Pharmacokinetics of a Novel Anagrelide Extended-Release Formulation in Healthy Subjects: Food Intake and Comparison With a Reference Product

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Abstract
Anagrelide is an established therapy for essential thrombocythemia. Common adverse effects have been linked to peak plasma concentrations of anagrelide and its 3OH metabolite. Our study was performed to investigate the pharmacokinetics (PK) of a novel anagrelide extended-release (AER) formulation and its active metabolites. Thirty healthy volunteers were randomized to receive either 2 mg AER (under fasting and fed conditions) or 2 mg commercially available reference product (CARP) in an open-label, 3-way crossover trial with washout periods of 6 days. Plasma concentrations of anagrelide and its active metabolites were assessed by tandem mass spectrometry. The PK differed significantly between all treatment periods. Bioavailability of AER was 55% of the CARP under fasting conditions and 60% under fed conditions. Cmax, AUCt, and AUC∞ were significantly higher and Tmax and T1/2 were significantly shorter after the CARP compared with AER. Food had a significant impact on the PK of AER, increasing the Cmax and AUCt while reducing the T1/2, plateau, and mean residence time. Both formulations were well tolerated, with a trend toward more frequently occurring adverse events after the CARP. The PK of AER and the CARP differed significantly in all parameters. Food enhanced the bioavailability of AER.

Keywords
anagrelide, bioavailability, drug delivery, extended release, healthy volunteers, platelets, pharmacokinetics

Anagrelide (imidazo-[2,1-b] quinazolin-2[3H]-one, 6,7-dichloro-1,5-dihydro, monohydro-chloride) is an established platelet-reducing treatment option for patients with essential thrombocythemia or thrombocythemia associated with myeloproliferative disorders who meet the defined risk criteria.¹ These risk criteria include age > 60 years, platelet count > 1000 × 10⁹/L, or history of thrombohemorrhagic events.² It was originally developed to inhibit platelet aggregation by inhibiting cyclic adenosine monophosphate phosphodiesterase 3 enzyme activity.³ However, at doses lower than the amount required to inhibit platelet aggregation, it reduces platelet counts.⁴,⁵ Anagrelide specifically blocks the differentiation and proliferation of megakaryocytes.⁶ Treatment with anagrelide reduced megakaryocyte number and volume and normalized ploidy in patients with essential thrombocythemia. Anagrelide did not change platelet survival in these patients.⁷ In vitro experiments demonstrated that anagrelide alters the transcriptional control of megakaryocyte gene expression.⁸,⁹

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Anagrelide Extended-Release Pharmacokinetics and Effects of Food EudraCT: 2008-005689-30
The efficacy of anagrelide was investigated in 2 randomized clinical trials,10,11 and response rates of approximately 80% were reported, depending on definitions and underlying diseases. The most common adverse effects of the drug included cardiovascular (palpitations, tachycardia, hypotension, dizziness, syncope, dyspnea, edema, congestive heart failure), headache, diarrhea, nausea, fatigue, and anemia.12

The risk of transformation to myelodysplastic syndromes or acute leukemia is not increased in anagrelide-treated patients.1,13,14

Immediate-release anagrelide formulations are well absorbed, with an approximate bioavailability of 75%, metabolized by cytochrome P450 1A2 to the active metabolite 3-hydroxy-anagrelide or 2-amino-5,6-dichloro-3,4-dihydroquinazolin (RL603) or inactive metabolites. The active metabolite is equally eliminated by conversion to inactive metabolites or by renal clearance.15 Food intake significantly delays absorption, decreases maximum concentrations but increases total drug exposure (area under the concentration curve, AUC) of anagrelide and its active metabolite.16

The rationale for developing an extended-release formulation of anagrelide is based on the results of a study investigating the pharmacokinetics (PK), pharmacodynamics, and adverse events of 2 marketed anagrelide formulations. The formulation with lower Cmax, delayed Tmax, and lower AUC was associated with fewer adverse events, as might be expected, however surprisingly without diminished clinical efficacy regarding platelet counts.17 Consequently, an extended-release formulation of anagrelide (AER) was developed with the goal of delivering a larger amount of drug (2 mg per dose) with a Cmax lower than that from immediate-release doses of 1 mg or preferably 0.5 mg, allowing for a dosing frequency down to once daily while keeping clinical efficacy. Such an AER formulation was achieved by an ER tablet based on polyacrylic acid (Carbomer, Carbopol 971) as the retarding matrix polymer.

