キネレット皮下注 100 mg シリンジ

＜アナキンラ（遺伝子組換え）＞

第 2 部（モジュール 2）
CTD の概要（サマリー）

2.6　非臨床試験の概要文及び概要表

2.6.7　毒性試験概要表

Swedish Orphan Biovitrum Japan 株式会社

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2.6.7 毒性試験概要表

2.6.7.1 毒性試験：一覧表

Type of study	Test system (species and strain)	Method of administration	Duration of dosing (frequency)	Dose (mg/kg/day)	GLP[a]	Testing facility	Study number	eCTD location
Single-dose toxicity								
Acute intravenous toxicity study of IL-1Ra in rats	Rat, CD® BR VAF/Plus®	i.v.	Single dose	0, 1.5, 15, 150, 720	Yes	[redacted]	SY05-T94-01	[4.2.3.1]
Acute intravenous toxicity study of IL-1Ra in cynomolgus monkeys	Monkey, cynomolgus (*Macaca fascicularis*)	i.v.	Single dose	0, 1.5, 15, 150	Yes	[redacted]	SY05-T94-02	[4.2.3.1]
Repeat-dose toxicity								
Subchronic (2 week, b.i.d.) subcutaneous toxicity study of IL-1Ra in Sprague-Dawley rats	Rat, Sprague-Dawley CD® (SD) BR	s.c.	2 weeks (b.i.d.)	0, 5, 20, 80	Yes	[redacted]	SY05-T89-10	[4.2.3.2]
Subchronic high-dose subcutaneous toxicity (q.i.d.) study of IL-1Ra in Sprague-Dawley rats	Rat, Sprague-Dawley	s.c.	2 weeks (q.i.d.)	0, 120	Yes	[redacted]	SY05-T89-12	[4.2.3.2]

Type of study	Test system (species and strain)	Method of administration	Duration of dosing (frequency)	Dose (mg/kg/day)	GLP[a]	Testing facility	Study number	eCTD location
Subcutaneous 6-month toxicity study in rats treated with anakinra, including 1-month interim and 1-month recovery groups	Rat, Sprague-Dawley	s.c.	1 and 6 months (b.i.d.)	0, 2, 20, 200	Yes	[redacted] Canada	SY05-T94-04	[4.2.3.2]
Intravenous (2 week) systemic toxicity study of IL-1Ra in Sprague-Dawley rats	Rat, Sprague-Dawley	i.v.	2 weeks	0, 0.047, 0.231, 1.17	Yes	[redacted]	SY05-T89-01	[4.2.3.2]
A subchronic (2 week) intravenous toxicity study of IL-1Ra in Sprague-Dawley rats	Rat, Sprague-Dawley	i.v.	2 weeks	0, 2, 5, 20	Yes	[redacted]	SY05-T89-02	[4.2.3.2]
A 4-week toxicity and toxicokinetic study of subcutaneously administered anakinra and orally administered methotrexate in rats	Rat, Crl:CD (SD)BR VAF/Plus	s.c. anakinra; p.o. MTX	4 weeks (q.d.)	Anakinra - 0, 1, 10, 100; MTX - 0, 0.04, 0.2	Yes	[redacted]	960048	[4.2.3.2]
Subchronic (2 week) intravenous toxicity study of IL-1Ra in Sprague-Dawley rats	Rat, Sprague-Dawley	i.v.	2 weeks	0, 3, 10, 30	Yes	[redacted]	SY05-T89-05	[4.2.3.2]

Type of study	Test system (species and strain)	Method of administration	Duration of dosing (frequency)	Dose (mg/kg/day)	GLP[a]	Testing facility	Study number	eCTD location
Subchronic (2 week) intravenous toxicity study of IL-1Ra in Sprague-Dawley rats	Rat, Sprague-Dawley	i.v.	2 weeks	0, 30	Yes	[redacted]	SY05-T89-09	[4.2.3.2]
A 2-week nose-only inhalation toxicity study of recombinant human IL-1Ra in the rat	Rat, Sprague-Dawley	Inhalation	2 weeks	0, 0.33, 3.2	Yes	[redacted]	SY05-T92-01	[4.2.3.2]
Subchronic (2 week, b.i.d) systemic toxicity study of IL-1Ra in male and female rhesus monkeys following repeated subcutaneous injections	Monkey, rhesus (*Macaca mulatta*)	s.c.	2 weeks (b.i.d.)	0, 5, 20, 80	Yes	[redacted]	SY05-T89-11	[4.2.3.2]
A 4-week toxicity study of IL-1Ra administered by subcutaneous injection to rhesus monkeys with a 4-week recovery period	Monkey, rhesus (*Macaca mulatta*)	s.c.	4 weeks	0,10, 100, 200	Yes	[redacted]	960040	[4.2.3.2]
1-week intravenous infusion study on IL-1Ra in male rhesus monkeys	Monkey, rhesus (*Macaca mulatta*)	i.v. (CI)	1 week	0, 150	Yes	[redacted]	SY05-T90-02	[4.2.3.2]

Type of study	Test system (species and strain)	Method of administration	Duration of dosing (frequency)	Dose (mg/kg/day)	GLP[a]	Testing facility	Study number	eCTD location
A range-finding (2 week) toxicity study of IL-1Ra in male cynomolgus monkeys following repeated intravenous injections	Monkey, cynomolgus (*Macaca fascicularis*)	i.v.	2 weeks	0, 0.1, 1, 10	Yes	[redacted]	SY05-T89-03	[4.2.3.2]
Subchronic systemic toxicity study with recovery period of IL-1Ra in rhesus monkeys	Monkey, rhesus (*Macaca mulatta*)	i.v.	2 weeks (b.i.d.)	0, 3, 10, 30	Yes	[redacted]	SY05-T89-08	[4.2.3.2]
Genotoxicity								
Salmonella/Mammalian -microsome plate incorporation mutagenicity assay (Ames test) and *Escherichia coli* WP2 *uvr*A reverse mutation assay with a confirmatory assay - IL-1Ra	*Escherichia coli*; *Salmonella typhimurium*	In vitro	N/A	0.1 to 5000 µg/plate (dose-range-finding assay); 33 to 5000 µg/plate (mutagenicity and confirmatory assays)	Yes	[redacted]	SY05-T92-13	[4.2.3.3.1]
In vitro mammalian cytogenetics test - IL-1Ra	CHO cells	In vitro	N/A	0.2 to 2000 µg/mL (initial assay); 250, 500, 1000, and 2000 µg/mL (repeat assay)	Yes	[redacted]	SY05-T92-15	[4.2.3.3.1]

