

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Lachmann HJ, Kone-Paut I, Kuemmerle-Deschner JB, et al. Use of canakinumab in the cryopyrin-associated periodic syndrome. *N Engl J Med* 2009;360:2416-25.

A Randomized Trial of Canakinumab in the Cryopyrin-Associated Periodic Syndrome

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Supplementary material: methods

Dose determination

Unusually, the dosing regimen used in this study was based on data from only four patients who had participated in an initial open-label study. [Lachmann et al 2009] Free interleukin 1 β (IL-1 β) is not quantifiable in serum, but treatment with the antibody enabled detection of complexed IL-1 β . A two-compartment mathematical model was generated that included the diffusion-exchange of canakinumab and IL-1 β between the tissue and plasma compartments, plus elimination rates for free canakinumab, free IL-1 β and the canakinumab-IL-1 β complexes from plasma. In healthy subjects this model predicted a constitutive IL-1 β production rate of 6 ng/day, and in patients with the Cryopyrin-Associated Periodic Syndrome (CAPS) increased IL-1 β production to a mean of 31 ng/day. Canakinumab treatment of the four patients with CAPS reduced the production rate of IL-1 β to normal levels within 8 weeks suggesting that IL-1 β production in these patients was mainly IL-1 β -driven. Modeling of this response predicted that a dosing schedule of 150 mg subcutaneously (s.c.) every 8 weeks should maintain most CAPS patients in a flare-free state, i.e. should enable sufficient canakinumab to be available to continue to bind newly produced IL-1 β and maintain free IL-1 β levels below the threshold associated with clinical evidence of active CAPS. This dose was selected for the present study. This approach was adopted due to constraints inherent in studying an extremely rare disease.

Study design

The study consisted of three parts (Fig 1). Part 1 was an open-label treatment period during which a single dose of canakinumab was administered to all patients. Part 2 was a double-blind withdrawal period, in which patients who showed a complete response in part 1 were randomly assigned to canakinumab or placebo every 8 weeks for up to 24 weeks. At the end of part 2 or on relapse, whichever occurred first, patients immediately entered the open-label part 3 of the study in which they received canakinumab every 8 weeks for a minimum of 16 weeks producing a total study duration of 48 weeks.

Patients

The study enrolled patients aged 4–75 years, body weight \geq 15 kg but <100 kg, with CAPS associated with an *NLRP3* mutation and who required treatment. However, patients using the following treatments in the specified period before the baseline visit were excluded: corticosteroids \geq 20 mg/day or >0.4 mg/kg for 1 week; colchicine, dapson or mycophenolate mofetil for 3 weeks; etanercept, leflunomide, thalidomide or ciclosporin for 4 weeks; adalimumab or intravenous immunoglobulin for 8 weeks; or infliximab, 6-mercaptopurine, azathioprine, cyclophosphamide or chlorambucil for 12 weeks. Females entering the study were prepubescent, postmenopausal,

sterilized or required to use effective contraception. Patients with HIV/AIDS, significant medical history of other conditions, history of recurrent infections or live vaccination within 3 months of the study, or who had an abnormality on their electrocardiogram were excluded from the study.

Determination of sample size

Power calculations indicated that at least 20 patients would be required in part 2 to demonstrate superiority of canakinumab over placebo. A sample size of 10 patients in each arm was estimated to have 90% power to detect treatment differences in relapse of 15% with canakinumab and 90% with placebo, using Fisher's exact test with a 0.05 two-sided significance level.

Efficacy assessments

Study visits occurred at screening and at weeks 0, 1, 2 (for patients with a partial response at week 1), 4 and 8, and 4-weekly thereafter up to week 48 (see Fig 1). At each study visit (except week 4), physicians assessed global disease activity and each of: urticarial skin rash; arthralgia; myalgia; headache/migraine; conjunctivitis; fatigue/malaise and other symptoms related or unrelated to CAPS. For each assessment, the physician selected the term which best described the patient's conditions from the following: absent = 1, minimal = 2, mild = 3, moderate = 4 or severe = 5.

Throughout the study, patients performed a global assessment of their symptoms together with assessments of each of: fever/chills; skin rash; joint/muscle pain; eye discomfort/redness; fatigue; headache and other symptoms. As for the physician assessment, for each assessment, patients selected one term which best described their condition from the following: absent = 1, minimal = 2, mild = 3, moderate = 4, or severe = 5.

