in detail in the corresponding section of the original document. All participants will be asked about their combined medications at each visit.

Outcomes

Primary outcomes

The primary outcome will be the incidence of APR within 72 hours after ZA infusion. Symptoms that appear will be recorded from the second day before ZA infusion to the third day postinfusion, including one or more of the following symptoms: fever (based on recorded body temperature), muscle pain, headache and joint pain (based on the recorded Visual Analogue Scale (VAS) score). Fever will be defined as the presence of an axillary temperature higher than 38°C (measured by the subject using a thermometer). Pain will be defined as an increase of more than one point in the VAS score (the subject will

perform and record VAS scores for muscle pain, headache and joint pain by themselves).

Secondary outcomes

The secondary outcome will be the occurrence time and severity of APR; the frequency and amount of acetaminophen usage within 72 hours after ZA infusion; changes in the white blood cells (WBC) count, the expression levels of C-reactive protein (CRP), IFN-γ and IL-6, and γδT-cell activation within 48 hours after infusion; and other AEs that occur within 14 days post-trial.

Participant timeline

The timeline of this study is shown in figure 1. Figure 2 depicts the detailed screening, enrolment, intervention, assessments, visits and data collection schedule for the participants.

To assess the incidence of APR, a recording will be made every day at 8:00 for 2 days prior to ZA administration and, every 4 hours thereafter, with the final recording obtained at 20:00. On the day of ZA administration, the subject's status will be immediately recorded after infusion (before 9:00) and then recorded every 3 hours thereafter, with the last record at 21:00. Furthermore, a record will be made at 9:00 and continued at 3 hours intervals until 21:00 on the first, second and third days after the infusion. In addition, the subjects must record the corresponding time and symptoms if the fever (as mentioned above) or pain occurs at other times.

For acetaminophen usage comparison between the pravastatin arm and placebo arm, records will be made within 72 hours after ZA infusion, including time, frequency and dosage. Venous blood will be collected at baseline and 48 hours post-administration to monitor and compare blood parameters, such as WBC, CPR, IFN-γ and IL-6. At the same time, 5 mL blood samples will be collected and separated with lymphocyte separation fluid to obtain PBMCs for the measurement of CD3, TCR-Vδ2 and TCR-Vγ9 expression levels on the surface of γδT cells.

Furthermore, serum transaminase, creatine kinase, serum calcium and creatinine clearance levels will be detected at the time of predosing and 48 hand 14 days post-trial to monitor the AEs related to pravastatin.

Sample size

This study adopts a real-world prospective non-randomised controlled clinical trial design method. Considering that there may be 4–6 factors associated with baseline variables (including intervention), the number of events per variable was selected to be 10.[16–18] After the adjustment of five factors, such as age, body weight, height, BMD and intervention, approximately (10×5=) 50 participants in each arm were deemed necessary. In addition, considering a 10% loss to follow-up, 55 participants will be needed for each arm for a total of 110 participants.

Blinding

Because this is a real-world clinical trial and participant allocation is based on the principle of shared decisions, the blinding method is not applicable.

Data collection and management

The assessments will be conducted in the Outpatient Department of Peking University Third Hospital, and the intervention process will be carried out in the Peking University Third Hospital Drug Clinical Trial Center. All the assessments will be carried out by trained and experienced clinicians. Participants will be seen at the screening period (visit 1), at the baseline assessment (visit 2), on day-2 ~day-1 (visit 3) and on days 0, 1, 2, 3 and 9 (figure 2). Once baseline assessments and shared decisions are completed, participants will be allocated to one of the two arms. Primary and secondary outcomes assessments will be repeated during the follow-up visits.

Before each visit, a phone call will be made to all participants to remind them of the visit date, with the date and time recorded. If any participant expresses the desire to withdraw from the study, she will be required to complete the 'end of study' visit. Data collected up to the withdrawal time will remain in the database and be included in the data analysis.