Type of study	Test system (species and strain)	Method of administration	Duration of dosing (frequency)	Dose (mg/kg/day)	GLP[a]	Testing facility	Study number	eCTD location
L5178Y thymidine kinase +/- mouse lymphoma mutagenesis assay with a confirmatory assay - IL-1Ra	Mouse lymphoma (L5178Y thymidine kinase +/-)	In vitro	N/A	67 to 5000 µg/mL	Yes	[redacted]	SY05-T92-14	[4.2.3.3.1]
Micronucleus cytogenetics assay in mice - IL-1Ra	Mouse, ICR	In vivo	Single dose	0, 500, 1000, 2000	Yes	[redacted]	SY05-T92-16	[4.2.3.3.2]
Carcinogenicity								
No carcinogenicity studies were performed.								
Reproductive and developmental toxicity								
Fertility and general reproduction (Segment I) study of IL-1Ra administered by subcutaneous injection to rats	Rat, Sprague-Dawley Crl:CD®BR VAF/Plus®	s.c.	Males: 64 days prior to mating to study day 85; Females: 15 days prior to mating, during gestation and lactation until the day prior to euthanasia	0, 12.5, 50, 200	Yes	[redacted]	SY05-T93-01	[4.2.3.5.1]

Type of study	Test system (species and strain)	Method of administration	Duration of dosing (frequency)	Dose (mg/kg/day)	GLP[a]	Testing facility	Study number	eCTD location
A study of fertility and early embryonic development to implantation of recombinant-methionyl human interleukin-receptor antagonist (r-metHuIL-1Ra) administered subcutaneously in rats	Rat, Sprague-Dawley Crl:CD VAF/Plus	s.c.	Males: 29 days prior to mating and throughout mating until one day prior to euthanization; Females: 14 days prior to mating to day 7 of gestation	0, 10, 100, 200	Yes	[redacted]	960044	[4.2.3.5.1]
Developmental toxicity study in rats with IL-1Ra	Rat, Sprague-Dawley Crl:CD BR VAF/Plus	s.c.	12 days (Day 6 to Day 17 of gestation)	0, 12.5, 50, 200	Yes	[redacted]	SY05-T92-03	[4.2.3.5.2]
Perinatal and postnatal study of IL-1Ra administered by subcutaneous injection to rats	Rat, Sprague-Dawley Crl:CD BR VAF/Plus	s.c.	15 days (Day 6 of gestation to Day 20 of lactation)	0, 12.5, 50, 200	Yes	[redacted]	SY05-T93-02	[4.2.3.5.3]
Developmental toxicity study in rabbits with IL-1Ra	Rabbit, New Zealand White	s.c.	13 days (Day 6 to Day 18 of gestation)	0, 12.5, 50, 200	Yes	[redacted]	SY05-T92-04	[4.2.3.5.2]
Studies in juvenile animals								

Type of study	Test system (species and strain)	Method of administration	Duration of dosing (frequency)	Dose (mg/kg/day)	GLP[a]	Testing facility	Study number	eCTD location
A feasibility study by subcutaneous injection in rats to determine the tolerability of the proposed high-dose level and confirm feasibility of once and twice daily dosing	Rat, Sprague-Dawley	s.c.	7 days (Day 4 to Day 10 postpartum for Group 1 [q.d.]) and 16 days (Day 4 to Day 19 postpartum for Group 2 [b.i.d.])	100 (Group 1) 200 (Group 2)	No	[redacted] United Kingdom	497888	[4.2.3.5.4]
A 6 week neonatal/juvenile study to determine the adverse effects on memory and learning functions in rats after subcutaneous injection followed by a 1-month recovery period	Rat, Sprague-Dawley	s.c.	6 weeks (38 days, b.i.d.)	0, 20, 60, 200	Yes	[redacted] United Kingdom	498001	[4.2.3.5.4]
Local tolerance								
No local tolerance studies have been conducted.								
Other toxicity studies								

Type of study	Test system (species and strain)	Method of administration	Duration of dosing (frequency)	Dose (mg/kg/day)	GLP[a]	Testing facility	Study number	eCTD location
Potential effects of IL-1Ra and PEG-sTNF-RI dosed singly and in combination on immune function in Fisher 344 rat	Rat, Fisher 344	s.c. (anakinra and TNF); i.v. (anti-asialo GM1 antibody); i.p. (cyclophosphamide)	21 days (b.i.d.)	Anakinra – 0, 20, 200; TNF – 5, 50; Anti-asialo GM1 antibody – 24 hours prior to scheduled sacrifice (Phase 1 only); Cyclophosphamide – 25, 4 days prior to sacrifice	Yes	[redacted]	100797	[4.2.3.7.2]
A study to determine the effects of 3-day continuous infusion of IL-1Ra on *E. coli* clearance and organ colonization in the rat	Rat, Tac: N(SD)fBR	i.v.	3 days	0, 173, 346 (CI)	Yes	[redacted]	SY05-T92-17	[4.2.3.7.3]
A study to determine the effects of 3-day continuous infusion of IL-1Ra on *Staphylococcus aureus 502A* clearance and organ colonization in the rat	Rat, Tac: N(SD)fBR	i.v.	3 days	0, 173, 346 (CI)	Yes	[redacted]	SY05-T92-18	[4.2.3.7.3]
A study to determine the effects of 3-day continuous infusion of IL-1R on *Listeria monocytogenes* clearance and organ colonization in the rat	Rat, Tac: N(SD)fBR	i.v.	3 days	0, 173, 346 (CI)	Yes	[redacted]	SY05-T92-19	[4.2.3.7.3]

