Table IV Numbers in discriminate analysis: actual rows by predicted columns

Three groups using 5 gene expression levels (Fig.5a)			
	HNL	Disease control	Normal control
HNL	22	2	0
Disease control	11	74	9
Normal control	1	1	32
Misclassified number: 24, accuracy: 84.2 %			
Two groups using 3 gene expression levels (Fig.5b)			
	HNL	Disease control	
HNL	20	4	
Disease control	17	77	
Misclassified number: 21, accuracy: 82.2 %			

Clinically, malignant lymphoma and leukemia are the most important disorders to be distinguished from HNL. A lymph node biopsy is often required for definitive diagnosis. CXCL10 expression may be enhanced in PBMC of the patients with these diseases because it was reported that CXCL10 expression was associated with the progression of leukemia and with the poor prognosis of lymphoma [17, 18]. In this study, we could distinguish HNL from these diseases by analyzing expression levels of other ISGs (Figs. 1 and 5). On the other hand, SLE presents the most challenging differential consideration, and sometimes its histologic presentation is almost identical to HNL [1, 2, 9]. Ishii et al. [16] reported that expression levels of *IFI27* and *EPSTI1* were increased in PBMC of SLE patients. GBP1 was expressed in lesional skin, and *IFI27*, *IFI44* and *IFI44L* were up-regulated in the synovium of patients with SLE [19, 20]. *IFI27* was also increased in PBMC of patients with Sjögren syndrome [21]. In our study, 4 ISGs, other than CXCL10, were up-regulated in SLE patients. Szturz et al. indicates that the pattern of serum cytokine levels in patients with HNL is similar to that of SLE patients [22]. These findings suggest that HNL and SLE are similar in the pathophysiology which includes immune responses mediated by type 1 IFNs.

Hundreds of ISGs were identified and different viruses are targeted by unique sets of ISGs. In addition, combined expression of pairs of ISGs showed additive antiviral effects [14]. We found that the expression levels of five ISGs (*IFI44L*, *CXCL10*, *GBP1*, *EPSTI1*, and *IFI27*) showed log-normal distribution and moderately positive correlation among them (r^2=0.28–0.60) (Figs. 1 and 2). These results suggest that the 5 genes are coordinately-induced in HNL. On the other hand, up-regulation of ISGs was similarly observed in patients with measles, varicella and other viral infections, and it was reported that GBP1, IFI27 and IFI44L could suppress hepatitis C virus replication [14, 23]. These findings indicate that HNL might be related with certain viral infections.

There are two kinds of ISGs, broad-acting effectors like interferon regulatory factor 1 (IRF1), retinoic acid-inducible gene-I (RIG-I), and melanoma differentiation-associated protein 5 (MDA5), and specific effectors which include IFI44L. It is known that unique sets of ISGs are important for specific antiviral effects [14]. The combination of up-regulated ISGs in HNL seemed to be a specific response induced by viral infections or autoantigens, which would be helpful for a non-invasive diagnosis for HNL.

There is a limitation for the availability of this model in daily routine for the diagnosis of HNL. Although it can not give the direct definitive diagnosis of HNL itself, the evaluation of the ISGs mRNA expression levels of peripheral blood seems to be helpful. Further research with more patients would be necessary for the early, non-invasive, and definitive diagnosis for HNL.

Acknowledgments We thank Department of Pathology, Faculty of Medicine, Fukuoka University, Japan, for the material support. The statistical analyses were advised by Junji Kishimoto at Kyushu University Hospital, Japan. This work was supported by a Grant-in-Aid for research on intractable diseases for Health and Labour Sciences Research Grants from the Ministry of Health, Labour and Welfare of Japan.

References

1. Bosch X, Guilabert A, Miquel R, Campo E. Enigmatic Kikuchi-Fujimoto disease: a comprehensive review. Am J Clin Pathol. 2004;122:141–52.
2. Hutchinson CB, Wang E. Kikuchi-Fujimoto disease. Arch Pathol Lab Med. 2010;134:289–93.
3. Yoshioka K, Miyashita T, Nakamura T, et al. Treatment of histiocytic necrotizing lymphadenitis (Kikuchi's disease) with prolonged fever by a single course of methylprednisolone pulse therapy without maintenance therapy: experience with 13 cases. Intern Med. 2010;49:2267–70.
4. Pileri SA, Facchetti F, Ascani S, et al. Myeloperoxidase expression by histiocytes in Kikuchi's and Kikuchi-like lymphadenopathy. Am J Pathol. 2001;159:915–24.
5. Pilichowska ME, Pinkus JL, Pinkus GS. Histiocytic necrotizing lymphadenitis (Kikuchi-Fujimoto disease): lesional cells exhibit an immature dendritic cell phenotype. Am J Clin Pathol. 2009;131:174–82.
6. Ohshima K, Shimazaki K, Kume T, et al. Perforin and Fas pathways of cytotoxic T-cells in histiocytic necrotizing lymphadenitis. Histopathology. 1998;33:471–8.
7. Ohshima K, Shimazaki K, Suzumiya J, et al. Apoptosis of cytotoxic T-cells in histiocytic necrotizing lymphadenitis. Virchows Arch. 1998;433:131–4.
8. Ohshima K, Karube K, Hamasaki M, et al. Apoptosis- and cell cycle-associated gene expression profiling of histiocytic necrotising lymphadenitis. Eur J Haematol. 2004;72:322–9.
9. Hu S, Kuo TT, Hong HS. Lupus lymphadenitis simulating Kikuchi's lymphadenitis in patients with systemic lupus erythematosus: a clinicopathological analysis of six cases and review of the literature. Pathol Int. 2003;53:221–6.
10. Sumiyoshi Y, Kikuchi M, Takeshita M, et al. Alpha-interferon in Kikuchi's disease. Virchows Arch B Cell Pathol Incl Mol Pathol. 1991;61:201–7.

11. Kubota M, Tsukamoto R, Kurokawa K, et al. Elevated serum interferon gamma and interleukin-6 in patients with necrotizing lymphadenitis (Kikuchi's disease). Br J Haematol. 1996;95:613–5.
12. Ikeda K, Yamaguchi K, Tanaka T, et al. Unique activation status of peripheral blood mononuclear cells at acute phase of Kawasaki disease. Clin Exp Immunol. 2010;160:246–55.
13. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc. 2008;3:1101–8.
14. Schoggins JW, Wilson SJ, Panis M, et al. A diverse range of gene products are effectors of the type I interferon antiviral response. Nature. 2011;472:481–5.
15. Ohshima K, Haraoka S, Takahata Y, et al. Interferon-gamma, interleukin-18, monokine induced by interferon-gamma and interferon-gamma-inducible protein-10 in histiocytic necrotizing lymphadenitis. Leuk Lymphoma. 2002;43:1115–20.
16. Ishii T, Onda H, Tanigawa A, et al. Isolation and expression profiling of genes upregulated in the peripheral blood cells of systemic lupus erythematosus patients. DNA Res. 2005;12:429–39.
17. Ansell SM, Maurer MJ, Ziesmer SC, et al. Elevated pretreatment serum levels of interferon-inducible protein-10 (CXCL10) predict disease relapse and prognosis in diffuse large B-cell lymphoma patients. Am J Hematol. 2012;87:865–9.
18. Lee Y, Chittezhath M, Andre V, et al. Protumoral role of monocytes in human B-cell precursor acute lymphoblastic leukemia: involvement of the chemokine CXCL10. Blood. 2012;119:227–37.
19. Nzeusseu Toukap A, Galant C, Theate I, et al. Identification of distinct gene expression profiles in the synovium of patients with systemic lupus erythematosus. Arthritis Rheum. 2007;56:1579–88.
20. Naschberger E, Wenzel J, Kretz CC, et al. Increased expression of guanylate binding protein-1 in lesional skin of patients with cutaneous lupus erythematosus. Exp Dermatol. 2011; 20:102–6.
21. Kimoto O, Sawada J, Shimoyama K, et al. Activation of the interferon pathway in peripheral blood of patients with Sjogren's syndrome. J Rheumatol. 2011;38:310–6.
22. Szturz P, Adam Z, Chovancová J, et al. Cytokine analysis in a patient with relapsing Kikuchi-Fujimoto disease. Leuk Lymphoma. 2012;53:743–5.
23. Itsui Y, Sakamoto N, Kakinuma S, et al. Antiviral effects of the interferon-induced protein guanylate binding protein 1 and its interaction with the hepatitis C virus NS5B protein. Hepatology. 2009;50:1727–37.

Safety and efficacy of canakinumab in Japanese patients with phenotypes of cryopyrin-associated periodic syndrome as established in the first open-label, phase-3 pivotal study (24-week results)

T. Imagawa[1], R. Nishikomori[2], H. Takada[3], S. Takeshita[1], N. Patel[4], D. Kim[4], K. Lheritier[5], T. Heike[2], T. Hara[3], S. Yokota[1]

[1]Department of Paediatrics, Yokohama City University, Yokohama, Japan; [2]Department of Paediatrics, Kyoto University, Kyoto, Japan; [3]Department of Paediatrics, Kyushu University, Fukuoka, Japan; [4]Novartis Pharmaceutical Corporation, East Hanover, NJ, USA; [5]Novartis Pharma AG, Basel, Switzerland.

Abstract

Objectives

Cryopyrin-associated periodic syndrome (CAPS), a rare hereditary auto-inflammatory disease, is associated with mutations in the NLRP3 gene resulting in elevated interleukin-1β (IL-1 β) release. CAPS generally occurs in early childhood with most patients presenting with periodic fever, skin rash, osteoarthropathy, aseptic meningitis, sensorineural hearing loss and optic neuritis. Canakinumab, a fully human anti-IL-1β monoclonal antibody which binds selectively to IL-1β, has demonstrated good efficacy with CAPS. This is the first study to evaluate the safety and efficacy of canakinumab in Japanese patients with CAPS.

Methods

In this open-label study, 19 Japanese CAPS patients aged ≥2 years received canakinumab either 150 mg s.c. or 2 mg/kg for patients with a body weight ≤ 40 kg every 8 weeks for 24 weeks. The primary objective was to assess the proportion of patients who were free of relapse at week 24.

Results

A complete response was achieved in 18 (94.7%) patients with some requiring a dose and/or a frequency adjustment to attain full clinical response. The majority of patients (14/18; 77.8%) were in remission, i.e. free of relapse at week 24. Auto-inflammatory disease activity as assessed by physician's global assessment declined from baseline to end of the study (score of absent in 10.5% at baseline versus *31.6% at end of the study). Two patients had serious adverse events (SAEs), which resolved with standard treatment. One patient reported a mild injection-site reaction. No malignancies or deaths were reported during the study.*

Conclusion

Canakinumab 150 mg s.c. every 8 weeks was well-tolerated, highly efficacious and offered a convenient dosing regimen for treating Japanese patients with CAPS.

Key words

canakinumab, cryopyrin-associated periodic syndrome, interleukin-1β, auto inflammatory syndromes

Tomoyuki Imagawa, MD, PhD
Ryuta Nishikomori, MD, PhD
Hidetoshi Takada, MD, PhD
Saoko Takeshita, MD
Neha Patel, MS
Dennis Kim, MD, MPH
Karine Lheritier, PhD
Toshio Heike, MD, PhD
Toshiro Hara, MD, PhD
Shumpei Yokota, MD, PhD

Please address correspondence and reprint requests to:
Dr Tomoyuki Imagawa,
Department of Paediatrics,
Yokohama City University Hospital,
3-9 Fukuura, Kanazawa,
Yokohama 236-0004, Japan.
E-mail: timagawa@gmail.com

Received on September 28, 2011; accepted in revised form on July 19, 2012.

Funding: this study was financially supported by an unrestricted grant from Novartis Pharma AG, Basel, Switzerland. ClinicalTrials.gov (identifier: NCT00991146).