The objective of our trial was to evaluate the pharmacokinetic profile of an AER formulation in comparison with the reference formulation (Xagrid®) and to test the effect of food intake on the pharmacokinetics of this novel formulation.

Methods

This trial was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. The Independent Ethics Committee of the Center for Pharmacology and Analysis, Pilsen, Czech Republic, approved the study prior to initiation. It was performed at the Center for Pharmacology and Analysis, Pilsen, Czech Republic. The trial was registered at the EudraCT database with the identifier 2008-005689-30

The design of a 3-way crossover study was chosen in accordance with the Committee for Proprietary Medicinal Products (CPMP; now the Committee for Medicinal Products for Human Use) Note for Guidance on modified-release oral and transdermal dosage forms.18 As the pharmacodynamic activity of anagrelide is related to the parent drug and its active metabolite, 3-hydroxyanagrelide, in compliance with the CPMP Note for Guidance on the Investigation of Bioavailability and Bioequivalence, the comparison of bioavailability was based on the plasma concentration of the parent drug and 3-hydroxyanagrelide.19 The PK of the other active metabolite, RL603, was investigated to further characterize distinct formulations.

Three different study periods were defined (see also Figure 1). In period A subjects in a fasting state were treated with a single dose of 2-mg AER tablets (AOP Orphan Pharmaceuticals AG, Austria). In period B subjects under fed conditions (after a high-fat breakfast containing 2 slices of toast with butter, 2 fried eggs, 2 strips of bacon, hash brown potatoes, and 240 mL of whole milk) were treated with a single dose of 2-mg AER tablets (AOP Orphan Pharmaceuticals AG, Austria). In study period C subjects were treated with
the commercially available reference product (CARP), 4 tablets of 0.5-mg anagrelide capsules under fasting conditions (Xagrid®, Shire Pharmaceutical Contracts Ltd., UK). Six different sequences of periods were generated, and healthy volunteers were randomly assigned to a sequence (ABC, ACR, BAC, BCA, CAB, CBA) using a computer random number generator. Thus, each subject was planned to complete each period. The total exposure of each subject was 6 mg of anagrelide. Standardized meals were provided for the fasting and the fed periods. In the fasting periods meals were served 11 hours before and 4, 6, 9, and 13 hours after drug intake. In the fed period, meals were served 11 and 0.5 hours before and 4, 6, 9, and 13 hours after drug intake. All subjects received 200 mL of water 1 hour before, at the time of, and 2 hours after study drug intake. After 4 hours subjects were allowed to drink water or tea ad libitum. Subjects were confined to the study ward 12 hours before until 24 hours after study drug intake. Caffeine (or other xanthines), grapefruit, and alcohol intake were forbidden 72 hours (48 hours regarding alcohol) before until 24 hours after each dosing.

Blinding of subjects or study personnel was not performed, because plasma concentrations are robust and objective measurements and blinding would be difficult because of the investigation of food effects. Laboratory personnel were blinded.

**Blood Sampling**

Blood samples were drawn predose and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, and 24 hours after study drug intake in the AER study periods and at predose and 0.33, 0.67, 1, 1.33, 1.67, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, and 24 hours after intake of the CARP. The total blood volume per subject was 456 mL. Blood samples were drawn into tubes containing K$_2$-ethylenediaminetetraacetic acid (EDTA), gently shaken, and centrifuged at 2300g for 6 minutes and within 5 minutes of sampling. After centrifugation the supernatant plasma was transferred into polypropylene tubes and stored at -75°C for further analysis.