Type of study	Test system (species and strain)	Method of administration	Duration of dosing (frequency)	Dose (mg/kg/day)	GLP[a]	Testing facility	Study number	eCTD location
An antigenicity study (19 day) of IL-1Ra in male rhesus monkeys following repeated intravenous injections	Monkey, rhesus (*Macaca mulatta*)	i.v.	19 days	10	Yes	[redacted]	SY05-T89-07	[4.2.3.7.1]
A 1-month toxicity study of r-metHuIL-1Ra and PEG r-metHu-sTNF-RI administered subcutaneously in cynomolgus monkeys with a 1-month recovery period	Monkey, cynomolgus	s.c. anakinra – daily; sTNF-RI – once weekly	1 month	Anakinra – 0, 100 mg/kg; sTNF-RI – 0, 1, 5, 25 mg/kg	Yes	[redacted] Japan	100484	[4.2.3.2]

Abbreviations: b.i.d., Twice daily; CI, Continuous infusion; eCTD, Electronic Common Technical Document; GLP, Good Laboratory Practice; GM1, Ganglioside-monosialic acid; IL,Interleukin; IL-1Ra, Interleukin-1 receptor antagonist; i.p., Intraperitoneal; i.v., Intravenous; MTX, Methotrexate; N/A, Not applicable; PEG r-metHu-sTNF-RI, Polyethylene glycosylated recombinant-methionyl human-soluble tumor necrosis factor-receptor I; PEG-sTNF-RI, Polyethylene glycosylated soluble tumor necrosis factor-receptor I; p.o., Peroral; q.d., Once daily; q.i.d., 4 times-a-day; r-MetHuIL-1Ra; Recombinant-methionyl human interleukin-1 receptor antagonist; s.c., Subcutaneous; sTNF-RI, soluble tumor necrosis factor-receptor type I; TNF, Tumor necrosis factor.

[a] "Yes" indicates study includes a GLP compliance statement.

2.6.7.2　トキシコキネティクス：トキシコキネティクス試験の一覧表

TK 試験は、［2.6.4.3.3.1］項、［2.6.4.3.3.2］項、［2.6.4.3.3.5］項、［2.6.4.3.3.6］項及び［2.6.5.4］項に記載している。

2.6.7.3　トキシコキネティクス：トキシコキネティクス試験成績の一覧

TK パラメータについては、［2.6.4.3.3.1］項、［2.6.4.3.3.2］項、［2.6.4.3.3.5］項、［2.6.4.3.3.6］項及び［2.6.5.4］項で詳細に記載している。

2.6.7.4 毒性試験：被験物質一覧

Test Article: Anakinra

Batch No.	Purity by RP-HPLC	Protein concentration	Study Number	Type of Study
Acceptable range	Main Peak >= 94%	200～240 mg/mL		
[redacted]	97%*	221 mg/mL*	498001	Juvenile rat study

RP-HPLC, High-Performance Liquid Chromatography-Reversed Phase
Study 498001 is a GLP compliant study whose report is regarded as evaluable data in Japan.
*, Certificate of Analysis of Batch [redacted]

2.6.7.5 単回投与毒性試験

Single-dose toxicity studies					**Test article:** Anakinra		
Test system (species/strain)	**Method of administration (vehicle/formulation)**	**Doses in mg/kg (test article concentration and lot no.)**	**Sex No. per group**	**Observed maximum nonlethal dose (mg/kg)**	**Approximate lethal dose (mg/kg)**	**Noteworthy findings**	**Study no.**
Rat/CD® BR VAF/Plus	Bolus i.v. injection of 8 mL/kg on Day 1 (10 mmol/L sodium citrate, 140 mmol/L NaCl and 0.1 % polysorbate 80 [w/w] in water; pH 6.5)	0, 1.5, 15, 150, 720 (0.188 mg/mL, L94-009; 1.88 mg/mL, L94-010; 18.8 mg/mL, L94-011; 90 mg/mL, L94-012)	M and F 5 each per group	-	>720 mg/kg	No deaths were observed at any time during this 14-day study. Highest dose tested was 720 mg/kg.	SY05-T94-01
Monkeys/ cynomolgus (*Macaca fascicularis*)	i.v. infusion of 8 mL/kg 4 times a week (10 mmol/L sodium citrate, 140 mmol/L NaCl and 0.1 % polysorbate 80 [w/w] in water; pH 6.5	0, 1.5, 15, 150 (0.188 mg/mL, L94-009; 1.88 mg/mL, L94-010; 18.8 mg/mL, L94-023)	F 2 (same 2 young adult females utilized at each dose level)	-	>150 mg/kg	No deaths were observed at any time during this 4-day study. Highest dose tested was 150 mg/kg. No noteworthy findings in clinical observations, food consumption, body weight, or necropsy assessments.	SY05-T94-02
Acute toxicity in repeat-toxicity studies							
Rat/CD (SD) BR Sprague-Dawley	s.c. once a day, 7 or 14 days	0, 120 mg/kg/day	M and F 10 each per group	–	>120 mg/kg	No deaths Inflammation at injection sites	SY05-T89-12
Monkey/rhesus	s.c. once a day, 14 days	0, 5, 20, 80 mg/kg/day	M and F 3 each per group	–	>80 mg/kg	No deaths Inflammation at injection sites	SY05-T89-11

Abbreviations: “-”, Not reported, no data; F, female; i.v., Intravenous; s.c. Subcutaneous; M, Male; NaCl, Sodium chloride; no., Number; w/w, Weight-to-weight equivalent.