Competing interests: none declared.

Introduction

Cryopyrin-associated periodic syndrome (CAPS) represents a group of rare inherited auto-inflammatory diseases and encompasses phenotypes of varying severity. An increase in severity is evident between phenotypes: familial cold auto-inflammatory syndrome (FCAS) is the mildest, while Muckle-Wells syndrome (MWS) is predominantly of intermediate severity, and neonatal-onset multisystem inflammatory disease (NOMID)/chronic infantile neurological cutaneous and articular syndrome (CINCA) is the most severe phenotype of CAPS. All phenotypes are characterised by urticaria-like rash, fever, variant degree of central nervous system and tissue inflammation, arthropathy, risk of development of amyloidosis (1) and other constitutional symptoms. CAPS is associated with mutations of the *NLRP3* gene encoding cryopyrin (2-6), an important component of inflammasome. Inflammasone activates caspase-1, leading to enhanced production of the cytokine interleukin-1beta (IL-1β) and subsequent inflammation (7, 8). The pathogenic role of IL-1β in CAPS has been demonstrated by the achievement of complete response after treatment with IL-1β inhibitors (9-13). Positive therapeutic effects of the IL-1 receptor antagonist and anakinra have been hampered by the need for frequent injections (14-17) associated with severe pain, which impairs the quality of life of patients, especially the paediatric population. Canakinumab (ACZ885, Ilaris®, Novartis Pharma), a fully human anti-IL-1β monoclonal antibody (18), has shown prolonged selective IL-1β inhibition (19, 20) and has demonstrated rapid (within hours), complete and sustained response in CAPS patients of mainly Caucasian origin without any consistent pattern of side effects (21). Canakinumab is approved by the US Food and Drug Administration (FDA) for FCAS and MWS (22) only and by EMA for treatment of all three phenotypes of CAPS (23).

At present, there are no approved therapies for CAPS in Japan. The present study was therefore conducted to evaluate safety and efficacy of canakinumab in Japanese paediatric and adult patients with CAPS. Herein we report the study data up to 24 weeks.

Materials and methods

Study design, patients and study definitions

This was an open-label, safety and efficacy study of canakinumab administered for 24 weeks (6 months) in Japanese patients diagnosed with FCAS, MWS or NOMID. Molecular diagnosis showed that 17 (89.5%) patients were positive for *NLRP3* mutations and two (10.5%) patients (one each with MWS and NOMID) were negative for the mutation. The study included an extension phase to provide canakinumab treatment to study patients until canakinumab is marketed in Japan. Two NOMID patients aged 2 and 3 years previously treated with anti-IL-1 agents (anakinra) were enrolled.

Patients received canakinumab 150 mg s.c. or 2 mg/kg for those patients with body weight ≤40 kg for every 8 weeks. In case of residual symptoms, stepwise increase of the dose up to 600 mg s.c. or 8 mg/kg s.c. (≤40 kg) and/or increased dosing frequency were allowed.

After a 6-hour washout period for those patients previously treated with anakinra, 19 patients were included. Ten had received anakinra prior to study initiation, of which five patients had reported a complete response, while the remaining had achieved partial response to anakinra. Patients requiring oral steroids, NSAIDs and/or disease-modifying anti-rheumatic drugs (DMARDs) were enrolled if they were on a stable dose (oral steroids: <20 mg/day or ≤0.4 mg/kg prednisone or prednisone equivalent, whichever applies) for at least 4 weeks prior to the screening visit. Steroid therapy was tapered after the first canakinumab treatment cycle (8 weeks between doses), at the discretion of the investigator. TNF-α inhibitors and IL-6 receptor blockers were not allowed during the study. Women of child bearing potential had to use an accepted form of contraception during the study and for at least 3 months after the last dose. Patients receiving live vaccine within 3 months before recruitment were excluded.

Complete response assessed at day 15 and day 29 was defined by (i) physician's global assessment of no or minimal auto-inflammatory disease (on a 5-point Likert scale ranging from absent, minimal, mild, moderate to severe) and assessment of no or minimal skin disease, and (ii) serological remission defined as serum CRP <1 mg/dL, and/or SAA <10 μg/mL. Patients who did not achieve (or maintain) complete response following canakinumab injection in any treatment period could receive a dose escalation (supporting Fig. 1). The possible step-wise up-titration regimens were: 300 mg s.c. (or 4 mg/kg for patients with a body weight ≤40 kg), 450 mg s.c. (or 6 mg/kg for patients with a body weight ≤40 kg), and 600 mg s.c. (or 8 mg/kg for patients with a body weight ≤40 kg).

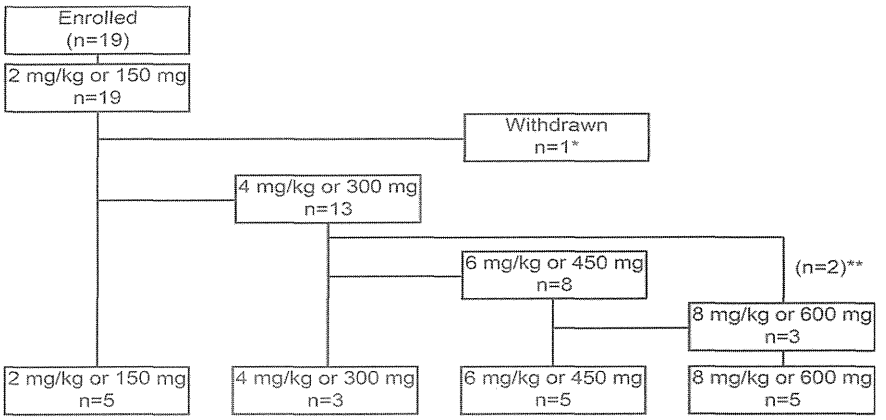
The primary efficacy endpoint was defined as the proportion of patients who did not experience a relapse at week 24. Relapse was defined as clinical relapse (physician's global assessment of both auto-inflammatory disease activity and assessment of skin disease, mild or greater) and serological relapse (serum CRP >3 mg/dL, and/or SAA >30 μg/mL).

Clinical improvement of the central nervous system (CNS) was assessed in NOMID patients only (defined as a mean weekly headache score [from the daily diary] <0.5 and a normal white cell count [≤15 cells/mm^3] in cerebrospinal fluid). Other key secondary endpoints included safety and tolerability of canakinumab, assessed by the occurrence of adverse events (AEs), serious AEs (SAEs) and immunogenicity.

This study was approved by the Independent Ethics Committee for each centre and performed in accordance to the ethical principles of the Declaration of Helsinki. All patients, parents or legal guardians (for patients aged <20 years) provided written informed consent.

Statistical analyses

Safety and full analysis set (efficacy analysis) included all patients who received at least one dose of study treatment. Only 19 patients were enrolled due to the low prevalence of CAPS, hence the estimation of statistical power was not applicable. Descriptive statistics were used to summarise demographics, baseline characteristics, efficacy and safety. Missing values were not imputed.



*One patient withdrew from this study by cancellation of the consent.

**Two patients needed two up titrations till Day 15 due to incomplete response to the first administration of canakinumab. Patients with incomplete response from the standard dosing regimen (2 mg/kg or 150 mg) received step-wise up-titration regimen. Patients who did not achieve complete response or had a relapse before the next planned administration received a dose up-titration.

Fig. 1. Patient disposition and dosing.

Results

Patients, demographic and baseline characteristics

A total of 19 CAPS patients (12 [63.2%] male/7 [36.8%] female) with a diagnosis of MWS (n=7; 36.8%) or NOMID (n=12; 63.2%) were enrolled in this study, of which 18 (94.7%) completed the 24-week study phase. One patient withdrew consent (Fig. 1). At study entry, there were 11 patients (57.9%) aged <16 years and eight patients (42.1%) aged 16 years or older. Median age was 14 years (range 2–48). Of 19 patients, five (26.3%) weighed >40 kg at baseline. Other key demographic and baseline characteristics are summarised in Table I.

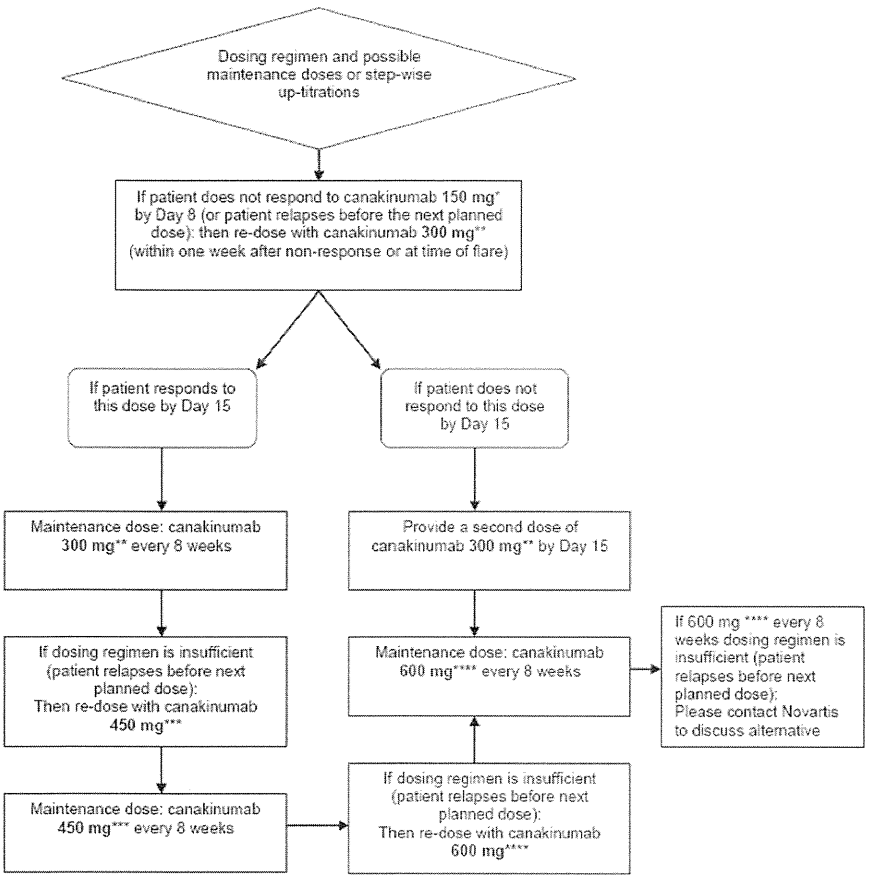
Treatment with canakinumab

At time of the 24-week analysis, the median treatment duration was 168 days (range 59–197 days) and patients received an average of 4.1 injections over 24 weeks of the study; 13 (68%) patients (MWS; n=4 and NOMID, n=9) received an up-titration of their dose, primarily due to absence of a complete response and in one patient the dose frequency was increased to every 6 weeks starting from day 49. In one NOMID patient aged 16 years, the

Table I. Baseline demographics and disease characteristics (safety population).

Characteristics	Canakinumab (n=19)
Sex, n (%)	
Male	12 (63.2)
Female	7 (36.8)
Age (years)	
Mean (SD)	14.8 (11.4)
Median (range)	14.0 (2–48)
≥2– <12 years, n (%)	8.0 (42.1)
≥12– <16 years, n (%)	3 (15.8)
≥16 years, n (%)	8 (42.1)
Weight (kg), n (%)	
≤40	14 (73.7)
>40	5 (26.3)
BMI (kg/m^2)	
Mean (SD)	17.6 (2.2)
Median (range)	17.2 (13.5–21.5)
Diagnosis, n (%)	
FCAS	0
MWS	7 (36.8)
NOMID	12 (63.2)
Molecular diagnosis of NLRP3 mutation, n (%)	19 (100.0)
Positive	17 (89.5%)
Negative	2 (10.5%)
Previous use of anakinra, n (%)	10 (52.6)
C-reactive protein (mg/dL) (normal value: <1mg/dL)	
Mean (SD)	4.52 (4.3)
Median (range)	3.3 (0.1–13.2)
Serum Amyloid A (μg/mL) (normal value: <10μg/mL)	
Mean (SD)	324.2 (364)
Median (range)	236 (2.6–1380)

BMI: body mass index; FCAS: familial cold autoinflammatory syndrome; MWS: Muckle-Wells syndrome; NOMID: neonatal-onset multisystem inflammatory disease; NLRP3: NOD-like receptor family, pyrin domain containing3; SD: standard deviation.