The original data will be traceable, real time, easy to read and accurate. All the research data collected from this study will be protected and properly maintained by the principal investigators. If data need to be corrected, the reason, the person who made the amendment and the date will be indicated, and the amendment traces will be kept. All electronic data will be stored and backed up on the local servers with a safe password, and inquirers will have to obtain permission prior to making amendments. Quality control (QC) and quality assurance systems will be established related to this study, with corresponding duties performed in strict agreement with the requirements of this study protocol and relevant laws and regulations. Members of the clinical research team will receive training on the trial protocol at the beginning of the study. Investigators should do the corresponding work carefully in accordance with the requirements of Standard Operating Procedure (SOP) and trial protocol, and record it in a timely, completely and standardised manner.

Potential and enrolled participants' personal information and outcome assessments will be kept confidential. The names and addresses of participants will not be recorded in the database, and any material information containing the participants' names will not be stored in any file. Participant identity will be kept strictly confidential by the research investigator.

Blood samples will be collected from all participants at the clinical centre. Then, plasma and serum will be analysed on the same day or stored at −80°C until further analysis according to the study objectives. Validated method will be used for the sample analysis. The detailed process and results will be provided in the method validation report and sample analysis report.

Statistical methods

Statistical analysis data sets

There will be three statistical analysis data sets, including the effective analysis population (EAP), preprotocol population (PP) and safety sets (SS). All participants enrolled in this study will be included in the intent-to-treat (ITT) analysis set. Participant information, including demographics and baseline characteristics, will be based on ITT. All participants with demographic data, baseline data and at least one APR datum will be included in the EAP. The efficacy analyses will be based on the EAP. Participants with no significant protocol deviation in the EAP will be included in the PP, which will be used in the primary outcome analyses. In addition, all participants who received treatment will be included in the SS, which will be used in the analysis of vital signs, laboratory tests, ECG and AEs.

Statistical methods for primary and secondary outcomes

Categorical data will be summarised according to frequency and percentage, and continuous data will be summarised using the mean, SD, median, maximum and minimum.

For the APR incidence analysis, differences in the APR score, body temperature and VAS score between the value at each time point and baseline will be calculated. Then, a t-test will be performed to compare whether differences exist in the mean values at each time between the two groups. In addition, differences between the two groups in the number of participants experiencing fever and pain will be evaluated using the χ^2 test at each time point.

Investigations will be conducted to assess changes in WBC, levels of CRP, IFN-γ and IL-6, and γδT-cell activation within 48 hours after ZA infusion in the two groups, including a comparison of the number of subjects who showed significant changes from baseline using the χ^2 tests and an assessment of the difference in mean values with t-tests. In addition, the number of participants who take acetaminophen and the dose regimen in the two groups will be compared using the χ^2 and t-tests, respectively.

Statistical analyses will be performed using SAS V.9.4 (SAS Institute North Carolina, USA). The demographic and baseline characteristics of the study participants by treatment arm will be summarised. The APR and safety analysis data set will include all participants enrolled in the trial. The frequency of each AE will be reported for participants stratified by intervention arm.

The statistical analysis unit will conduct statistical work according to the statistical analysis plan and SOP, and review the data process. The relevant documents for data processing will be locked in time after approval, and the statistical analysis report will be written.

Adverse event reporting and harms

This clinical trial will not consider additional steering committees; the principal investigators will direct the study. All researchers will discuss the study progress regularly. If a severe AE occurs, whether it is the first report or a follow-up report, the investigator will immediately fill in the *Severe Adverse Event Report Form* of the National Medical Products Administration (China). In addition, AEs will be reported to the ethics committee, the Beijing Municipal Medical Products Administration and the Bureau of Medical Administration of National Health Commission of the People's Republic of China.

AEs will be collected by assessing signs, symptoms or laboratory examinations. In addition, participants will be asked about AEs regularly after the beginning of the study, regardless of whether the event is related to the study drug. All AEs after the participants sign the ICF until the last follow-up visit will be fully recorded in the original medical record. Notably, AEs may be drug-related (such as fever) or OP-related (such as a fracture). The recorded content will include a description of the AE, start and end times, duration, severity, relationship with the study drug, measures and outcomes. All serious AEs (SAEs) will be recorded in the SAEs report form. SAEs should be reported in a timely manner according to the principle of Good Clinical Practice when SAE information is temporarily incomplete, with a subsequent supplement report form after more information is obtained.