2.6.7.6 反復投与毒性試験：重要な試験以外の試験

A 4-week toxicity study of IL-1Ra administered by subcutaneous injection to rhesus monkeys, with a 4-week recovery period						Test article: Anakinra		
Species/strain: Monkey/rhesus	Duration of dosing: 4 weeks					Study no.: 960040		
Initial age: 2.5 to 6 years	Duration of postdose: 4 weeks					Location in eCTD: 4.2.3.2		
Date of first dose: N/A	Method of administration: s.c. bolus injection, once daily					GLP compliance: Yes		
Special features: N/A	Vehicle/formulation: 10 mmol/L citrate, 140 mmol/L NaCl, 0.5 mM EDTA, 0.1 % Tween 80 at pH 6.5							
No observed adverse effect level: 100 mg/kg (compared to 1 mg/kg in human)								
Daily dose (mg/kg)	0 (Control), 10, 100, 200							
No. of animals/sex/dose	3 M and 3 F for Groups 2 and 3; 5 M and 5 F for Groups 1 and 4							
Dose (mg/kg)	0 (Group 1)		10 (Group 2)		100 (Group 3)		200 (Group 4)	
Toxicokinetics:								
Sampling day	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
AUC_{0-24} (ng·h/mL)	N/A	N/A	32490	57150	344800	375600	670700	606400
$AUC_{0-\infty}$ (ng·h/ml)	N/A	N/A	32510	N/A	347100	N/A	677000	N/A
C_{max} (ng/mL)	N/A	N/A	5841	7849	40660	38390	59580	56040
Terminal $t_{½}$ (h)	N/A	N/A	1.96	2.72	N/A	1.07	N/A	0.92
Noteworthy findings								
Died or sacrificed moribund	3/3 at Day 29; 2/2 at Day 57		3/3 at Day 29		2/3[a] at Day 29		3/3 at Day 29; 2/2 at Day 57	
Body weight (%)	–	–	–	–	–	–	–	–
Food consumption (%)	–	–	–	–	–	–	–	–
Clinical observations								
Skin lesions	–	–	–	–	–	–	5/10	
Inflammation at injection site	–	–	–	–	–	–	5/10	
Ophthalmoscopy	–	–	–	–	–	–	–	–
Electrocardiography	–	–	–	–	–	–	–	–
Hematology	–	–	–	–	–	–	–	–
Serum chemistry	–	–	–	–	–	–	–	–
Urinalysis	–	–	–	–	–	–	–	–

A 4-week toxicity study of IL-1Ra administered by subcutaneous injection to rhesus monkeys, with a 4-week recovery period									
Species/strain: Monkey/rhesus	Duration of dosing: 4 weeks						Test article: Anakinra		
Organ weights	Brain	–	–	–	–	–	–	–	–
	Testes	–	–	-	–	–	–	–	–
Histopathology		–	–	–	–	–	–	Infiltrate of lymphocytes and vacuolated histiocytes (lymphohistiocytic inflammation) at skin lesions	
Additional examinations	Anti-drug antibody (made relative to control)	-		-		-		-	
Postdose evaluation: No. evaluated		-		-		-		-	

Abbreviations: "-", Not reported, no data; AUC_{0-24}, Area under the curve from time 0 to 24 hours; $AUC_{0-\infty}$, Area under the curve from time 0 to infinity; b.i.d., Twice daily; $C_{max,}$ Maximum concentration; eCTD, Electronic Common Technical Document; EDTA, Ethylenediamine tetraacetic acid; F, Female; GLP, Good Laboratory Practice; IL-1Ra, Interleukin-1 receptor antagonist; M, Male; NaCl, Sodium chloride; no., Number; N/A, Not applicable; s.c., Subcutaneous; $t_{½}$, Half-life.

[a] 1 male animal in Group 3 died on Day 6 from gastric dilatation. The death of this animal was not considered to be treatment related. Additional studies (see Study 100484 and Module 2.6.4 and Module 2.6.5) in primates using this dose or a higher dose have not produced mortality.

Notes: The rhesus monkeys in the active treatment groups were exposed to anakinra approximately in proportion to the administered dose. No gender difference in the pharmacokinetics of anakinra was observed. There was no accumulation of mean plasma concentrations after multiple doses of anakinra for 28 days in the 100 and 200 mg/kg groups. A moderate accumulation of mean plasma concentrations on Day 28 was observed for the 10-mg/kg group. The accumulation could be attributed to the decrease in the systemic clearance of anakinra in the presence of antibodies. The incidence of seroreactivity to anakinra in rhesus monkeys was low.

2.6.7.7 反復投与毒性試験：重要な試験

2.6.7.7.1 ラットを用いた 2 週間反復皮下投与毒性試験

Subchronic (2 week, b.i.d.) subcutaneous toxicity study of interleukin-1 receptor antagonist (IL-1Ra) in Sprague-Dawley rats					Test article: Anakinra			
Species/strain: Rat/CD (SD) BR Sprague-Dawley		Duration of dosing: 14 days			Study no.: SY05-T89-10			
Initial age: 56 days (M)/67 days (F)		Duration of postdose: N/A			Location in eCTD: 4.2.3.2			
Date of first dose: Day 1, [redacted], 19[redacted]		Method of administration: s.c. bolus injection, b.i.d.			GLP compliance: Yes			
Special features: N/A		Vehicle/formulation: 10 mmol/L citrate, 140 mmol/L NaCl; 0.5 mmol/L EDTA; pH 6.5						
No observed adverse effect level: 80 mg/kg/day								
Daily dose (mg/kg)		0 (Control)		5		20		80	
No. of animals		M: 10	F: 10	M: 10	F: 9	M: 10	F: 9	M: 10	F: 10
Noteworthy findings									
Died or sacrificed moribund		–	–	–	–	–	–	–	–
Body weight (%)		–	–	–	–	–	–	–	–
Food consumption (%)		–	–	–	–	–	–	–	–
Clinical observations	Injection site hemorrhage incidence	3/10	2/10	2/10 ns	1/9 ns	3/10 ns	3/9 ns	6/10 ns	2/10 ns
	Injection site subacute inflammation	1/10	1/10	0/10 ns	0/9 ns	1/10 ns	1/9 ns	2/10 ns	2/10 ns
Ophthalmoscopy		–	–	–	–	–	–	–	–
Hematology		–	–	–	–	–	–	–	–
Serum chemistry		–	–	–	–	–	–	–	–
Urinalysis		–	–	–	–	–	–	–	–
Relative organ weights	Brain	–	–	–	–	D[a]	–	D[a]	–
	Testes	–	–	D[a]	–	D[a]	–	D[a]	–
Histopathology		–	–	–	–	–	–	–	–

Abbreviations: "-", Not reported, no data;, b.i.d., twice daily; D, Decrease; eCTD, Electronic Common Technical Document; EDTA, Ethylenediamine tetraacetic acid; F, Female; GLP, Good Laboratory Practice; IL-1Ra, Interleukin-1 receptor antagonist; M, Male; s.c., Subcutaneous; N/A, Not applicable; NaCl, sodium chloride;ND, Not determined; no., Number; ns, Not significant.
[a] $p < 0.05$