* canakinumab 150 mg s.c. for patients whose body weight is > 40 kg (or 2 mg/kg for patients with a body weight ≤ 40 kg)
** canakinumab 300 mg s.c. (or 4 mg/kg for patients with a body weight ≤ 40 kg)
*** canakinumab 450 mg s.c. (or 6 mg/kg for patients with a body weight ≤ 40 kg)
**** canakinumab 600 mg s.c. (or 8 mg/kg for patients with a body weight ≤ 40 kg)
There is currently no long-term safety information for doses greater than 600 mg s.c. available.
The above outlined decision tree may be applied to those patients who either did not achieve a complete response by Day 8 or Day 15 or to those patients who relapse prior to their next scheduled dose.

Supporting Fig. 1. Alternative dosing regimen for CAPS patients who do not experience sufficient symptomatic relief.

canakinumab dose was escalated to the highest dose of 600 mg. Four patients (8–25 years) with baseline body weight ≤40kg received a dose escalation to 8 mg/kg.

Proportionally higher mean last doses of canakinumab were required in patients ≤40 kg (n=12) *versus* >40 kg (n=6) at 6 mg/kg and 250 mg, respectively; in patients weighing >40 kg, the canakinumab dose administered was 350 and 150 mg for NOMID and MWS, respectively.

Efficacy

Relapse assessment. Overall, protocol-defined complete response was achieved in 18 (94.7%) patients. One patient achieved a complete response by day 148. This patient achieved clinical remission by day 29, but the inflammatory markers remained elevated until day 148. One non-responder patient achieved clinical remission, but the patient's CRP and SAA levels remained above normal during the study; however there was a significant decrease by week 24 compared to baseline. Some patients required either a dose escalation and/or a frequency adjustment to attain full clinical response (supporting Fig. 1); 15 (78.9%) patients achieved a complete response within 15 days, 2 patients were up-titrated within 29 days, and 1 patient by day 148. At week 24, the majority of patients (n=14/18 [77.8%]) were in remission, *i.e.* free of relapse (Table II).

Table II. Relapse at week 24 in MWS and NOMID patients (full analysis set).

Characteristics	Canakinumab n=19 n (%)
Number of complete responders by week 24	
Total	18 (94.7)
Day 15*	15 (78.9)
Day 29*	2 (10.5)
Day 148*	1 (5.3)
Relapse at week 24	4 (22.2)
No relapse at week 24	14 (77.8)
MWS patients	6 (85.7)
NOMID patients	8 (72.7)
No clinical/serological relapse at week 24	12 (66.7)
Discontinue prematurely prior to week 24	1 (5.6)

*Patients requiring either a dose and/or a frequency adjustment to attain full clinical response.
MWS: Muckle-Wells syndrome; NOMID: neonatal-onset multisystem inflammatory disease.

Of 12 NOMID patients, 11 achieved complete response by week 24 and nine achieved a complete response by day 15; one achieved complete response with dose adjustment by day 29 and one by day 148. Three (27.3%) out of the 11 complete responders (all NOMID patients) had a relapse at week 24. All patients with MWS (n=7) achieved complete response by week 24, though one patient had a relapse at week 24. All except one patient achieved complete response with canakinumab. All prior responders to anakinra also achieved a complete response with canakinumab.

Auto-inflammatory disease activity

The severity of auto-inflammatory disease activity as assessed by physician's global assessment declined from baseline to the end of the treatment period. This decrease in disease activity was apparent in all the individual symptom components including assessments of skin disease, headache/migraine, conjunctivitis and fatigue/malaise (Fig. 2).

Inflammatory markers

Canakinumab treatment induced a rapid decline in CRP levels within 15 days (Fig. 3a). Overall, mean CRP levels decreased by 2.94±2.99 mg/dL (38% decrease) from baseline to end of the study, day 169 (4.52 mg/dL *vs.* 1.19

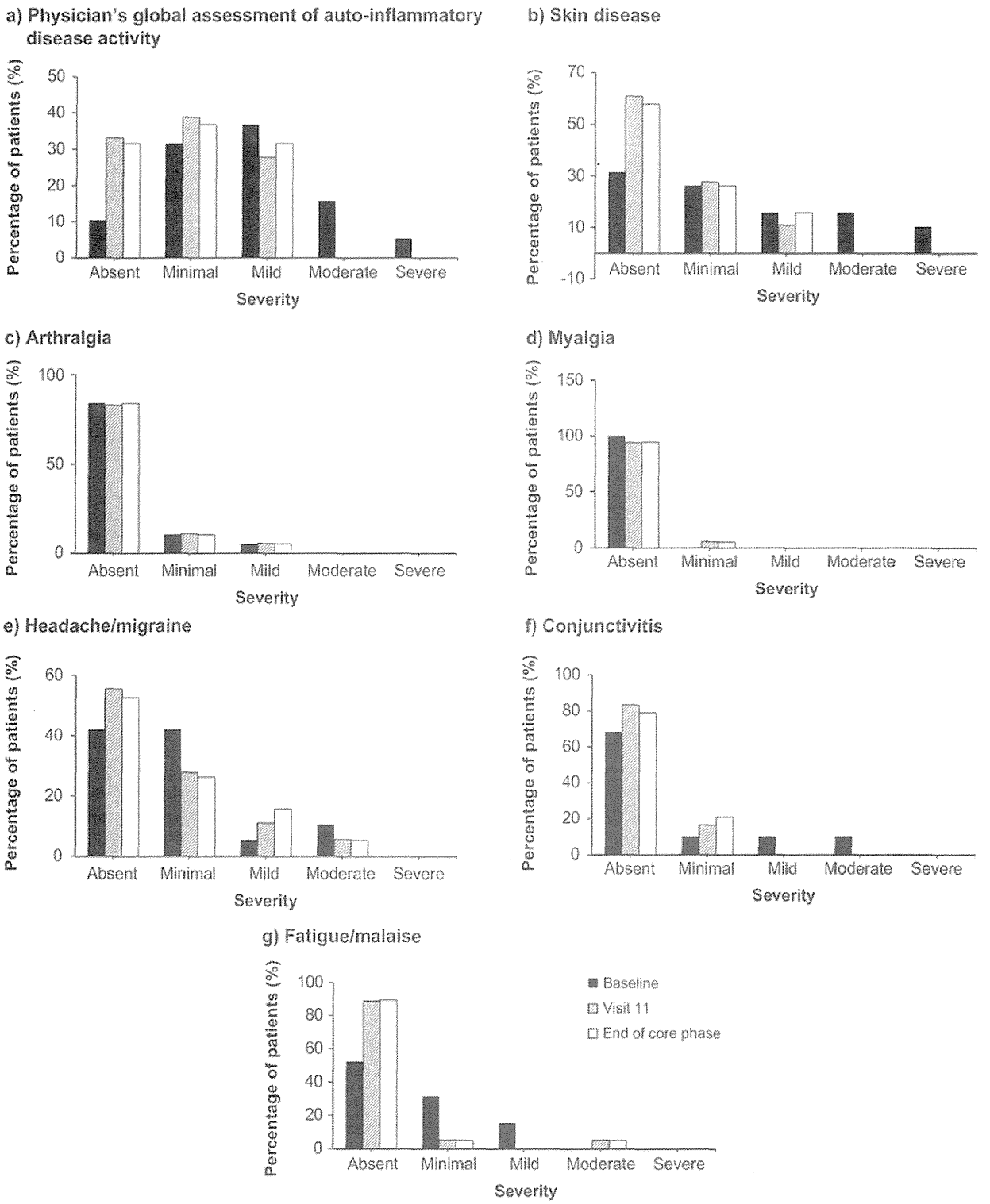


Fig. 2. Summary of assessment of auto-inflammatory disease activity (full analysis set).

mg/dL). A similar trend was observed for mean serum SAA level, which decreased from baseline to end of the study (324.19 μg/mL *vs.* 54.71 μg/mL) (Fig. 3b). On day 57, there was an increase in CRP and SAA levels, however this was driven by measurements from three patients whose mean values were near normal at other time points.

Immunogencity

Of the 19 patients, three were detected with anti-canakinumab binding antibodies during one of the post-dose assessments. However, no anti-canakinumab antibodies were detected afterwards.

Specific assessments in NOMID patients

A protocol-defined CNS remission was achieved in 33.3% (n=4/12) of the NO-

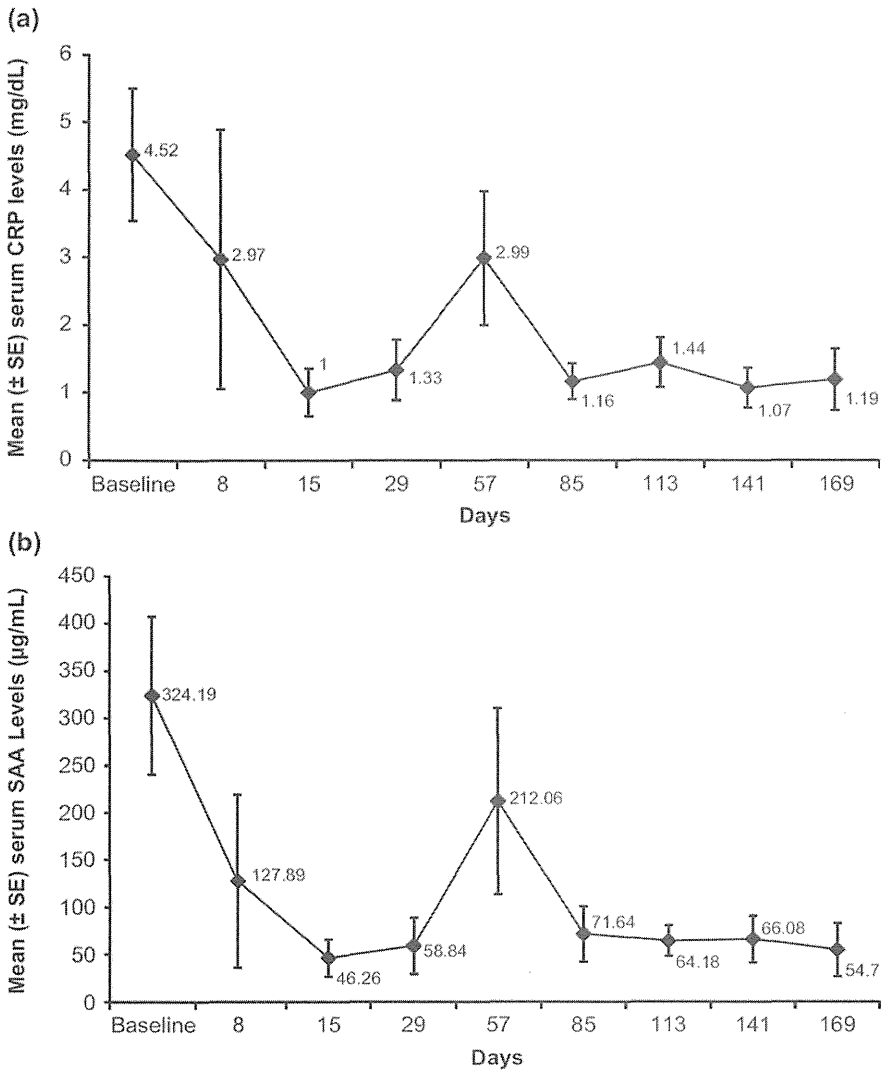


Fig. 3. (a) Serum CRP level across time points (full analysis set); (b) Serum SAA levels across time points.