**Pharmacokinetics**

Plasma concentrations of anagrelide, 3-hydroxyanagrelide, and RL603 were determined using high-pressure liquid chromatography–tandem mass spectrometry (HPLC-MS/MS). Blood samples were collected in EDTA tubes (Greiner, Kremmünster, Austria), Plasma aliquots were frozen at -70°C until analysis and analyzed within 8 weeks of sampling. The internal standard was D4-anagrelide (source: Technical University Vienna), which was dissolved in dimethyl sulfoxide (DMSO; HPLC grade, Fluka, St. Gallen, Switzerland) and diluted with acetonitrile (gradient grade, Merck, Darmstadt, Germany) for the internal standard solution. Anagrelide (Rolf Sachse GmbH, Berlin, Germany) and its metabolites (source: Technical University of Vienna, Vienna, Austria) were dissolved in DMSO/MeOH (Merck, Darmstadt, Germany), and a calibration curve was constructed with 8 concentrations ranging from 0.05 to 25 ng/mL. The applied system consisted of an HPLC pump PE Series 200 Micro Pump (Perkin Elmer, Waltham, Massachusetts), a sample injector PE Series 200 Auto-Sampler (Perkin Elmer), a column oven HP1100 (Agilent, Ratingen, Germany) and Jetstream 2 Plus (W.O. Electronics, Vienna, Austria), a Mass Spectrometer API 5000 (PE Sciex, Concord, Ontario, Canada), and an HPLC column Synergy Polar RP, 4 × 2 mm and 100 × 2 mm ID (Phenomenex, Torrance, California). For analysis, samples were thawed in a 20°C–25°C water bath, 0.1 mL was transferred in vials, and 0.15 mL of internal standard solution was added for protein precipitation. After centrifugation at 4000 g for 2 minutes, 200 μL was transferred into new vials. In a rotation vacuum centrifuge, the organic part of the liquid phase evaporated (20 minutes, of which 15 minutes was at 45°C; pressure, 100 mbar; security pressure, 110 mbar). Thereafter, 20 μL was injected into the HPLC-MS/MS system using electrospray ionization in positive mode. The transitions 256 to 199 m/z for anagrelide, 216 to 199 m/z for 3-OH anagrelide, 272 to 199 m/z for RL603, and 260 to 201 m/z for D4 anagrelide were monitored in multiple reaction monitoring mode. Mobile phase A consisted of 50 mM formic acid in water and mobile phase B of 50 mM formic acid in MeOH (all Merck). Chromatographic separations were achieved using a Synergy Polar RP column (4 × 2 mm and 100 × 2 mm ID, Phenomenex) at a temperature of 50°C. Total run time per sample was 6 minutes at a flow rate of 0.8 mL/min, using a gradient (0–4.5 minutes, 25%–85% B) followed by isocratic elution (4.5–5.2 minutes, 95% B) and equilibration (5.2–6 minutes, 25% B). The retention times were approximately 2.7 minutes for 3-OH anagrelide, 3.5 minutes for RL603, and 3.9 minutes for anagrelide and D4-anagrelide. The autosampler was cooled to 5°C. Data processing, statistics and calculations were done using the software Analyst 1.4.2. (Applied Biosystems, Foster City, California). The interbatch coefficient of variation ranged between 8.7% and 10.5% for anagrelide and its metabolites. All laboratory analyses were conducted in accordance with the Good Laboratory Practice guideline.20

Noncompartmental methods were used for PK analysis. Calculated parameters include maximum plasma concentration (C$_{max}$), time of maximum plasma concentration (T$_{max}$), area under the plasma concentration–time curve from 0 hours to the last measurable concentration estimate (AUC$_{t}$) or extrapolated
until infinity ($AUC_{\infty}$), and half-life of drug elimination during the terminal phase ($t_{1/2}$).

**Safety**

For safety assessment vital signs, including heart rate, blood pressure, and body temperature, were measured before dosing and 1, 3, 5, 8, 12, and 24 hours thereafter. Safety laboratory diagnostics including blood counts, blood chemistry, and urinalysis were performed during the screening and follow-up visits. Another pregnancy test was performed during the follow-up visit. All occurring adverse events were documented throughout the study.

**Statistical Analysis**

The statistical analysis was carried out in full agreement with the CPMP Note for Guidance on the Investigation of Bioavailability and Bioequivalence\(^{19}\) and CPMP Points to Consider on Multiplicity Issues in Clinical Trials.\(^{21}\) In this trial the variance balanced “Williams design” for comparing 3 treatments was applied, which consists of 6 sequences and 3 periods (see Figure 1).

Coefficients of variation (CV) for the PK parameters of anagrelide have not been reported previously. The sample size was calculated assuming an intrasubject CV of 20% for the more variable parameter $C_{\text{max}}$. Twenty-six subjects would be needed to show bioequivalence with a power of 90% (bioequivalence range, 0.80–1.25; bioequivalence test/reference ratio between 0.95 and 1.05). The sample size was increased to 30 subjects considering the study design, which involved 6 sequences, and to account for potential dropouts.