2.6.7.7.2 ラットを用いた 2 週間反復皮下投与毒性試験（高用量）

Subchronic high-dose subcutaneous toxicity (q.i.d.) study of interleukin-1 receptor antagonist (IL-1Ra) in Sprague-Dawley rats				Test article: Anakinra
Species/strain: Rat/CD (SD) BR Sprague-Dawley		Duration of dosing: 7 or 14 days		Study no.: SY05-T89-12
Initial age: ~2 months (53 days old)		Duration of postdose: N/A		Location in eCTD: 4.2.3.2
Date of first dose: [redacted], 19[redacted]		Method of administration: s.c. bolus injection, (q.i.d., at approximately 6-hour intervals)		GLP compliance: Yes
Special features: N/A		Vehicle/formulation: 10 mmol/L sodium phosphate, 150 mmol/L NaCl, 0.1 mmol/L EDTA; pH 7.0		
No observed adverse effect level: 120 mg/kg/day				
Daily dose (mg/kg)		0 (Control)	120	
No. of animals		M: 10	F: 10	M: 10	F: 10
Noteworthy findings				
Died or sacrificed moribund		0	1	0	0
Body weight (%)		–	–	–	–
Food consumption (%)		–	–	–	–
Clinical observations	Chronic inflammation (trace to mild) at injection site – Day 15 sacrifice	0/10	0/10	3/10	4/10
Ophthalmoscopy		–	–	–	–
Hematology		–	–	–	–
Serum chemistry		–	–	–	–
Urinalysis		–	–	–	–
Organ weights (%)		–	–	–	–
Histopathology		–	–	–	–
Anti-drug antibody[a]		N/A		8/10	
Body temperature		–	–	–	–
Ophthalmology		–	–	–	–

Abbreviations: "-", not reported, no data; eCTD, Electronic Common Technical Document; EDTA, Ethylenediamine tetraacetic acid; F, Female; GLP, Good Laboratory Practice; IL-1Ra, Interleukin-1 receptor antagonist; M, Male; N/A, Not applicable; NaCl, Sodium chloride; no., Number; q.i.d., 4 times daily; s.c., Subcutaneous.

[a] The anti-drug antibody determination was non-GLP compliant.

2.6.7.7.3 ラットを用いた 6 ヵ月間反復皮下投与毒性試験

A subcutaneous 6-month toxicity study in rats treated with anakinra, including a 1-month interim and 1 month recovery groups	Test article: Anakinra
Species/strain: Rat/Sprague-Dawley	Study no.: SY05-T94-04
Special features: 1-month, 6-month, and recovery periods	Location in eCTD: 4.2.3.2
No observed adverse effect level: 20 mg/kg/day, (approximately 20 times the approximate clinical dose of 1 mg/kg/day).	GLP compliance: Yes
Conclusions: Chronic s.c. administration of anakinra at doses of 2, 20, or 200 mg/kg/day (b.i.d.) for 6 months revealed a slight increase in body weight and food consumption (males only). Increases in urine protein, a slight increase in mixed cell infiltration at the injection site, chronic progressive nephropathy, and increases in kidney and liver weights were seen primarily at 200 mg/kg/day. All of these effects were considered reversible.	

Abbreviations: b.i.d., Twice daily; eCTD, Electronic Common Technical Document; GLP, Good Laboratory Practice; no., Number; s.c., Subcutaneous.

Study details are presented in [2.6.6.3.3]

2.6.7.7.4 ラットを用いた 2 週間反復静脈内投与毒性試験

Intravenous (2 week) systemic toxicity study of interleukin-1 receptor antagonist (IL-1Ra) in Sprague-Dawley rats							Test article: Anakinra	
Species/strain: Rat/CD (SD) BR Sprague-Dawley		Duration of dosing: 14 days					Study no.: SY-05-T89-01	
Initial age: Young adults		Duration of postdose: N/A					Location in eCTD: 4.2.3.2	
Date of first dose: [redacted], 19[redacted]		Method of administration: Bolus i.v. injection of 1 mL/kg					GLP compliance: Yes	
Special features: N/A		Vehicle/formulation: Phosphate-buffered saline (10 mmol/L sodium phosphate, 150 mmol/L NaCl; pH 7)						
No observed adverse effect level: 1.17 mg/kg/day								
Daily dose (mg/kg)	0 (Control)		0.047		0.231		1.170	
No. of animals	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Noteworthy findings								
Died or sacrificed moribund	0	0	0	0	0	0	0	0
Body weight (%)	–	–	–	–	–	–	–	–
Food consumption (%)	–	–	–	–	–	–	–	–
Clinical observations	–	–	–	–	–	–	–	–
Ophthalmoscopy	–	–	–	–	–	–	–	–
Hematology	–	–	–	–	–	–	–	–
Serum chemistry	–	–	–	–	–	–	–	–
Urinalysis	–	–	–	–	–	–	–	–
Gross pathology	–	–	–	–	–	–	–	–
Histopathology	–	–	–	–	–	–	–	–

Abbreviations: "-", Not reported, no data; eCTD, Electronic Common Technical Document; F, Female; GLP, Good Laboratory Practice; IL-1Ra, Interleukin-1 receptor antagonist; i.v., Intravenous; M, Male; N/A, Not applicable; NaCl, Sodium chloride; no., Number.

2.6.7.7.5 ラットを用いた 2 週間反復静脈内投与毒性試験

A subchronic (2 week) intravenous toxicity study of interleukin-1 receptor antagonist (IL-1Ra) in Sprague-Dawley rats							Test article: Anakinra	
Species/strain: Rat/Crl:CD (SD) BR	Duration of dosing: 14 days						Study no.: SY05-T89-02	
Initial age: 7 to 9 weeks	Duration of postdose: N/A						Location in eCTD: 4.2.3.2	
Date of first dose: [redacted], 19[redacted]	Method of administration: Bolus i.v. injection; 1 mL/kg Groups 2 and 3; 2 mL/kg Groups 1						GLP compliance: Yes	
Special features: N/A	Vehicle/formulation: Phosphate-buffered saline; pH 7.0							
No observed adverse effect level: N/A								
Daily dose (mg/kg)	0 (Control)		2.0		5.0		20.0	
No. of animals	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5
Noteworthy findings								
Died or sacrificed moribund	0	0	0	0	0	0	0	0
Body weight (%)	–	–	–	–	–	–	I	–
Food consumption (%)	–	–	–	–	–	–	–	–
Clinical observations	–	–	–	–	–	–	–	–
Hematology	–	–	–	–	–	–	–	–
Serum chemistry	–	–	–	–	–	–	–	–
Urinalysis	–	–	–	–	–	–	–	–
Organ weights (%)	–	–	–	–	–	–	–	–
Necropsy	–	–	–	–	–	–	–	–
Histopathology	–	–	–	–	–	–	–	–

Abbreviations: "-", Not reported, no data; eCTD, Electronic Common Technical Document; F, Female; GLP, Good Laboratory Practice; I, Increase; IL-1Ra, Interleukin-1 receptor antagonist; i.v., Intravenous; M, Male; N/A, Not applicable; no., Number.