MID patients by day 8 and in 41.7% (n=5/12) at the end of study; 9/12 patients had CNS remission at week 24 (with just the headache score). Lumbar puncture was only performed in 7/12 patients, of which five were in CNS remission based on the headache score and normal white cell count. A CNS relapse was reported in two (16.7%) patients on day 57 and in one patient (9.1%) on day 113. Of the three patients with a protocol-defined CNS relapse, one was uptitrated from 4mg/kg to 6mg/kg due to a concomitant clinical and serological relapse. In the other two patients, no uptitration was performed for CNS relapse. In these three patients, there was no association between the CNS relapse and clinical flare. The results of key cerebrospinal fluid assessments in NOMID patients were available in only 6 patients, who had both baseline and week 24 values. In these patients (n=6), mononuclear cells (lymphocytes, macrophages, monocytes) remained unchanged or elevated slightly from baseline to week 24 (normal values: adult ≤5 WBC/mm^3, newborns ≤20 WBC/mm^3). Absolute neutrophils which markedly reduced in two NOMID patients remained largely unchanged in the other three patients, even though it was elevated in one patient at week 24 compared with baseline. None of these patients reported headache, but they were noted to have elevated CRP and/or SAA levels. In addition to elevated SAA levels, one patient had physician's global assessment of autoinflammatory disease activity above minimal and had a relapse at week 24.

Table III. Most frequently occurring (>10%) adverse events regardless of study drug relationship (safety population).

Primary system organ class/ preferred term	Canakinumab n=19 n (%)
Total patients with AEs	18 (94.7)
Gastrointestinal disorders	7 (36.8)
Abdominal pain upper	2 (10.5)
Diarrhoea	2 (10.5)
Stomatitis	2 (10.5)
General disorders and administration site conditions	3 (15.8)
Infections and infestations	16 (84.2)
Nasopharyngitis	7 (36.8)
Gastroenteritis	6 (31.6)
Upper respiratory tract infection	3 (15.8)
Nervous system disorders	2 (10.5)
Respiratory, thoracic and mediastinal disorders	5 (26.3)
Rhinorrhoea	3 (15.8)
Cough	2 (10.5)
Skin and subcutaneous tissue disorders	6 (31.6)
Acne	2 (10.5)
Dry skin	2 (10.5)
Urticaria	2 (10.5)
Vascular disorders	2 (10.5)
Hypertension	2 (10.5)

A patient with multiple occurrences of an AE is counted only once in the AE category. A patient with multiple adverse events within a primary system organ class is counted only once in the total row. AE: adverse event.

Safety

Overall, 18 (94.7%) patients experienced at least one AE. The most commonly reported AEs (≥15% of patients) were nasopharyngitis (n=7, 36.8%), gastroenteritis (n=6, 31.6%), upper respiratory tract infection (n=3, 15.8%), and rhinorrhea (n=3, 15.8%). Twelve (63.2%) patients reported AEs, which were suspected to be study drug-related (Table III). The majority of AEs were mild (n=13, 68.4%) or moderate (n=3, 15.8%) in severity. Severe AEs of diffuse vasculitis and pneumonia were each reported in one (5.3%) patient. All but one MWS patient experienced at least one AE. Nasopharyngitis was reported in a higher proportion of NOMID patients (n=6, 50%) compared to MWS patients (n=1, 14.3%). All other AEs in NOMID and MWS patients occurred at similar frequencies or in less than three patients in each group. Two patients had serious AEs, which were suspected to be treat-

ment-related (Parvovirus infection and Epstein-Barr virus infection [n=1] and pneumonia [n=1]), but resolved with standard treatment. Of the 19 patients, only one reported a mild injection-site reaction. No deaths were reported during the study. Higher canakinumab s.c. doses (>150mg or 2mg/kg q8wks) did not appear to be associated with a differential safety profile.

Discussion

The present study confirms the clinical and serological efficacy of canakinumab in a Japanese population of paediatric and adult CAPS patients presenting with the most severe NOMID and MWS-phenotypes. Eighteen (94.7%) out of 19 patients enrolled in this study have achieved a complete response with some patients requiring either a dose and/or a frequency adjustment to attain full clinical response. For most patients (78.9%), irrespective of CAPS phenotype, a complete response was achieved with the standard subcutaneous canakinumab dose (13), *i.e.* 150 mg (>40 kg body weight) or 2 mg/kg (≤40 kg body weight) every 8 weeks.

All clinical symptoms frequently observed in CAPS patients such as inflammation of skin, eyes, bones, joints and meninges accompanied by recurrent fever, severe fatigue, myalgia and headache, showed an improvement during canakinumab treatment. Improvement in clinical outcomes with canakinumab therapy such as autoinflammatory disease activity, and reduction in the levels of acute phase proteins such as CRP and SAA confirms the pivotal role of IL-1β and its inhibition in CAPS.

The sustained effects of canakinumab on patient's clinical symptoms have been associated with its mean terminal half-life of 26 days and a possibly disease-modifying effect through autocrine down-regulation of IL-1β production (19). The canakinumab administration schedule of one injection every 8 weeks and the low incidence of injection-site reactions, as previously observed in other phase II and III canakinumab CAPS studies (21, 25, 26), may be beneficial, especially to paediatric patients.

In the present study, individualised up-titration in patients with an incomplete response proved to be a safe and an efficacious approach for the majority of patients achieving a complete response within one month. Patients with incomplete response, as shown by changes in clinical symptoms (headache, fever or rash according to CAPS) and raised inflammatory marker levels (elevated CRP >3 mg/dL, and/or SAA >30 μg/mL), had initially received canakunimab titrated up to 8 mg/kg. The dosage interval was shortened by up to four weeks if patients failed to achieve a complete response. There was no clear correlation between the genotype, phenotype, and treatment response. The mean dose requirement for patients ≤40 kg was found to be proportionally higher (6 mg/kg) than for those with a body weight >40 kg (250 mg). In the group of patients with a body weight >40kg, the NOMID patient subgroup required a higher mean dose compared to the MWS patient subgroup, in line with the level of severity of the disease.

At baseline, 12 NOMID patients presented with CNS symptoms that included headache and pleocytosis and 9 showed improvement in these symptoms by week 24. Patients showed no significant changes, either worsening or improvement, based on audiogram and neurological or ophthalmic assessments. Two patients showed normalisation in auditory acuity and one patient showed normalisation in visual acuity. There were no organic changes observed on magnetic resonance imaging (MRI). This may be attributed to the fact that the observation period was relatively short and approximately 53% of patients were pre-treated with anakinra at the time of the study entry.

In the present study, no patients discontinued due to unsatisfactory therapeutic effect, suggesting that an effective individual canakinumab dosing regimen was determined. The safety profile was comparable to that observed in previous canakinumab studies (21, 24), with no new or unexpected safety findings. Consistently with previous studies and other biologics, infections were the most frequent AEs and the patients responded well to standard therapy. There were no deaths, discontinuations nor dose adjustments/or interruptions due to AEs. In 3 out of 19 patients, anti-canakinumab binding antibodies were detected in one of the post-dose visits, however these patients showed no evidence of immunogenicity related AEs or impaired efficacy. The overall safety profile observed in previous canakinumab studies in CAPS was confirmed in this Japanese population including the paediatric and NOMID sub-populations.

The present study has limitations, including the small size of the patient population, the non-controlled design and the relatively short-term observation period, each of which were addressed in previous studies. Additionally, the small sample size and short follow-up period did not allow detailed assessment of side effects related to anti-IL-1 therapy such as malignant disease and autoimmunity. Long-term observation with a large population is needed to address these issues (27).

Conclusion

Canakinumab 150 mg s.c. dosed every 8 weeks proved to be efficacious and provided a convenient dosing regimen for treating Japanese patients with CAPS. Higher canakinumab doses in younger patients and in adult patients with more severe CAPS disease were efficacious in achieving a complete response and were well tolerated without any evidence of increased AEs. While these results for the treatment of CAPS with canakinumab for up to 197 days are encouraging, the long-term safety of canakinumab in CAPS patients will be further evaluated in this ongoing study.

Acknowledgements

The authors would like to thank Novartis Pharma AG, Switzerland, for their financial support. Moreover, they would like to thank co-investigators: Takako Miyamae, Masako Kikuchi, Toshitaka Kizawa and Tomo Nozawa, Yokohama City University, Japan; Takahiro Yasumi, Kyoto University, Japan; Kenji Ihara and Takehiko Doi, Kyushu University, Japan. We also thank Heike Schwende, Novartis Pharma, AG, Basel for editorial assistance and

Kalyan Pulipaka and Raghuraj Puthige, Novartis Healthcare Pvt. Ltd, India for medical writing support.