Blood samples were collected for measurement of the concentration of the acute-phase reactants, C-reactive protein (CRP) and serum amyloid-A protein (SAA), at screening and during each study visit except week 4. Blood samples, which remained blinded to study treatment, were analyzed centrally so that the normal range remained constant for all samples (<10 mg/L for both CRP and SAA) regardless of study location.

Assessment of adverse events

Adverse events (AEs) were graded as mild, moderate and severe based on the investigator's decision.

Throughout this study, infectious AEs were recorded in two different ways. Firstly information on AEs was collected by investigators and was reported by selecting the appropriate description from a list of Medical Dictionary for Regulatory Activities (MedDRA[®]) terms. In addition, in order to ensure that all infections were reported, even if they fell outside MedDRA[®] definitions, investigators were asked to record any suspected infectious AEs on a special Case Report Form (CRF).

Assessment of laboratory abnormalities

Blood samples collected at weeks 0 and 1, and at 2-monthly intervals (weeks 8, 16, 24, 32, 40 and 48) were assessed for hematologic markers (hemoglobin, hematocrit, white blood cells, red blood cells and platelets) and biochemical markers (albumin, alkaline phosphatase [ALP], total bilirubin, calcium, chloride, cholesterol, creatinine, creatinine phosphokinase, gamma glutamyl transferase [GGT], glucose, lactate dehydrogenase, inorganic phosphorus, α -amylase, potassium, total protein, serum aspartate aminotransferase [AST], serum alanine aminotransferase [ALT], sodium,

triglycerides, urea/blood urea nitrogen and uric acid). Notable abnormalities in laboratory assessments occurring in parts 1, 2 and 3, of the study were summarized.

Assessment of immunogenicity

Immunogenicity was assessed by a sensitive and validated Biacore® binding assay. Canakinumab was immobilized on the sensor chip, ensuring exposure of its potentially immunogenic CDR regions. Serum samples were added and binding of putative anti-canakinumab antibodies was measured in real time, by surface plasmon resonance spectroscopy (Biacore 3000/A100 instrument). A signal was defined as screening-positive when above a defined threshold negative cut-off (NCO) value. The NCO was calculated for each assay run from the mean of 25 individual lots of human serum + 2SD. Positive signals were further investigated in a confirmatory assay after pre-incubation with canakinumab. A signal was considered specific when pre-incubation with canakinumab inhibited the proportion of the signal above the NCO by at least 30%. As a high serum concentration of canakinumab could potentially produce false-negative results, anti-human IgG depleted anti-idiotypic anti-canakinumab sheep serum was used to mimic a strong, mild, or weak immune response in validation of the Biacore assay. Canakinumab was then spiked at different concentrations and samples were analyzed. A weak immune response can be detected up to canakinumab concentrations of 27 µg/mL, a medium immune response can be detected up to concentrations of 111 µg/mL and a strong immune response can be detected up to concentrations of 425 µg/mL. These results combined with the serum concentration of canakinumab present in the patient samples were considered when interpreting the immunogenicity data.

Patient disposition

Forty-one patients were screened for entry into the study, six of whom were ineligible (see Fig. 2). Thus, 35 patients entered part 1 and received treatment. Of these, 31 patients entered part 2 and were randomized to receive canakinumab or placebo. All patients in the canakinumab group completed part 2 and then entered part 3 of the study. Of the patients randomized to placebo in part 2, four completed part 2 while the other 12 discontinued due to an unsatisfactory therapeutic effect. All 31 patients who entered part 2, proceeded to part 3 and 29 patients completed part 3.

Supplementary material: results

No safety issues emerged from hematologic monitoring, urinalysis or other assessments. Table 1 summarizes the abnormalities that were detected. A very mildly elevated eosinophil count was detected in four patients during canakinumab therapy (11.4% of the 35 patients), and one patient had a notably high white blood cell count in the placebo group. These were determined to be not clinically significant by the investigators at the relevant centers. Biochemical abnormalities were uncommon, and no abnormality was reported by more than three patients.

Supplementary material: reference

Lachmann HJ, Lowe P, Felix SD, et al. In vivo regulation of interleukin-1 beta in patients with cryopyrin associated periodic syndromes. *J Exp Med* 2009;206:1029-36.

Figure 1: Study Design

The time point for entering part 3 was based on completion of part 2 or relapse.

*For patients who completed part 2, part 3 had a duration of 16 weeks, with patients receiving two injections of canakinumab. For patients who relapsed in part 2, part 3 had a duration of up to 40 weeks, with patients receiving canakinumab injections every 8 weeks.