Logarithmically transformed $AUC_{t}$, $AUC_{\infty}$, and $C_{\text{max}}$ and untransformed $T_{\text{max}}$ of anagrelide and the measured metabolites were evaluated by analysis (ANOVA) of variance. Bioavailability was assessed using ANOVA for the Williams design with 3 formulations. Standard conditions for bioequivalence evaluation were applied, including ANOVA, consequent analysis of estimated marginal means for treatments, and parametric 90% confidence intervals based on estimated marginal means for treatment ratios A/C and B/A. These procedures are equal to Schuirmann’s Two One-Sided Tests procedure for logarithmically transformed data. Pairwise comparisons based on the estimated marginal means display mean difference (point estimate) for factor levels (drug form). The Wilcoxon rank sum test was applied for the nonparametrical $T_{\text{max}}$ bioequivalence testing after reduction to the standard model.

Safety data are presented by descriptive statistics.

All calculations were performed using commercially available statistical software (Microsoft Excel, IBM SPSS).

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**Figure 2.** Plasma concentrations of anagrelide (upper) and 3-hydroxyanagrelide (lower) after intake of $4 \times 0.5$-mg commercially available reference product (CARP) tablets in fasting condition and 2 mg of anagrelide extended release (AER) in fed or fasting conditions are presented. Means ± standard deviations are presented ($n = 28$).

**Results**

Thirty healthy subjects with a median age of 30 years (range, 18–47 years) and a median body mass index of 23.6 kg/m$^2$ (range, 19.6–26.9 kg/m$^2$) were included in the study. Two healthy volunteers had to be excluded during the second washout phase because of adverse events as described below. Thus, 28 subjects, 14 women and 14 men, completed the study.

**Plasma Concentrations**

Arithmetic mean plasma concentrations of anagrelide and 3-hydroxyanagrelide for the 2 formulations and for AER under fasted and fed conditions are shown in Figure 2. As expected, the CARP was more rapidly absorbed than the AER. Interestingly, a plasma concentration curve with 2 peaks could be observed for the test substance under fed conditions. Individual plasma curves are presented in Figure 3. The almost uniform
absorption pattern of the CARP and the reduced but sustained absorption pattern of the AER formulations and the effect of food on absorption were well recognizable.

**Pharmacokinetics**

Pharmacokinetic data of anagrelide, 3-hydroxyanagrelide, and RL603 are presented in Tables 1–3. The complete set of PK parameters of anagrelide was obtained in 15 subjects in the fasting-condition period, in 26 subjects in the fed-condition period, and in all 28 healthy volunteers of the reference period. In some subjects $T_{1/2}$ and $AUC_{\infty}$ could not be calculated because of concentration irregularities (i.e., plateau phases or increase toward the end; Figure 3).

The full set of 3-hydroxyanagrelide was obtained in 10 subjects under fasting conditions, in 26 subjects under fed conditions, and in 28 subjects of the reference period. Regarding RL603, full PK data were available for 7 healthy volunteers under fasting conditions, 23 under fed conditions, and all 28 subjects taking the CARP. Again, reliable estimations were not feasible in all healthy volunteers because of plasma concentration irregularities.

**Bioavailability and Comparison of PK**

PK parameters differed significantly between AER and the CARP ($P < .001$ for all tests) and were not within the predefined margins of 0.8–1.25. However, because of high intrasubject variability with coefficient of variation > 20% for all parameters, the power of statistical testing did not reach 80%.

Food had an impact on almost all PK parameters under evaluation. The calculated point estimates of PK parameters of anagrelide and both active metabolites are presented in Table 4.

Relative bioavailability of anagrelide, calculated as the ratio of $AUC_{\infty}$ between AER and the CARP, was 60.5% under fed conditions and 55% under fasting conditions.

**Safety**

Two of 30 subjects withdrew from the study during washout phase 2 (after the second dosing) because of adverse events (AEs). One withdrew because of an elevation of liver function parameters. The second subject refused to continue because of experiencing AEs (headaches and dizziness). The adverse event profile and tolerability of the substances in general were in good accordance with the summary of product characteristics. Forty-six AEs occurred throughout the trial, among them with descending frequency: headache, palpitations, dizziness, tachycardia, chills, vomiting, extrasystoles, and hematoma, as well as alterations of laboratory parameters (elevation of alanine aminotransferase, aspartate aminotransferase, and bilirubin). As shown in Table 5, palpitations, extrasystoles, and tachycardia mainly occurred after intake of the CARP. No serious AEs and no unexpected adverse drug reaction or clinically significant adverse event occurred. There was a trend toward an increasing number of AEs in the following ascending order: AER fasting state, AER fed state, CARP. There were approximately
twice as many AEs in the CARP period as in the AR fasting-state period.

Discussion
This study investigated the PK of an anagrelide extended-release formulation and compared its PK with a marketed reference product. The study revealed marked differences. Furthermore, a pronounced effect of food on the PK of the AER was detected.