References

1. AKSENTIJEVICH I, D PUTNAM C, REMMERS EF *et al.*: The clinical continuum of cryopyrinopathies: novel CIAS1 mutations in North American patients and a new cryopyrin model. *Arthritis Rheum* 2007; 56: 1273-85.
2. AGANNA E, MARTINON F, HAWKINS PN *et al.*: Association of mutations in the NALP3/CIAS1/PYPAF1 gene with a broad phenotype including recurrent fever, cold sensitivity, sensorineural deafness, and AA amyloidosis. *Arthritis Rheum* 2002; 46: 2445-52.
3. DODÉ C, LE DÛ N, CUISSET L *et al.*: New mutations of CIAS1 that are responsible for Muckle-Wells syndrome and familial cold urticaria: a novel mutation underlies both syndromes. *Am J Hum Genet* 2002; 70: 1498-506.
4. HOFFMAN HM, MUELLER JL, BROIDE DH, WANDERER AA, KOLODNER RD: Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome. *Nat Genet* 2001; 29: 301-5.
5. FELDMANN J, PRIEUR AM, QUARTIER P *et al.*: Chronic infantile neurological cutaneous and articular syndrome is caused by mutations in CIAS1, a gene highly expressed in polymorphonuclear cells and chondrocytes. *Am J Hum Genet* 2002; 71: 198-203.
6. AKSENTIJEVICH I, NOWAK M, MALLAH M *et al.*: De novo CIAS1 mutations, cytokine activation, and evidence for genetic heterogeneity in patients with neonatal-onset multisystem inflammatory disease (NOMID): a new member of the expanding family of pyrin-associated autoinflammatory diseases. *Arthritis Rheum* 2002; 46: 3340-8.
7. DINARELLO CA: Mutations in cryopyrin: bypassing roadblocks in the caspase 1 inflammasome for interleukin-1beta secretion and disease activity. *Arthritis Rheum* 2007; 56: 2817-22.
8. GATTORNO M, TASSI S, CARTA S *et al.*: Pattern of interleukin-1beta secretion in response to lipopolysaccharide and ATP before and after interleukin-1 blockade in patients with CIAS1 mutations. *Arthritis Rheum* 2007; 56: 3138-48.
9. YAMAZAKI T, MASUMOTO J, AGEMATSU K *et al.*: Anakinra improves sensory deafness in a Japanese patient with Muckle-Wells syndrome, possibly by inhibiting the cryopyrin inflammasome. *Arthritis Rheum* 2008; 58: 864-8.
10. LESLIE KS, LACHMANN HJ, BRUNING E *et al.*: Phenotype, genotype, and sustained response to anakinra in 22 patients with autoinflammatory disease associated with CIAS-1/NALP3 mutations. *Arch Dermatol* 2006; 142: 1591-7.
11. ROSS JB, FINLAYSON LA, KLOTZ PJ *et al.*: Use of anakinra (Kineret) in the treatment of familial cold autoinflammatory syndrome with a 16-month follow-up. *J Cutan Med Surg* 2008; 12: 8-16.
12. HOFFMAN HM, THRONE ML, AMAR NJ *et al.*: Efficacy and safety of rilonacept (interleukin-1 Trap) in patients with cryopyrin-associated periodic syndromes: results from two sequential placebo-controlled studies. *Arthritis Rheum* 2008; 58: 2443-52.
13. WITTKOWSKI H, KUEMMERLE-DESCHNER B, AUSTERMANN J *et al.*: MRP8 and MRP14, phagocyte-specific danger signals, are sensitive biomarkers of disease activity in cryopyrin-associated periodic syndromes. *Arthritis Rheum Dis* 2011; 70: 2075-81.
14. MAKSIMOVIC L, STIRNEMANN J, CAUX F *et al.*: New CIAS1 mutation and anakinra efficacy in overlapping of Muckle-Wells and familial cold autoinflammatory syndromes. *Rheumatology* (Oxford) 2008; 47: 309-10.
15. O'CONNELL SM, O'REGAN GM, BOLGER T *et al.*: Response to IL-1-receptor antagonist in a child with familial cold autoinflammatory syndrome. *Pediatr Dermatol* 2007: 24: 85.
16. HOFFMAN HM: Rilonacept for the treatment of cryopyrin-associated periodic syndromes (CAPS). *Expert Opin Biol Ther* 2009; 9: 519-31.
17. GOLDBACH-MANSKY R, SHROFF SD, WILSON M *et al.*: A pilot study to evaluate the safety and efficacy of the long-acting interleukin-1 inhibitor rilonacept (interleukin-1 Trap) in patients with familial cold autoinflammatory syndrome. *Arthritis Rheum* 2008; 58: 2432-42.
18. LACHMANN HJ, LOWE P, FELIX SD *et al.*: In vivo regulation of interleukin 1beta in patients with cryopyrin-associated periodic syndromes. *J Exp Med* 2009; 206: 1029-36.
19. CHURCH LD, MCDERMOTT MF: Canakinumab, a fully-human mAb against IL-1beta for the potential treatment of inflammatory disorders. *Curr Opin Mol Ther* 2009; 11: 81-9.
20. Novartis. Ilaris prescribing information. http://wwwpharmausnovartiscom/product/pi/pdf/ilarispdf2010, Accessed Feb 17. http://wwwpharmausnovartiscom/product/pi/pdf/ilarispdf
21. 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.
22. http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/DrugandBiologicApprovalReports/PriorityNDAandBLAApprovals/UCM090995.pdfhttp://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/DrugandBiologicApprovalReports/PriorityNDAandBLAApprovals/UCM090995.pdf accessed July 2010.
23. http://www.ema.europa.eu/pdfs/human/opinion/illaris_44782909en.pdfhttp://www.ema.europa.eu/pdfs/human/opinion/illaris_44782909en.pdf.accessed 2010.
24. NEVEN B, PRIEUR AM, QUARTIER DIT MAIRE P.: Cryopyrinopathies: update on pathogenesis and treatment. *Nat Clin Pract Rheumatol* 2008; 4: 481-9.
25. KUEMMERLE-DESCHNER JB, RAMOS E, BLANK N *et al.*: Canakinumab (ACZ885, a fully human IgG1 anti-IL-1β mAb) induces sustained remission in pediatric patients with cryopyrin-associated periodic syndrome (CAPS). *Arthritis Res Ther* 2011; 13: R34 [Epub ahead of print].
26. KUEMMERLE-DESCHNER JB, HACHULLA E, CARTWRIGHT R *et al.*: Two-year results from an open-label, multicentre, phase III study evaluating the safety and efficacy of canakinumab in patients with cryopyrin-associated periodic syndrome across different severity phenotypes. *Ann Rheum Dis* 2011; 70: 2095-102.
27. FDA DRUG SAFETY COMMUNICATION: Early communication about an ongoing safety review of Tumor Necrosis Factor (TNF) blockers (marked as Remicade, Embrel, Humira, and Cimzia).

Mod Rheumatol (2013) 23:425–429
DOI 10.1007/s10165-012-0769-8

REVIEW ARTICLE

Guidance on the use of canakinumab in patients with cryopyrin-associated periodic syndrome in Japan

Shumpei Yokota · Ryuta Nishikomori · Hidetoshi Takada · Masako Kikuchi · Tomo Nozawa · Taichi Kanetaka · Toshitaka Kizawa · Takako Miyamae · Masaaki Mori · Toshio Heike · Toshiro Hara · Tomoyuki Imagawa

Received: 18 June 2012 / Accepted: 5 September 2012 / Published online: 20 October 2012

Abstract Cryopyrin-associated periodic syndrome (CAPS) is an orphan disease with incidence of about one in 1,000,000 persons. This autoinflammatory disease develops in the neonatal period or early childhood, with various inflammatory symptoms occurring repeatedly throughout the patient's lifetime. It is caused by abnormality of the NLRP3 protein which mediates the intracellular signal transduction mechanism of inflammatory processes, resulting in continuous overproduction of interleukin (IL)-1β, which induces chronic inflammation and progressive tissue damage. Definitive diagnosis of CAPS is difficult, and treatment has also been difficult because of a lack of effective medications in Japan. Clinical studies of human anti-human IL-1β monoclonal antibody (canakinumab) treatment were conducted in Japan, and approval was granted for therapeutic use of canakinumab for CAPS in September 2011. Similar to other biological drugs, canakinumab is clinically highly effective. However, sufficient attention to the method of use and adverse drug reactions is necessary. This guidance describes the use of canakinumab in Japan for CAPS in relation to exclusion criteria, method of use, evaluation criteria, and adverse drug reactions.

Keywords Canakinumab · Cryopyrin-associated periodic syndrome · Human anti-human IL-1β monoclonal antibody · Interleukin-1β

S. Yokota (✉) · M. Kikuchi · T. Nozawa · T. Kanetaka · T. Kizawa · T. Miyamae · M. Mori · T. Imagawa
Department of Pediatrics, Yokohama City University School of Medicine, 3-9, Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan
e-mail: syokota@med.yokohama-cu.ac.jp

R. Nishikomori · T. Heike
Department of Pediatrics, Graduate School of Medicine, Kyoto University, Kyoto, Japan

H. Takada · T. Hara
Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Introduction

Cryopyrin-associated periodic syndrome (CAPS) is an autoinflammatory disease that develops in the neonatal period or early childhood, with various inflammatory symptoms. Patients experience recurrent rash, articular symptoms, fever, and headache associated with chronic meningitis, as well as progressive visual and auditory impairment. Many patients have poor prognosis, and a large proportion develop amyloidosis.

CAPS is classified into the following three types according to its symptoms: familial cold autoinflammatory syndrome (FCAS), Muckle–Wells syndrome (MWS), and neonatal-onset multisystem inflammatory disease (NOMID). All types are associated with overproduction of interleukin (IL)-1β, which induces inflammation [1, 2] and chronic tissue damage. The overproduction is caused by a mutation of the *NLRP3* gene [3–6], which mediates responses to infectious agents, tissue damage, and intracellular proteins derived from apoptosis.

The incidence of CAPS is about one in 1,000,000 persons. Definitive diagnosis of CAPS is difficult, and treatment has also been difficult because of a lack of effective medications in Japan. Therefore, clinical studies of human anti-human IL-1β monoclonal antibody (canakinumab) treatment were conducted in Japan, and approval was granted for therapeutic use of canakinumab for CAPS in

September 2011. However, canakinumab can also suppress physiological inflammation by neutralizing the activity of IL-1β. Although canakinumab is clinically highly effective, adverse drug reactions will be carefully monitored.

This guidance describes the use of canakinumab for treatment of CAPS in Japan in relation to exclusion criteria, method of use, evaluation criteria, and adverse drug reactions.

Overview of canakinumab

Canakinumab (Ilaris®; Novartis Pharma K.K.) is a recombinant human immunoglobulin G1 monoclonal antibody against human IL-1β expressed in mouse hybridoma SP2/0-Ag14 cells. It neutralizes the activity of IL-1β by binding to IL-1β and inhibiting binding of IL-1β to its receptor [7]. Clinical studies conducted in Japan and other countries have demonstrated that canakinumab promptly improves various inflammation-related symptoms and abnormal laboratory values in CAPS patients by inhibiting the action of IL-1β [8, 9], and that these effects persist for long time [10].

Canakinumab was approved for treatment of CAPS in the USA and Europe in 2009, and had been approved in at least 50 countries as of August 2011. In Japan, the phase I clinical study in healthy volunteers began in December 2006, and the phase III clinical study in CAPS patients began in October 2009. Canakinumab was designated as an orphan drug in August 2010. Based on the results of the Japanese phase I and phase III clinical studies and overseas clinical studies, canakinumab was approved for treatment of CAPS in September 2011.

Guideline for canakinumab treatment of CAPS patients

Indications and use

Canakinumab is indicated for treatment of CAPS. CAPS is classified according to its severity into three types: FCAS [11], MWS [12], and NOMID [13]. Patients with FCAS, which is considered to be a mild form, experience urticarial rash, fever, conjunctivitis, and other symptoms due to cold stimuli. MWS and NOMID are classified according to differences in the timing of onset and severity of symptoms, but there are no other specific differences between these types. Symptoms include fever, urticarial rash, headache, central nervous system inflammation, arthritis, and amyloidosis, depending on the severity. Inflammatory indices such as C-reactive protein (CRP) and serum amyloid A (SAA) are elevated. If these common findings and the following characteristic findings are observed, CAPS should be suspected, specialists who have experience in the prescription of canakinumab should be consulted, and *NLRP3* genetic testing should be performed.

The age of onset of FCAS [11] is just after birth or in early infancy in about 95 % of cases. Inflammatory episodes including rash, fever, and arthralgia occur repeatedly following cold exposure. Inflammatory reactions may last less than 24 h. Conjunctivitis occurs during inflammatory episodes, but hearing loss, periorbital edema, lymphadenopathy, and serositis are not observed. Concomitant amyloidosis is rare. The *NLRP3* gene is mutated in most patients.

The age of onset of MWS [12] is usually during infancy, but some patients develop the disease in childhood or adolescence. Abnormality of the *NLRP3* gene is detected in 65–75 % of cases. Inflammatory symptoms occur repeatedly due to stress, and persist for almost 3 days. Patients experience fever, rash, arthritis, myalgia, headache, conjunctivitis, and uveitis. Sensorineural hearing loss or hearing impairment occurs in 50–70 % of patients, and renal failure due to amyloidosis occurs in about 25 %.

Inflammatory symptoms occur continuously and repeatedly from soon after birth in NOMID [13]. About half of patients have low birth weight. Patients experience fever, urticarial rash, arthritis, headache, conjunctivitis, and episcleritis almost every day. Headache associated with chronic aseptic meningitis, vomiting, and irritability can also occur. Hydrocephalus, sensorineural hearing loss, psychomotor retardation, growth disorders, joint disorders, and amyloidosis develop in the long term. Joint disorders during the developmental stage lead to gait disturbance. About 20 % of patients have poor prognosis before the age of 20 years, and many patients experience progression to amyloidosis.

Contraindications and careful administration of canakinumab are presented in Table 1. There have been no cases of discontinuation of canakinumab to date.

Predose testing

Patients should be carefully screened for common infections, especially otitis media, sinusitis, and respiratory tract infections (including bronchiectasis). Patients must also be screened for tuberculosis with an interview and the following tests: chest X-ray, tuberculin reaction, chest computed tomography (CT), and QuantiFERON® (QFT). As chest X-ray and tuberculin reaction do not always give a definitive diagnosis, chest CT and/or QFT should be performed as necessary. Chest CT is necessary for all pediatric patients. Patients with history of tuberculosis or a suspected tuberculosis infection should be evaluated by physicians with experience in the treatment of tuberculosis, including pulmonologists and radiologists.