2.6.7.7.6 ラットを用いたアナキンラの皮下投与及びメトトレキサートの経口投与による 4 週間反復投与毒性試験

A 4-week toxicity and toxicokinetic study of subcutaneously administered anakinra and orally administered methotrexate in rats	Test article: Anakinra
Species/strain: Rat/Crl:CD (SD)BR VAF/Plus	Study no.: 960048
Special features: Assess the interactive effects of administration of s.c. anakinra and oral MTX.	Location in eCTD: 4.2.3.2
No observed adverse effect level: 100 mg/kg/day for anakinra given alone.	GLP compliance: Yes
Conclusions: Based on clinical and anatomical pathology results, following administration of MTX (orally) or anakinra (s.c.) alone or in combination to female Crl:CD (SD)BR VAF/Plus rats for 4 weeks, the NOAEL for MTX was 0.04 mg/kg/day; co-administration of anakinra had no effect on the changes induced by MTX, except for histiocytic inflammation in the spleen, observed with MTX alone, but was absent in animals given MTX in combination with anakinra. The NOAEL for systemic toxicity for anakinra given alone was 100 mg/kg/day; local observations of increased chronic active inflammation were present at the s.c. injection site for Groups 5 and 9 (100 mg/kg/day anakinra).	

Abbreviations: eCTD, Electronic Common Technical Document; GLP, Good Laboratory Practice; MTX, Methotrexate; NOAEL, No observed adverse effect level; no., Number; s.c., Subcutaneous.

Study details are presented in [2.6.6.3.6].

2.6.7.7.7 ラットを用いた 2 週間反復静脈内投与毒性試験

Subchronic (2 week) intravenous toxicity study of interleukin-1 receptor antagonist (IL-1Ra) in Sprague-Dawley rats							Test article: Anakinra	
Species/strain: Rat/Crl:CD (SD/BR)		Duration of dosing: 14 days					Study no.: SY05-T89-05	
Initial age: 67 days (M); 74 days (F)		Duration of postdose: N/A					Location in eCTD: 4.2.3.2	
Date of first dose: [redacted], 19[redacted]		Method of administration: Bolus i.v. injection; 1 mL/kg/day					GLP compliance: Yes	
Special features: N/A		Vehicle/formulation: 10 mmol/L sodium phosphate, 150 mmol/L NaCl, 0.10 mmol/L EDTA in water; pH 7.0						
No observed adverse effect level: 10 mg/kg/day for injection site toxicity, 30 mg/kg/day for systemic toxicity								
Daily dose (mg/kg)		0 (Control)		3.0		10.0		30.0	
No. of animals		M: 20	F: 20	M: 10	F: 10	M: 10	F: 10	M: 20	F: 20
Noteworthy findings									
Died or sacrificed moribund		0	0	0	0	0	0	1[a]	1[a]
Body weight (%)		–	–	–	–	–	–	–	–
Food consumption (%)		–	–	–	–	–	–	–	–
Clinical observations	Perivascular inflammation (trace to moderate) at injection site (Day 15 sacrifice)	1/10	3/10	2/10	1/10	3/10	0/10	5/11	4/10
	Perivascular inflammation at injection site (Day 43 sacrifice)	0/10	0/10	–	–	–	–	0/9	0/10
Ophthalmoscopy		–	–	–	–	–	–	–	–
Hematology		–	–	–	–	–	–	–	–
Serum chemistry		–	–	–	–	–	–	–	–
Urinalysis		–	–	–	–	–	–	–	–
Organ weights (%)		–	–	–	–	–	–	–	–
Necropsy		–	–	–	–	–	–	–	–
Histopathology		–	–	–	–	–	–	–	–

Abbreviations: "-", Not reported, no data; eCTD, Electronic Common Technical Document; EDTA, Ethylenediamine tetraacetic acid; F, Female; GLP, Good Laboratory Practice; IL-1Ra, Interleukin-1 receptor antagonist; i.v., Intravenous; M, Male; N/A, Not applicable; NaCl, Sodium chloride; no., Number.

[a] Deaths of 2 animals (1 male, 1 female) in the high-dose recovery group occurred during anesthesia for blood collection and were considered incidental mortalities, not related to treatment.

2.6.7.7.8 ラットを用いた 2 週間反復静脈内投与毒性試験

Subchronic (2 week) intravenous toxicity study of interleukin-1 receptor antagonist (IL-1Ra) in Sprague-Dawley rats					Test article: Anakinra
Species/strain: Rat/Crl:CD (SD) BR	Duration of dosing: 14 days		Duration of postdose: N/A		Study no.: SY05-T89-09
Initial age: 56 or 66 days	Method of administration: i.v. bolus injection (1 mL/kg)				Location in eCTD: 4.2.3.2
Date of first dose: [redacted], 19[redacted]	Vehicle/formulation: 10 mmol/L sodium phosphate, 150 mmol/L NaCl, 0.1 mmol/L EDTA in water; pH 7.0				GLP compliance: Yes[a]
Special features: N/A	No observed adverse effect level: 30 mg/kg/day				
Daily dose (mg/kg/day)		0 (Control)		30	
No. of animals		M: 10	F: 10	M: 10	F: 10
Noteworthy findings					
Died or sacrificed moribund		0	0	0	0
Body weight (%)		–	–	–	–
Food consumption (%)		–	–	–	–
Clinical observations		–	–	–	–
Ophthalmoscopy		–	–	–	–
Hematology		–	–	–	–
Serum chemistry		–	–	–	–
Urinalysis		–	–	–	–
Organ weights (%)		–	–	–	–
Necropsy		–	–	–	–
Histopathology	Trace, subacute inflammation at i.v. injection site	–	–	1	1
Body temperatures		–	–	–	–

Abbreviations: "-", Not reported, no data; eCTD, Electronic Common Technical Document; EDTA, Ethylenediamine tetraacetic acid; F, Female; GLP, Good Laboratory Practice; IL-1Ra, Interleukin-1 receptor antagonist; i.v., Intravenous; M, Male; N/A, Not applicable; NaCl, Sodium chloride; no., Number.