The pharmacokinetics of the CARP were published previously. This allows external validation of our results and the applied assay. In a phase 1 study in healthy volunteers the $C_{\text{max}}$ after intake of 1 mg anagrelide was 5.08 ng/mL, and an $AUC_{\infty}$ of 13.5 ng·h/mL was reported, which, when assuming dose-proportional PK, corresponds well with the current trial ($C_{\text{max}}$, 10.7 ng/mL; $AUC_{\infty}$, 31.3 ng·h/mL). Another trial measured a $C_{\text{max}}$ of approximately 10 ng/mL after intake of 2 mg of anagrelide, with peak concentrations occurring after 1 hour. Similarly $T_{\text{max}}$ and $T_{\frac{1}{2}}$ also agreed well with published data.

The $C_{\text{max}}$ and the $AUC_{\infty}$ of the active metabolites were highest for the CARP. Food intake increased levels of anagrelide and its metabolites compared with intake of AER under fasting conditions. Okamoto et al reported a $C_{\text{max}}$ of 2-amino-5,6-dichloro-3,4-dihydroquinazolin of 1.4 ng/mL after treatment with...
Table 4. Point Estimates of Anagrelide

| Parameter       | AER vs CARP | AER Fed vs Fasting | AER vs CARP | AER Fed vs Fasting | AER vs CARP | AER Fed vs Fasting |
|-----------------|-------------|--------------------|-------------|--------------------|-------------|--------------------|
| AUCt (μg·h/L)   | 0.32 (0.28–0.36) | 1.73 (1.53–1.95) | 0.28 (0.26–0.32) | 1.79 (1.63–1.95) | 0.31 (0.27–0.35) | 1.87 (1.65–2.12) |
| Cmax (μg/L)     | 0.08 (0.06–0.09) | 3.2 (2.7–3.8) | 0.09 | 3 | 0.19 | 2.37 |
| Tmax (h)        | 8.0 (5.8–10.1) | 1.3 (3.4–0.9) | 10.3 | −3.9 (−6.2 to −1.7) | 13.0 | −6.1 (−7.9) |

Point estimates (90% confidence intervals) of PK parameter are presented. Except for the MRT of the metabolite RL603, no PK parameter lies within the predefined margins (0.8–1.25). Thus, PK parameters are significantly different between AER and the commercially available reference product (CARP), and food intake has a significant impact on the PK of AER (P < .001 for all tests); n = 28.

Table 5. Adverse Events in Different Treatment Periods

| Symptom or Laboratory Value | AER Fasting | AER Fed | CARP | Total Incidence |
|-----------------------------|-------------|---------|------|----------------|
| Headache                    | 4           | 8       | 9    | 21             |
| Dizziness                   | 1           | 3       | 5    | 9              |
| Palpitations                | 0           | 1       | 8    | 9              |
| Vomiting                    | 0           | 0       | 1    | 1              |
| Extrasystoles               | 0           | 0       | 1    | 1              |
| Tachycardia                 | 0           | 0       | 1    | 1              |
| Chills                      | 0           | 0       | 1    | 1              |
| Hematoma                    | 1           | 0       | 0    | 1              |
| ALT elevation               | 0           | 1       | 0    | 1              |
| ALT, AST, and bilirubin elevation | 0   | 0       | 1    | 1              |
| Total                       | 6           | 13      | 27   | 46             |

n = 30. AER, anagrelide extended release; CARP, commercially available reference product.

0.5 mg of anagrelide, which is in agreement with our results.22 The Cmax of 3-hydroxyanagrelide in this trial was much higher (19 ng/mL) compared with an approximate Cmax of 8 ng/mL in another trial investigating the PK after 2 mg of anagrelide.17 However, assuming dose-proportional PK, it is very comparable to the reported PK of a trial using 1 mg of anagrelide as treatment.16

The main goal of this trial was to determine the bioavailability of AER and the effects of food ingestion on the PK. The bioavailability, calculated as the ratio of the geometric means of the AUC∞ of the test substance under fed conditions, was approximately 60% of CARP and 55% under fasting conditions. Of note, the difference in the AUC1, which is the AUC of the first 24 hours, was substantial. The extremely slow absorption of the AER under fasting conditions increased the T1/2 to 21 hours, which could consequently lead to an overestimation of the AUC∞.