Table 1 Contraindications and careful administration of canakinumab

Contraindications	Careful administration
Patients with serious infection (Infection may worsen)	Patients with infection or suspected infection (Infection may worsen)
Patients with active tuberculosis (Symptoms may worsen)	Patients with history of tuberculosis or suspected tuberculosis infection (Tuberculosis may be activated)
Patients with history of hypersensitivity to any of the ingredients of canakinumab	Patients with history of recurring infection (Infection may recur)
	Immunocompromised patients (Infection may be induced)

Canakinumab should only be given after the administration of an antituberculous drug in patients who meet any of the followings criteria:

- Patients with shadows consistent with or indicative of old tuberculosis on chest imaging
- Patients with history of treatment for tuberculosis (including extrapulmonary tuberculosis)
- Patients strongly suspected of having tuberculosis infection in a tuberculin test or interferon gamma response test (QFT)
- Patients with history of close contact with a tuberculosis patient

Dosage and administration (Fig. 1)

Canakinumab is usually administered at 2 mg/kg for CAPS patients with body weight ≤40 kg or 150 mg for body weight >40 kg, every 8 weeks as a single dose via subcutaneous injection.

If satisfactory clinical response (resolution of rash and other generalized inflammatory symptoms) has not been achieved, the dose should be gradually increased as appropriate. Maximum dose is 8 mg/kg for body weight ≤40 kg or 600 mg for body weight >40 kg [10].

If the patient experiences relapse within 8 weeks after an administration with the maximum dose, an increase of dosing frequency of up to every 4 weeks can be considered.

The dose may be adjusted according to the condition.

Treatment evaluation

Remission criteria (clinical and serological remission) and relapse criteria (clinical and serological relapse) were used in the Japanese clinical studies to evaluate the therapeutic effects of canakinumab in patients with CAPS (Table 2). Clinical remission and relapse were evaluated using the following five levels of symptoms: absent, minimal, mild, moderate, and severe. These five levels of evaluations should be evaluated based on physician's assessment because there are no criteria for each level.

Evaluations of adverse reactions

In clinical studies in Japan, adverse drug reactions occurred in 12 of 19 subjects (63.2 %). Common adverse reactions were nasopharyngitis in three subjects (15.8 %) and stomatitis in two subjects (10.5 %). In clinical studies in other countries, adverse drug reactions occurred in 68 of 169 subjects (40.2 %). Common adverse reactions were headache in seven subjects (4.1 %), weight gain in seven subjects (4.1 %), vertigo in six subjects (3.6 %), and bronchitis in five subjects (3.0 %).

Canakinumab may affect the inflammatory and immunological reactions to viruses, bacteria, and *Mycobacterium tuberculosis* by inhibiting the action of IL-1β, which may lead to worsening of infection. In clinical studies conducted in Japan and other countries, infections including upper respiratory tract infection were reported frequently, and some of these infections were serious. Patients should therefore be carefully monitored for the occurrence, recurrence, and exacerbation of infection during canakinumab therapy.

Immunization during canakinumab therapy

Inactivated vaccines may be administered during canakinumab therapy. Live vaccines should not be given, because a risk of developing infection cannot be ruled out. It is desirable to administer necessary vaccines prior to canakinumab therapy.

Caution

Canakinumab must be used with strict adherence to the indications and contraindications. It is recommended that canakinumab be used by physicians with appropriate

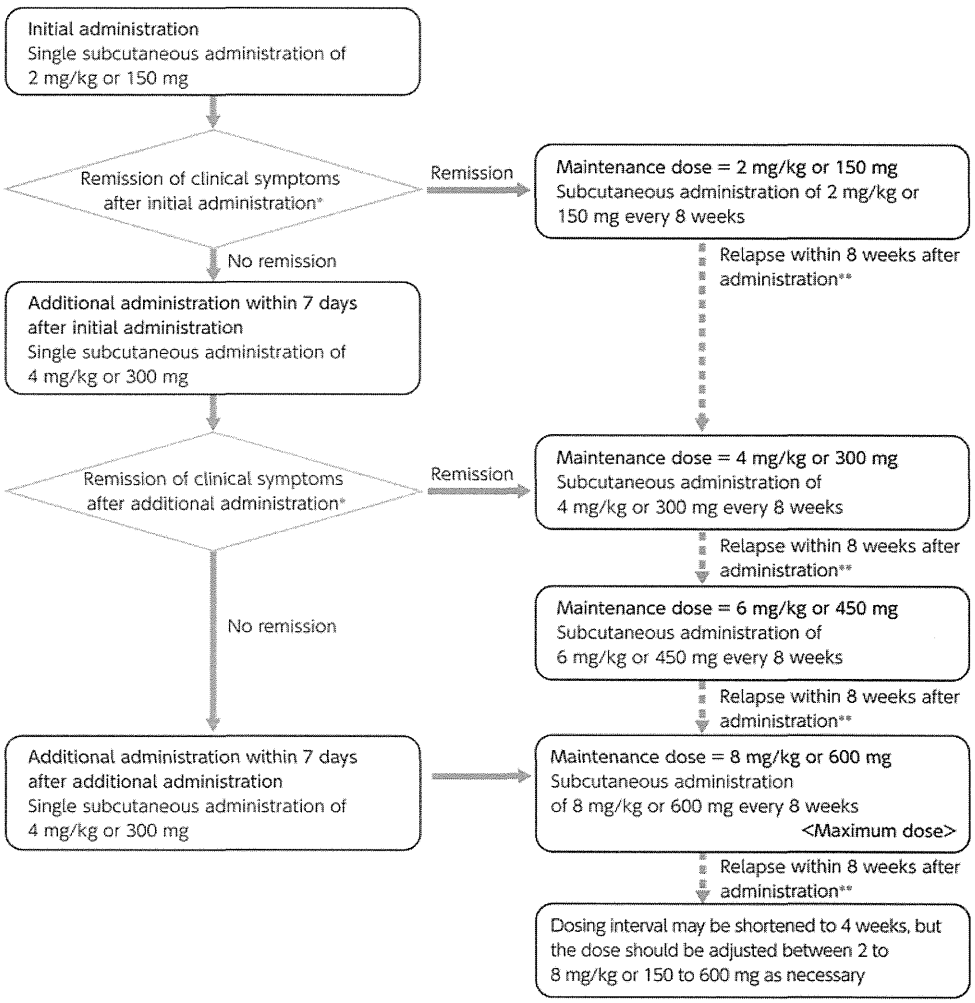


Fig. 1 Dosing regimen of canakinumab (Ilaris®) for CAPS patients [10]. Dosing regimen for CAPS patients who do not experience sufficient symptomatic relief. If sufficient clinical effects are not observed with the initial dose, the dose should be increased as shown until clinical effects are observed. The dose at which effects are observed should be the maintenance dose. * Criteria for remission in Japanese clinical studies. ** Criteria for relapse in Japanese clinical studies (modified from Ilaris® Product Information, Novartis Pharma K.K.)

Table 2 Criteria for remission and relapse used in clinical studies in Japan to evaluate the therapeutic effects of canakinumab in patients with CAPS

Remission: If the following criteria are all met, the disease is considered to have remitted	Relapse: If the following criteria are both met, the disease is considered to have relapsed
Clinical remission	Clinical relapse
Overall evaluation of autoinflammatory disease activity by physicians is minimal or lower Evaluation of skin disease is minimal or lower	Overall evaluation of autoinflammatory disease activity by physicians is mild or higher, or overall evaluation of autoinflammatory disease activity by physicians is minimal and evaluation of skin disease is mild or higher
Serological remission	Serological relapse
CRP is less than 10 mg/L (=1 mg/dL) or SAA is less than 10 mg/L (=10 μg/mL)	CRP is higher than 30 mg/L (=3 mg/dL) or SAA is higher than 30 mg/L (=30 μg/mL)

Evaluation grades are in 5 levels: absent, minimal, mild, moderate, and severe. These five levels of evaluations should be evaluated based on physician's assessment because there are no criteria for each level

education, in cooperation with physicians who have experience in the treatment of CAPS. It is the responsibility of pediatricians to optimize the effects of pharmaceutical products and minimize adverse drug reactions.

Acknowledgments The Japanese version of this work was published as a similar report in the Journal of the Japanese Pediatric Society.

Conflict of interest None.

References

1. Dinarello CA. Mutations in cryopyrin: bypassing roadblocks in the caspase 1 inflammasome for interleukin-1beta secretion and disease activity. Arthritis Rheum. 2007;56:2817–22.
2. Gattorno M, Tassi S, Carta S, et al. Pattern of interleukin-1beta secretion in response to lipopolysaccharide and ATP before and after interleukin-1 blockade in patients with CIAS1 mutations. Arthritis Rheum. 2007;56:3138–48.
3. Aganna E, Martinon F, Hawkins PN, et al. Association of mutations in the NALP3/CIAS1/PYPAF1 gene with a broad phenotype including recurrent fever, cold sensitivity, sensorineural deafness, and AA amyloidosis. Arthritis Rheum. 2002;46:2445–52.
4. Dodé C, Le Dû N, Cuisset L, et al. New mutations of CIAS1 that are responsible for Muckle–Wells syndrome and familial cold urticaria: a novel mutation underlies both syndromes. Am J Hum Genet. 2002;70:1498–506.
5. Hoffman HM, Mueller JL, Broide DH, Wanderer AA, Kolodner RD. Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle–Wells syndrome. Nat Genet. 2001;29:301–5.
6. Feldmann J, Prieur AM, Quartier P, et al. Chronic infantile neurological cutaneous and articular syndrome is caused by mutations in CIAS1, a gene highly expressed in polymorphonuclear cells and chondrocytes. Am J Hum Genet. 2002;71:198–203.
7. Church LD, McDermott MF. Canakinumab, a fully-human mAb against IL-1beta for the potential treatment of inflammatory disorders. Curr Opin Mol Ther. 2009;11:81–9.
8. 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.
9. Kuemmerle-Deschner JB, Ramos E, Blank N et al.: Canakinumab (ACZ885, a fully human IgG1 anti-IL-1ß mAb) induces sustained remission in pediatric patients with cryopyrin-associated periodic syndrome (CAPS). Arthritis Res Ther. 2011;13(1):R34 (Epub ahead of print).
10. Kuemmerle-Deschner JB, Hachulla E, Cartwright R, et al. Two-year results from an open-label, multicentre, phase III study evaluating the safety and efficacy of canakinumab in patients with cryopyrin-associated periodic syndrome across different severity phenotypes. Ann Rheum Dis. 2011;70(12):2095–102.
11. Hoffman HM, Wanderer AA, Broide DH. Familial cold autoinflammatory syndrome: phenotype and genotype of an autosomal dominant periodic fever. J Allergy Clin Immunol. 2001;108:615–20.
12. Muckle TJ, Wells M. Urticaria, deafness, and amyloidosis: a new heredo-familial syndrome. Q J Med. 1962;31:235–48.
13. Prieur AM, Griscelli C, Lampert F, Truckenbrodt H, Guggenheim MA, Lovell DJ, et al. A chronic, infantile, neurological, cutaneous and articular (CINCA) syndrome. A specific entity analysed in 30 patients. Scand J Rheumatol Suppl. 1987;66:57–68.