2.6.7.7.9 ラットを用いた 2 週間反復吸入投与毒性試験

A 2-week nose-only inhalation toxicity study of recombinant human interleukin-1 receptor antagonist (IL-1Ra) in the rat							Test article: Anakinra	
Species/strain: Rat/Crl:CD® Br	Duration of dosing: 14 days (exposed 2 h/day, 7 days/week)						Study no.: SY05-T92-01	
Initial age: 7 weeks (M); 8 weeks (F)	Duration of postdose: N/A						Location in eCTD: 4.2.3.2	
Date of first dose: [redacted], 19[redacted]	Method of administration: Inhalation (nose only)						GLP compliance: Yes	
Special features: N/A	Vehicle/formulation: 10 mmol/L citrate, 140 mmol/L NaCl, 0.5 mmol/L EDTA, 0.1 % Tween 80 (pH 6.5)							
No observed adverse effect level: 3.2 mg/kg/day								
Daily dose (mg/kg/day; calculated)	0 (air control)		0 (vehicle control)		0.33		3.2	
Daily dose (mg/m³ measured)	0		0		8.7		86	
No. of animals	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Noteworthy findings								
Died or sacrificed moribund	0	0	0	0	1[a]	0	0	0
Body weight (%)	–	–	–	–	–	–	–	–
Food consumption (%)	–	–	–	–	–	–	–	–
Clinical observations	–	–	–	–	–	–	–	–
Hematology	–	–	–	–	–	–	–	–
Serum chemistry	–	–	–	–	–	–	–	–
Urinalysis	–	–	–	–	–	–	–	–
Organ weights (%)	–	–	–	–	–	–	–	–
Necropsy	–	–	–	–	–	–	–	–
Histopathology	–	–	–	–	–	–	–	–
IgM anti-drug antibody incidence (Day 7)[b]	1/10		0/10		1/20		0/20	
IgM anti-drug antibody incidence (Day 14)[b]	0/10		0/10		0/19		1/20	
IgG anti-drug antibody incidence (Day 7)[b]	0/10		0/10		0/20		0/20	
IgG anti-drug antibody incidence (Day 14)[b]	0/7		0/10		0/19		6/20	

Abbreviations: "-", Not reported, no data; eCTD, Electronic Common Technical Document; EDTA, Ethylenediamine tetraacetic acid; F, Female; GLP, Good Laboratory Practice; IgG, Immunoglobulin G; IgM, Immunoglobulin M; IL-1Ra, Interleukin-1 receptor antagonist; M, Male; N/A, Not applicable; NaCl, Sodium chloride; no., Number.

[a] The death on Day 8 was attributed to excessive blood loss as a result of a blood sampling.

[b] Both genders combined.

2.6.7.7.10 サルを用いた 2 週間反復皮下投与毒性試験

Subchronic (2 week, b.i.d.) systemic toxicity study of IL-1Ra in male and female rhesus monkeys following repeated subcutaneous injections							Test article: Anakinra	
Species/strain: Monkey/rhesus		Duration of dosing: 14 days					Study no.: SY05-T89-11	
Initial age: Mature at study initiation		Duration of postdose: N/A					Location in eCTD: 4.2.3.2	
Date of first dose: [redacted], 19[redacted]		Method of administration: Subcutaneous bolus injections (b.i.d.) 0.4 ml/kg/injection					GLP compliance: Yes	
Special features: N/A		Vehicle/formulation: 10 mmol/L sodium citrate, 140 mmol/L NaCl, 0.5 mmol/L EDTA in water; pH 6.5						
No observed adverse effect level: 5 mg/kg/day for injection site toxicity, 80 mg/kg/day for systemic toxicity								
Daily dose (mg/kg)		0 (Control)		5		20		80	
No. of animals		M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Noteworthy findings									
Died or sacrificed moribund		0	0	0	0	0	0	0	0
Body weight (%)		–	–	–	–	–	–	–	–
Food consumption (%)		–	–	–	–	–	–	–	–
Clinical observations		–	–	–	–	–	–	–	–
Ophthalmoscopy		–	–	–	–	–	–	–	–
Electrocardiography		–	–	–	–	–	–	–	–
Hematology		–	–	–	–	–	–	–	–
Serum chemistry		–	–	–	–	–	–	–	–
Urinalysis		–	–	–	–	–	–	–	–
Organ weights (%)		–	–	–	–	–	–	–	–
Necropsy		–	–	–	–	–	–	–	–
Histopathology	Chronic inflammation (trace) at injection site	1/3	0/3	0/3	1/3	1/3	2/3	3/3	2/3
Body temperature		–	–	–	–	–	–	–	–

Abbreviations: "-", Not reported, no data; b.i.d., Twice daily; eCTD, Electronic Common Technical Document; EDTA, Ethylenediamine tetraacetic acid; F, Female; GLP, Good Laboratory Practice; IL-1Ra, Interleukin-1 receptor antagonist; M, Male; N/A, Not applicable; NaCl, Sodium chloride; no., Number.