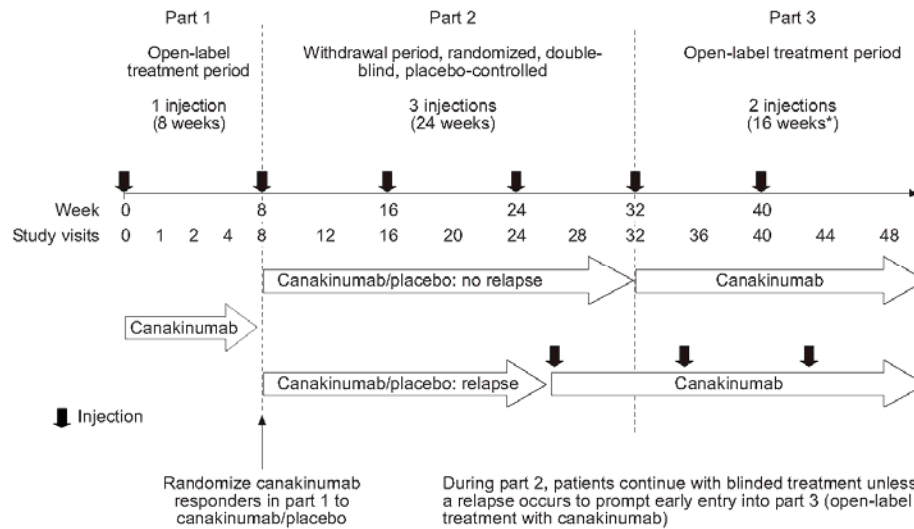


Fig. 2. Study Disposition, Showing Numbers of Patients Who Entered and Completed Each Stage, and Reasons for Study Withdrawal.