PEDIATRICS INTERNATIONAL Official Journal of the Japan Pediatric Society

Pediatrics International (2013) **55**, 801–802 doi: 10.1111/ped.12225

Brief Report

Development of Kawasaki disease in a patient with PFAPA

Takahito Ninomiya, Hidetoshi Takada, Yusaku Nagatomo, Etsuro Nanishi, Hazumu Nagata, Kenichiro Yamamura, Takehiko Doi, Kazuyuki Ikeda and Toshiro Hara

Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Abstract Periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis syndrome (PFAPA) is one of the autoinflammatory diseases of unknown etiology characterized by regularly recurrent fever episodes with attacks lasting 3–6 days every 3–8 weeks associated with at least one of the three cardinal clinical signs: aphthous stomatitis, pharyngitis, and cervical adenitis. Kawasaki disease (KD) is an acute, self-limited systemic vasculitis that occurs predominantly in infants and young children. In most KD patients, i.v. immunoglobulin leads to a rapid amelioration of clinical symptoms and significantly decreases the risk of coronary artery aneurysms. Although the etiology of KD is still unknown, it was reported that innate immunity was activated in the patients. Described herein is a patient with PFAPA who developed KD. This is the first report of KD development in a PFAPA patient. The association between KD and PFAPA may represent a genetic predisposition to dysregulated innate immune response.

Key words: aphthous stomatitis, innate immunity, Kawasaki disease, periodic fever, pharyngitis and cervical adenitis syndrome.

Case report

A 2-year-old girl was admitted to hospital because of fever, peripheral rash, cervical lymphadenopathy, injection of bulbar conjunctiva and peripheral edema. She had been receiving 150 mg of cimetidine every day since she was 1 year old after being diagnosed as having periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis syndrome (PFAPA).[1,2] The manifestation of her PFAPA was typical and fulfilled the diagnostic criteria.[3] Usually, her fever attacks were accompanied by cervical lymphadenopathy, enlarged tonsils, and pharyngitis. The last fever attack of PFAPA occurred 1 month before her admission, although there were no fever attacks for 2 months before it (Fig. 1).

At the time of admission, her clinical signs fulfilled the diagnostic criteria of Kawasaki disease (KD; Fig. 1),[4] and the manifestation lacked the primary symptoms of PFAPA including enlarged tonsils and pharyngitis. Laboratory results were as follows: white blood cell count, 17 560/mm^3 (neutrophils 43.9%); platelet count, 238 000/mm^3; and C-reactive protein (CRP) concentration, 4.69 mg/dL. On days 3 and 5 of illness, 2 g/kg i.v. immunoglobulin (IVIG) was given (Fig. 1).[5,6] The symptoms seemed to gradually subside thereafter. Although the symptoms such as injection of bulbar conjunctiva, peripheral edema and rash subsided, high fever and cervical lymphadenopathy continued with elevated serum CRP level. Her high fever persisted even after infliximab on day 6 and additional IVIG therapy on day 7.

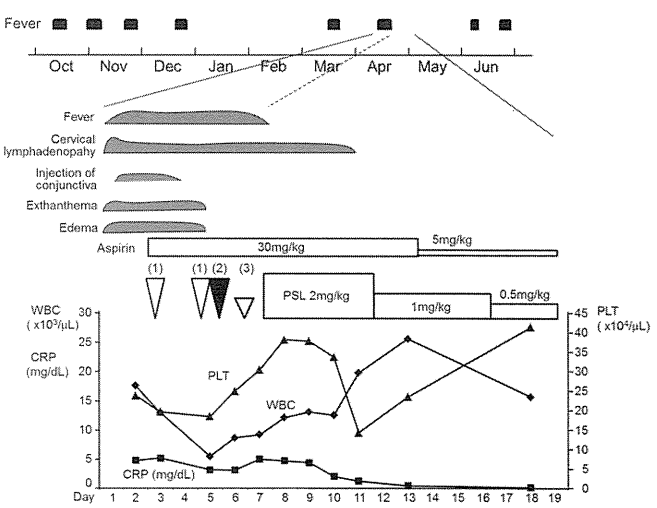


Fig. 1 Clinical course. The patient had Kawasaki disease (KD) in the course of recurrent fever attacks of periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis syndrome (PFAPA). Although the symptoms of KD other than fever and cervical lymphadenopathy subsided after treatment with i.v. immunoglobulin (IVIG) and infliximab, high fever continued. After starting corticosteroid, fever subsided rapidly. 1, IVIG (2 g/kg); 2, infliximab (5 mg/kg); 3, IVIG (1 g/kg); CRP, C-reactive protein; PLT, platelet count; PSL, XXX; WBC, white blood cell count.

The patient had slightly enlarged palatine tonsils without injection or exudates and mild cervical lymphadenopathy other than high fever, and she had no aphthous stomatitis, at this point. We suspected that her fever originated from PFAPA rather than from KD, because other symptoms of KD had subsided.

Correspondence: Hidetoshi Takada, MD PhD, Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, 3-1-1, Maidashi Higashi-ku, Fukuoka 810-8582, Japan. Email: takadah@pediatr.med.kyushu-u.ac.jp

Received 12 May 2013; revised 26 July 2013; accepted 10 September 2013.

Therefore, 2 mg/kg per day prednisolone (PSL) was started from day 8 of the illness. Nonetheless, because we could not completely deny the possibility of KD as the cause of her fever, PSL was used according to the Clinical Guideline for Medical Treatment of Acute Stage Kawasaki Disease from Scientific Committee, the Japanese Society of Pediatric Cardiology and Cardiac Surgery, 2012 (Fig. 1).

Shortly after starting corticosteroid treatment, rapid decline of fever was observed as we expected (Fig. 1). The patient had neither coronary complication during or after the course of the disease nor recurrence of KD. The patient still had PFAPA attacks at 4 years of age.

Discussion

This is the first report of a PFAPA patient who developed KD. Broderick *et al.* reported that four children with a history of KD developed PFAPA.[7] They noted that the incidence of four patients with PFAPA (4.7%) among 84 KD patients in San Diego County, seemed to be higher than expected, because the morbidity of PFAPA was estimated to be 9–34 in 100 000 children younger than 18 years. Therefore, they suggested that these patients might have a genetic propensity toward altered immune response and autoinflammatory syndrome. The present study supports their results with regard to the primary association of KD and PFAPA, because in the present patient, development of PFAPA was independent of the possible secondary dysregulation of innate immunity caused by KD.

Adenotonsillectomy or oral cimetidine has been reported to be effective in PFAPA, and a PFAPA attack can be treated with PSL (0.5–2.0 mg/kg), suggesting that the symptoms are caused by inflammatory cytokines, which are the product of dysregulated innate immunity rather than of infectious etiology.[2] PSL was dramatically effective for the fever in the present patient when the other symptoms of KD had subsided as a result of IVIG treatment, which led us to make a diagnosis of PFAPA rather than KD as the cause of fever at that time.

In contrast to PFAPA, T-helper 1 (Th1) cells do not seem to play an important role in the pathogenesis of KD.[8] Ikeda *et al.* reported an increased NOD-like receptor (NLR) mRNA level in the peripheral blood of KD patients.[8] In addition, Nishio *et al.* reported that *in vivo* injection of NLR ligand into mice caused KD-like coronary artery lesions.[9] These results suggest that dysregulated innate immune response plays an important role in the pathophysiology of KD. In contrast, epidemiologic data suggest the presence of host genetic factors in KD.[10]

We therefore suggest that the association between KD and PFAPA may represent a genetic predisposition to dysregulated innate immune response, although it cannot be denied that KD could have occurred in this PFAPA patient by chance. The recent increased awareness of disorders of innate immunity including PFAPA, may lead to this combination being recognized more commonly.

References

1 Marshall GS, Edwards KM, Butler J, Lawton AR. Syndrome of periodic fever, pharyngitis, and aphthous stomatitis. *J. Pediatr.* 1987; **110**: 43–6.
2 Stojanov S, Lapidus S, Chitkara P *et al.* Periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA) is a disorder of innate immunity and Th1 activation responsive to IL-1 blockade. *Proc. Natl Acad. Sci. U.S.A.* 2011; **108**: 7148–53.
3 Thomas KT, Feder HM Jr, Lawton AR, Edwards KM. Periodic fever syndrome in children. *J. Pediatr.* 1999; **135**: 15–21.
4 Kawasaki T. [Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children]. *Arerugi* 1967; **16**: 178–222.
5 Newburger JW, Takahashi M, Gerber MA *et al.* Diagnosis, treatment, and long-term management of Kawasaki disease: A statement for health professionals from the Committee on Rheumatic Fever, Endocarditis and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. *Circulation* 2004; **110**: 2747–71.
6 Durongpisitkul K, Gururaj VJ, Park JM, Martin CF. The prevention of coronary artery aneurysm in Kawasaki disease: A meta-analysis on the efficacy of aspirin and immunoglobulin treatment. *Pediatrics* 1995; **96**: 1057–61.
7 Broderick L, Tremoulet AH, Burns JC, Bastian JF, Hoffman HM. Recurrent fever syndromes in patients after recovery from Kawasaki syndrome. *Pediatrics* 2011; **127**: e489–93.
8 Ikeda K, Yamaguchi K, Tanaka T *et al.* Unique activation status of peripheral blood mononuclear cells at acute phase of Kawasaki disease. *Clin. Exp. Immunol.* 2010; **160**: 246–55.
9 Nishio H, Kanno S, Onoyama S *et al.* Nod1 ligands induce site-specific vascular inflammation. *Arterioscler. Thromb. Vasc. Biol.* 2011; **31**: 1093–9.
10 Onouchi Y. Genetics of Kawasaki disease: What we know and don't know. *Circ. J.* 2012; **76**: 1581–6.

J Infect Chemother (2013) 19:1210–1213
DOI 10.1007/s10156-013-0629-2

CASE REPORT

Neonatal hemophagocytic lymphohistiocytosis associated with a vertical transmission of coxsackievirus B1

Mitsuharu Fukazawa · Takayuki Hoshina · Etsuro Nanishi · Hisanori Nishio · Takehiko Doi · Shouichi Ohga · Toshiro Hara

Received: 3 March 2013 / Accepted: 27 May 2013 / Published online: 12 June 2013

Abstract Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening syndrome characterized by fever, cytopenias, hepatosplenomegaly, and coagulopathy with the background of hypercytokinemia. Early diagnosis and etoposide therapy are not established for affected newborns. An afebrile infant suffered from apnea 4 days after birth, showing leukocytosis, thrombocytopenia, coagulopathy, and cerebrospinal fluid pleocytosis. Serum levels of ferritin and sIL-2R were high. Bone marrow studies revealed activated/hemophagocytosing macrophages. Coxsackievirus B1 (CB1) was isolated from the throat and stool. Serum anti-CB1 antibody titers were elevated in the patient (4 → 16; 6 → 43 days after birth) and mother (128; 10 days after delivery). Normal expressions of perforin and CD107a precluded inherited HLH. The vertically transmitted CB1-HLH was successfully treated without administration of corticosteroid, cyclosporine, or etoposide. Serum cytokine levels showed dominant expression of monokines (IL-1β/6/8, and TNF-α) but not IFN-γ, which is the central player of inherited HLH. The cytokine profile might represent a unique pathophysiology of enterovirus-driven neonatal HLH.

Keywords Neonate · Hemophagocytic syndrome · Enterovirus · Cytokine · Interferon-γ · Interleukin-10

M. Fukazawa · T. Hoshina (✉) · E. Nanishi · H. Nishio · T. Doi · S. Ohga · T. Hara
Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi Higashi-ku, Fukuoka 812-8582, Japan
e-mail: hoshina@pediatr.med.kyushu-u.ac.jp

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening syndrome characterized by high fever, cytopenias, hepatosplenomegaly, coagulopathy, and bone marrow hemophagocytosis [1]. The familial form of HLH (FHL) arises from the genetic defects of *PRF1*, *UNC13D*, *STX11*, *STXBP2*, and *RAB27A*, which are responsible for the cytotoxic function of T cells and natural killer (NK) cells. The acquired form of HLH occurs in association with infection, malignancy, or autoimmunity. Although HLH patients may have a continuum of genetic predisposition [2], uncontrolled immune activation, mostly triggered by infectious agents, accounts for the common pathway to hypercytokinemia in both forms of HLH. Newborn onset of HLH is difficult to diagnose because of uncovered fever and perinatal infections with a variety of pathogens. The major etiologies of HLH in neonatal cases comprise FHL and herpes simplex virus (HSV) infection, both of which predict poor outcomes [3]. Enterovirus is the other causative agent of neonatal HLH, which does not always lead to an aggressive course [4–6]. The virus-dependent pathogenesis and prognosis of neonatal HLH have not been clarified. We herein report a neonatal case of coxsackievirus B1 (CB1)-driven, secondary HLH. The pathophysiology of neonatal HLH is discussed with special reference to cytokine profiles.