It was reported previously that food increases the AUC of the CARP by approximately 15%, the Tmax from 1.5 to 4 hours, but reduced the Cmax from 5.1 to 4.5 ng/mL after intake of 1 mg anagrelide.16 Thus, food effects appear formulation specific. The European Medicines Agency (EMA) note for guidance on modified-release oral and transdermal forms suggests to investigate potential influences of food on the bioavailability of modified-release formulations. The design of this study was based on the suggestions presented in Appendix 1 of this document.18

Some conclusions may be drawn from the plasma concentration–time curve. First, a biphasic absorption pattern can be recognized in the plasma concentration–time curves (Figure 2). Second, food, in this study a high-fat breakfast consisting of 2 eggs, 2 strips of bacon, hash brown potatoes, and 240 mL of whole milk, enhances the absorption of the study drug significantly. Furthermore the flat plasma concentration curve
suggests very slow absorption, which may be caused by slow release of the drug from the formulation, probably by slow disintegration of the extended-release tablet. This disintegration may be facilitated by meals, which may enhance bioavailability. Similarly to our data, an increase of $C_{\text{max}}$ and $T_{\text{max}}$ under fed conditions is frequently observed in extended-release formulations.\(^{23-26}\) It was demonstrated that the intragastric location and the time of gastric emptying, both affected by food intake, influence the PK of extended-release tablets.\(^{26}\) Moreover, the pharmaceutical properties of extended-release formulations may be responsible for interactions with food. The PK of 2 extended-release nifedipine formulations with distinct pharmaceutical properties was investigated. However, only one formulation showed significant interactions with food intake.\(^{27,28}\)

In the note for guidance published by the CPMP, it is suggested that in case of a significant effect of food on the PK, a suggestion for alternative dosing should be made.\(^{18}\) However, as the dose of anagrelide is titrated, this does not seem necessary. On the other hand, it seems plausible that patients should implement a daily routine to reduce the variability in absorption of the AER, that is, intake of the AER after breakfast and after dinner.

Safety was acceptable in all 3 periods and with regard to both study treatments. There was a trend toward less frequent AEs for the test substance compared to the CARP. It was reported previously that plasma concentrations of anagrelide (specifically the active 3-hydroxy metabolite) may be accountable for occurring AEs and that lower concentrations may offer an improved safety profile without necessarily being less effective.\(^{17}\) However, the trial was not powered to determine the effects of anagrelide extended release on AE frequency. If high peak concentrations are responsible for the occurrence of AEs, the flat plasma concentration curve of the AER may be attractive for further investigation.

There are some limitations to our study. First, not all data were available for the calculation of PK parameters, and there was a large intrasubject CV%. As shown in Figure 2, under fasting conditions, absorption of AER was highly variable, and no common absorption patterns were recognizable. This caused problems in the calculation of some PK parameters. For instance, in some subjects increasing plasma concentrations or plateaus between 12 and 24 hours were measurable. Thus, calculation of $T_{1/2}$ or $k_e$, and consequently $AUC_{\infty}$ was not feasible in all subjects. This reduced the sample size in the PK calculations under fasting conditions. Under fed conditions, this problem was minor. After treatment with CARP, because of the immediate-release formulation, absorption patterns were uniform, and these problems did not occur at all. The observation period for the group taking AER under fasting condition was too short because at the 24-hour point, plasma concentrations of anagrelide or its metabolites were frequently measurable. The performed sample-size calculation suggested that 26 subjects were needed to achieve a power of 90% in statistical testing. We did not reach a power of 80% in statistical testing because of the large intrasubject CV. Moreover, the chosen study design did not allow investigation of the pharmacodynamic effects of the study drug. Petrides et al reported that although lower $C_{\text{max}}$ and $AUC_{\infty}$ were measured, platelet numbers did not change significantly.\(^{17}\) As the dose for each patient needs to be found by titration of the drug, it is possible that equal efficacy but lower peak concentrations may be achieved by using AER. Clinical trials addressing this question are currently being performed (NCT01230775).

Conclusions
In conclusion, we report reduced bioavailability for the anagrelide extended-release formulation, which is enhanced by prior intake of a high-fat breakfast, compared with the CARP.

Declaration of Conflicting Interests
P.E.P. receives speaker and consultation honoraria from AOP. R.W. and C.K. are employees at AOP. The study was funded by AOP Orphan.

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Author Contributions
P.E.P. and R.W. designed the study. All authors were involved in statistical analysis and/or interpretation of the results. C.S. and B.J. drafted the article. All authors critically reviewed and approved the manuscript.

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