DISCUSSION

APR usually occurs within 3 days after ZA administration. Therapeutic options mainly involve symptomatic treatment with NSAIDs. However, the efficacy is unsatisfactory. Moreover, people with OP are generally elderly individuals with insufficient liver and kidney function. Thus, AEs related to NSAIDs are more likely to occur due to the correspondingly prolonged half-life. Therefore, how to prevent the occurrence of APR has attracted more attention in recent years.

According to the studied mechanism of ZA and statins, it is hoped that statins can be taken in advance to reduce APR. In vivo experiments have demonstrated that this hypothesis is feasible.[14] However, all clinical trials related to statins used to prevent APR yielded negative results.[4 5 7 19 20] This phenomenon may be because the free statin concentration could not reach the effective concentration required to block the activation of γδT cells in vitro. Compared with fluvastatin (PPB rate ≥98%), pravastatin is more likely to reach the effective concentration in vivo due to its reduced PPB rate in humans (50%). Considering that the peak concentration of pravastatin is 69 ng/mL after administration of 40 mg, the free concentration is 34.5 ng/mL.[21] Since the plasma concentration is proportional to the oral dose, it can be estimated that the free concentration of pravastatin in vivo is above 69 ng/mL after the administration of 80 mg. According to previous results from in vitro experiments, with an effective concentration of pravastatin of 45 ng/mL, we believe that the administration of 80 mg can reduce the γδT-cell activation to block APR.[14]

The FDA has approved the usage of pravastatin at a maximum daily dose of 80 mg, while the current maximum daily dose in China is 40 mg. However, several clinical trials have demonstrated that there were no significant differences in ALT levels or other AEs between the 80 mg and control groups or the 40 mg group, regardless of whether chronic liver disease was present.[22–26] Thus, this clinical trial will be safe for the 3 day administration period, considering that the enrolled subjects will not have abnormal liver or kidney function and that the participants will be fully informed of the precautions.

Nevertheless, the study also has some limitations. First, since this clinical trial is a real-world study, there are limitations in data collection and quality and data bias. Strict QC is required to ensure the integrity of the data as much as possible. Second, the sample size is relatively small and only female participants will be included. Thus, the results cannot be extrapolated to men with OP.

However, despite these limitations, our research may provide certain clinical data to support a more reasonable clinical use of pravastatin to prevent AEs associated with ZA infusion. This exploratory clinical trial could aid the development of new recommendations on the clinical use of pravastatin in conjunction with ZA for OP treatment.

ETHICS AND DISSEMINATION

Ethical approval has been obtained from Peking University Third Hospital Medical Science Research Ethics Committee. There are no interim analyses or extra auditing planned for this study beyond the continuous monitoring by the investigators. However, all protocol amendments will be submitted to the ethical committees. Any modifications will be updated at ClinicalTrails.gov by the principal investigator. The study's results will be published in scientific peer-reviewed journals with open access.

Acknowledgements We would like to acknowledge the Ethics Committee and Drug Clinical Trial Center of Peking University Third Hospital for supporting this research project.

Contributors CS is the chief investigator, contributed to the study design and led the development of the proposal and protocol. DL is the co-investigator and contributed to the study and protocol development. HL contributed to the protocol revision process. GH and RL made contributions to the study design. QL made contributions to the study design and drafted the original manuscript. Outcome assessment and data analysis will be performed by QL. LT made contributions to the statistical analyses and sample size calculation. DF, GD and MZ will conduct the clinical trial. All authors have read and approved the final manuscript.

Funding This work was supported by the National Key Research and Development Program of China (2020YFC2009004), a grant from the Beijing Medical and Health Foundation (B20083FN), the PKU-Baidu Fund (2020BD014) and the National Natural Science Foundation of China (81874010).

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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