Case report

A vigorous male infant weighing 2,260 g at 35 weeks of gestation was born by cesarean section. His mother had complained of abdominal pain the day before delivery and developed a fever on the delivery day. The infant was

urgently delivered because placental abruption was suspected by the findings of transvaginal ultrasonography. His father and brother became febrile on the day of delivery. The infant developed apnea 4 days after birth. The laboratory findings showed leukocytosis, thrombocytopenia, coagulopathy, and neutrophil pleocytosis (483/μl) in the cerebrospinal fluid (CSF). Bacterial cultures of the patient's blood and CSF were negative. Administration of antimicrobial agents and anticoagulant therapy with heparin did not control the apnea. The patient was transferred to our hospital for further investigation and intensive care.

On admission, the infant showed apnea and jitteriness, but not fever. There were no physical anomalies or skin eruptions. The cardiopulmonary sounds were normal. The liver and spleen were not enlarged, and his neurological reflexes were unremarkable. Complete blood cell counts showed a leukocyte count of 33.97 × 10^9/l with 38 % segmented neutrophils and 34 % lymphocytes, a hemoglobin concentration of 12.3 g/dl, and a platelet count of 55 × 10^9/l. Blood chemistries showed a lactate dehydrogenase level of 8,426 U/l [reference range (rr), 119–229], triglyceride level of 35 mg/dl (rr, 30–149), ferritin level of 35,404 ng/ml (rr, 39.9–465.0), and soluble IL-2 receptor level of 1,830.5 U/l (rr, 206.0–713.0). Coagulation studies showed a fibrinogen level of 66 mg/dl (rr, 150–400), a prothrombin time of 21.7 s (rr, 10.0–13.5), and a fibrinogen/fibrin degradation product level of 138.4 μg/ml (rr, <5.0). Both the proportion (0.77 %) and activity (6.8 % lysis; rr, 20.8–40.8) of NK cells were depressed. The proportion of activated $CD8^+$ T cells was 5 %. Bone marrow aspiration revealed appreciable numbers of activated and/or hemophagocytosing macrophages (Fig. 1, upper). Because hepatosplenomegaly emerged after admission, the patient fulfilled the diagnostic guideline for HLH [3]. Flow cytometric analyses showed normally expressed protein levels of perforin and CD107a in $CD56^+$ cells and those of MUNC13-4 and STX11 in platelets. There were no metabolic diseases or immunodeficiencies of known cause. These results precluded the genetic cause of HLH. CB1 was isolated from cultured specimens of pharyngeal swabs and the stool of the patient. A CSF polymerase chain reaction (PCR) analysis was positive for enterovirus. PCR analyses of the peripheral blood were all negative for HSV, cytomegalovirus, and Epstein–Barr virus (EBV). The

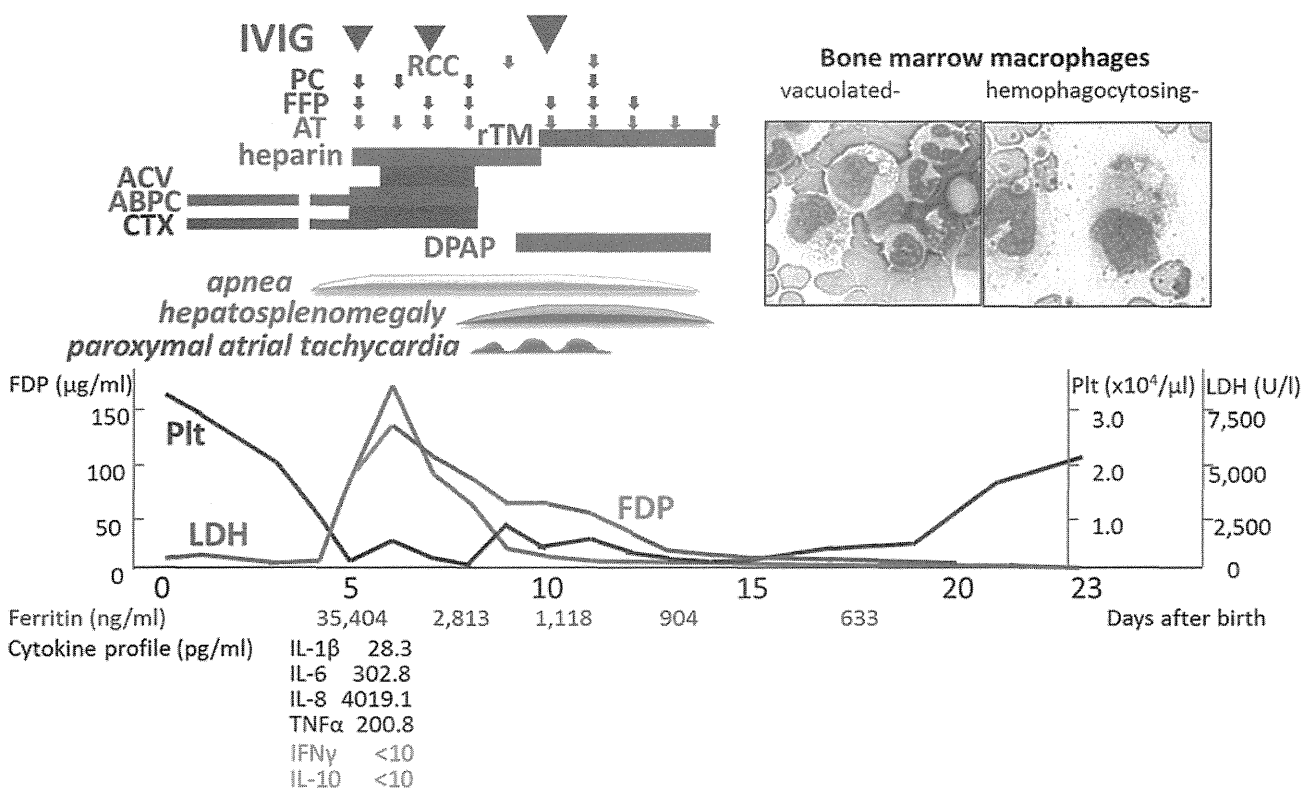


Fig. 1 Treatment course of newborn infant who developed a vertical transmission of coxsackievirus B1-driven hemophagocytic lymphohistiocytosis (HLH). At the diagnosis of HLH, high levels of monokines [interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α] and undetectable levels of interferon (IFN)-γ and IL-10 (<10 pg/ml) were determined assessed by cytometric bead array kit (BD Biosciences: detectable range, 20–5,000 pg/ml). There was no increased proportion of activated T cells in peripheral blood at diagnosis. *ABPC* ampicillin, *ACV* acyclovir, *AT* antithrombin, *CTX* cefotaxime, *DPAP* demand positive airway pressure, *FDP* fibrinogen/fibrin degradation product, *FFP* fresh frozen plasma, *IVIG* intravenous immunoglobulin, *LDH* lactate dehydrogenase, *PC* platelet concentrates, *Plt* platelet, *RCC* red cell concentrates, *rTM* recombinant thrombomodulin

neutralizing antibody titer against CB1 was elevated four-fold in the paired sera of infant (4 on the 6th day of life and 16 on the 43rd day of life) and that in his mother was 128 on the 10th day after delivery. Although the CB1-neutralizing antibody titers of the patient might be affected by blood transfusion, the patient was diagnosed as having vertically transmitted CB1-driven HLH because CB1 was isolated from pharyngeal swabs and stool of the patient. Serum cytokine concentrations were assessed by cytometric bead array kit (BD Biosciences Pharmingen, San Diego, CA, USA; detectable range, 20–5,000 pg/ml). At the diagnosis of HLH, high levels of interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α (28.3, 302.8, 4,019.1, and 200.8 pg/ml, respectively) and undetectable levels of interferon (IFN)-γ and IL-10 (<10 pg/ml) were determined (Fig. 1). Respiratory assists were required for the patient because of repeated events of paroxysmal atrial tachycardia. High-dose immunoglobulin (1 g/kg/dose, twice) and anticoagulant therapy with recombinant thrombomodulin in addition to blood transfusions successfully controlled the disease. The infant was discharged from the hospital 22 days after birth. He exhibited normal growth and development without any recurrence of HLH at 11 months of age.

Discussion

Enteroviruses cause severe neonatal infections, including meningoencephalitis and myocarditis. Group B coxsackieviruses and echovirus 11 are the most frequent agents [7]. Five of nine neonatal cases of enterovirus-associated HLH are shown in detail in Table 1 [4, 5]. No serotypes of enterovirus were identified in these reported cases. All patients, including three afebrile infants, were symptomatic within 5 days of birth. Four of these five patients survived without the administration of etoposide, although the other patient, who underwent etoposide-based immunotherapy, died. The prognoses in the enterovirus-associated cases appeared to be better than those observed in the cases of neonatal-onset FHL or HSV-HLH. Early diagnosis and etoposide therapy have improved the outcome of patients with FHL or EBV-HLH. Prompt administration of immunosuppressive and antiviral drugs appear to be recommended for the effective control of HLH in young infants [6]. However, we should pay more attention to the toxicity of etoposide in the early newborn infants. High-dose intravenous immunoglobulin therapy has been successfully used mainly in virus-associated HLH. It induces phagocyte Fc receptor blockade, downregulates immunoglobulin synthesis and immune stimulation, and can reduce the immunological response and prevent the excessive cytokine release involved in HLH [8]. Clinical resolution was

Table 1 Reported cases of neonatal hemophagocytic lymphohistiocytosis (HLH) associated with an enterovirus infection

Pt, sex	Pregnancy course	Birth				Mother		Patient			Treatment	Outcome	Reference
		GA (weeks)	BW (g)	Mode of delivery	Asphyxia	Days at onset before delivery	Manifestation	Days at onset after birth	Manifestation	Virus isolation			
1, M	Normal	39	2,900	VD	No	0	Diarrhea	0	Fever	Blood, CSF	tr, Ig	Alive	[4]
2, F	Normal	41	3,120	C-sec	No	14	Fever, chill	0	Not doing well	Blood, CSF	tr, Ig	Alive	[5]
3, M	Normal	37	4,010	C-sec	No	0	Fever, chill	3	Fever	Blood	tr, Ig, LT	Alive	[5]
4, M	Normal	38	3,550	VD	No	NR	NR	5	Cyanosis	Blood	tr, CT x	Death	[5]
5, M	Normal	35	2,260	C-sec	No	0	Fever	4	Apnea	Pharynx, stool CSF	tr, Ig	Alive	Our case

Other neonatal cases with enterovirus-associated HLH were reported by Suzuki et al. ($n = 1$) [6] and Imashuku ($n = 4$) (Eur J Pediatr 2005;164:315–319). However, clinical information pertaining to the previous five cases was not fully described in the reports

Pt patient, *GA* gestational age, *BW* birth weight, *tr* transfusions, *Ig* intravenous immunoglobulin, *NR* not recorded, *C-sec* cesarean section, *VD* vaginal delivery, liver transplantation, *CTx* etoposide-based chemotherapy