CR, complete response; *one patient relapsed on the last day of part 2

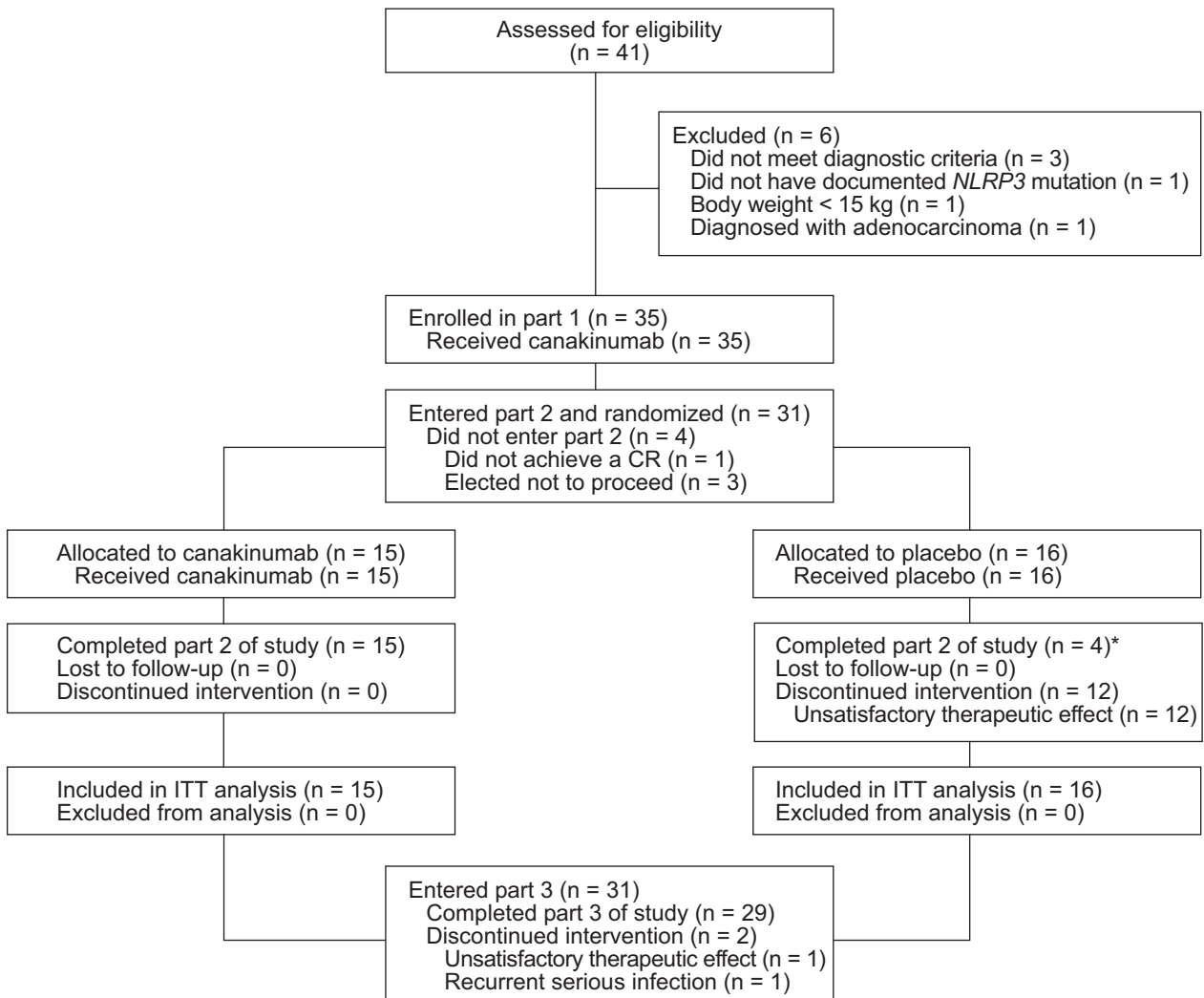


Table 1. Notable Laboratory Abnormalities Reported in Part 1, Part 2 and Part 3.

Laboratory test, number (%) with abnormality	Criterion for abnormality	Part 1 (n = 35)	Part 2		Part 3 (n = 31)
			Canakinumab (n = 15)	Placebo (n=16)	
Hemoglobin	≥20 g/L decrease from baseline	0	0	0	0
	<100 g/L (≥16 years) or <85 g/L (<16 years)	0	0	0	0
Platelets	<LLN	0	0	0	0
White cells	≤0.8 x LLN	0	0	0	0
	≥1.2 x ULN	0	0	1 (6.3%)	0
Neutrophils	≤0.9 x LLN	0	0	0	0
	≥1.2 x ULN	0	0	0	0
Eosinophils	≥1.1 x ULN	2 (5.7%)	1 (6.7%)	0	1 (3.2%)
Lymphocytes	<LLN	0	0	0	0
	≥1.1 x ULN	0	0	0	0
ALT	≥3 x ULN	1 (2.9%)	0	0	1 (3.2%)
	≥5 x ULN	1 (2.9%)	0	0	1 (3.2%)
	≥10 x ULN	0	0	0	0
AST	≥3 x ULN	2 (5.7%)	0	0	1 (3.2%)
	≥5 x ULN	1 (2.9%)	0	0	1 (3.2%)
	≥10 x ULN	0	0	0	0
Total bilirubin	>ULN	0	1 (6.7%)	0	1 (3.2%)
	≥1.5 x ULN	0	0	0	0
	≥1.5 x ULN if ALT and/or AST ≥3 x ULN	0	0	0	0
Albumin	<LLN	0	0	0	0
ALP	>ULN	1 (2.9%)	0	1 (6.3%)	1 (3.2%)
GGT	>3 x ULN	0	0	0	0
Creatinine (serum)	≥1.5 x ULN	0	0	0	0
Creatinine clearance*	25% decrease (≥16 years)	1 (3.1%)	1 (7.7%)	1 (6.7%)	2 (7.1%)
Potassium	≤3.0 mmol/L (≥16 years) or ≤3.5 mmol/L (<16 years)	0	0	0	0
	≥5.5 mmol/L	0	0	0	0
Sodium	≤130 mmol/L	0	0	0	0
	≥150 mmol/L	0	0	1 (6.3%)	0

Calcium	<LLN (≥16 years)	0	1 (7.7%)	1 (6.7%)	1 (3.6%)
	≥1.2 x ULN (≥16 years)	0	0	0	0
Protein urine dipstick	≥ ++ (≥16 years), ≥ + or trace (<16 years)	0	1 (8.3%)	0	1 (3.7%)

ALP, alkaline phosphatase; ALT, serum alanine aminotransferase; AST, serum aspartate aminotransferase; GGT, gamma glutamyl transferase; LLN, lower limit of normal; ULN, upper limit of normal

*Assessed using the Cockcroft-Gault formula for men:

creatinine clearance (mL/min) = $[(140 - \text{age (years)}) \times \text{weight (kg)}] / (\text{serum creatinine } (\mu\text{mol/L}) / 88.4 \text{ (mg/dL)} \times 72]$

and for women:

creatinine clearance (mL/min) = $[(140 - \text{age (years)}) \times \text{weight (kg)}] / (\text{serum creatinine } (\mu\text{mol/L}) / 88.4 \text{ (mg/dL)} \times 72) \times 0.85$