2.6.7.7.11 サルを用いた 1 週間反復静脈内投与毒性試験

1 week intravenous infusion study on IL-1Ra in male rhesus monkeys				Test article: Anakinra
Species/strain: Monkey/rhesus	Duration of dosing: 7 days			Study no.: SY05-T90-02
Initial age: Mature at study initiation	Duration of postdose: N/A			Location in eCTD: 4.2.3.2
Date of first dose: [redacted] 19[redacted]	Method of administration: i.v. infusion (5 mL/hour)			GLP compliance: Yes
Special features: N/A	Vehicle/formulation: 10 mmol/L citrate, 140 mmol/L NaCl, 0.5 mM EDTA, pH 6.5; 200 mg/mL			
No observed adverse effect level:150 mg/kg/day				
Daily dose (mg/kg/day)	0 (Control)		150	
No. of animals	M: 2	F: 0	M: 3	F: 0
Noteworthy findings				
Died or sacrificed moribund	0	N/A	0	N/A
Body weight (%)	–	N/A	–	N/A
Food consumption (%)	–	N/A	–	N/A
Clinical observations	–	N/A	–	N/A
Hematology	–	N/A	–	N/A
Serum chemistry	–	N/A	–	N/A
Organ weights (%)	–	N/A	–	N/A
Necropsy	–	N/A	–	N/A
Histopathology	–	N/A	–	N/A

Abbreviations: "-", Not reported, no data; eCTD, Electronic Common Technical Document; EDTA, Ethylenediamine tetraacetic acid; F, Female; GLP, Good Laboratory Practice; IL-1Ra, Interleukin-1 receptor antagonist; i.v., Intravenous; M, Male; N/A, Not applicable; NaCl, sodium chloride;no., Number.

2.6.7.7.12 サルを用いた 2 週間反復静脈内投与毒性試験

A range-finding (2 week) toxicity study of interleukin-1 receptor antagonist (IL-1Ra) in male cynomolgus monkeys following repeated intravenous injections						Test article: Anakinra	
Species/strain: Monkey/cynomolgus	Duration of dosing: 2 weeks					Study no.: SY05-T89-03	
Initial age: Mature at study initiation	Duration of postdose: N/A					Location in eCTD: 4.2.3.2	
Date of first dose: [redacted] 19[redacted]	Method of administration: Bolus i.v. of 1 mL/kg					GLP compliance: Yes	
Special features: N/A	Vehicle/formulation: 10 mmol/L sodium phosphate, 150 mmoL/L NaCl, 0.10 mmol/L EDTA in water; pH 7.0						
No observed adverse effect level: 10 mg/kg/day							
Daily dose (mg/kg)	0 (Control)		0.1		1.0		10.0	
No. of animals	M: 3	F: 0	M: 3	F: 0	M: 3	F: 0	M: 3	F: 0
Noteworthy findings								
Died or sacrificed moribund	0	N/A	0	N/A	0	N/A	0	N/A
Body weight (%)	–	N/A	–	N/A	–	N/A	–	N/A
Food consumption (%)	–	N/A	–	N/A	–	N/A	–	N/A
Clinical observations	–	N/A	–	N/A	–	N/A	–	N/A
Hematology	–	N/A	–	N/A	–	N/A	–	N/A
Serum chemistry	–	N/A	–	N/A	–	N/A	–	N/A
Urinalysis	–	N/A	–	N/A	–	N/A	–	N/A
Necropsy	–	N/A	–	N/A	–	N/A	–	N/A
Histopathology	–	N/A	–	N/A	–	N/A	–	N/A

Abbreviations: "-", Not reported, no data; eCTD, Electronic Common Technical Document; EDTA, Ethylenediamine tetraacetic acid; F, Female; GLP, Good Laboratory Practice; IL-1Ra, Interleukin-1 receptor antagonist; i.v., Intravenous; M, Male; N/A, Not applicable; NaCl, Sodium chloride; no., Number.

2.6.7.7.13 サルを用いた 2 週間反復静脈内投与毒性試験及び 4 週間回復性試験

Subchronic systemic toxicity study with recovery period of interleukin-1 receptor antagonist (IL-1Ra) in rhesus monkeys						Test article: Anakinra		
Species/strain: Monkey/rhesus		Duration of dosing: 14 days				Study no.: SY05-T89-08		
Initial age: Mature at study initiation		Duration of postdose: 4 weeks				Location in eCTD: 4.2.3.2		
Date of first dose: [redacted], 19[redacted]		Method of administration: i.v. bolus injections b.i.d.				GLP compliance: Yes		
Vehicle/formulation: 10 mmol/L sodium phosphate, 150 mmol/L NaCl, 0.10 mmol/L EDTA in water; pH 7.0								
Special features: N/A		No observed adverse effect level: 30 mg/kg/day						
Daily dose (mg/kg/day)	0 (Control)		3		10		30	
No. of animals	M: 6	F: 6	M: 3	F: 3	M: 3	F: 3	M: 6	F: 6
Noteworthy findings								
Died or sacrificed moribund	1[a]	1[a]	0	0	0	0	1	0
Body weight (%)	–	–	–	–	–	–	–	–
Food consumption (%)	–	–	–	–	–	–	–	–
Clinical observations	–	–	–	–	–	–	–	–
Ophthalmoscopy	–	–	–	–	–	–	–	–
Electrocardiography	–	–	–	–	–	–	–	–
Hematology	–	–	–	–	–	–	–	–
Serum chemistry	–	–	–	–	–	–	–	–
Urinalysis	–	–	–	–	–	–	–	–
Organ weights (%)	–	–	–	–	–	–	–	–
Necropsy	–	–	–	–	–	–	–	–
Histopathology	–	–	–	–	–	–	–	–

Abbreviations: "-", Not reported, no data; b.i.d., Twice daily; eCTD, Electronic Common Technical Document; EDTA, Ethylenediamine tetraacetic acid; F, Female; GLP, Good Laboratory Practice; IL-1Ra, Interleukin-1 receptor antagonist; i.v., Intravenous; M, Male; N/A, Not applicable; NaCl, Sodium chloride; no., Number.

[a] Unscheduled sacrifice.

2.6.7.8 In Vitro 遺伝毒性試験

2.6.7.8.1 細菌を用いる復帰突然変異試験

Salmonella/mammalian-microsome plate incorporation mutagenicity assay (Ames test) and *Escherichia coli* WP2 uvrA reverse mutation assay with a confirmatory assay - interleukin-1 receptor antagonist (IL-1Ra)		Test article: Anakinra
Test for induction of: Back mutations at selected loci	No. of independent assays: 3	Study no.: SY05-T92-13
Strains: *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538); *Escherichia coli* (WP2 *uvrA*)	No. of replicate cultures: 3	Location in eCTD: 4.2.3.3.1
Metabolizing system: Aroclor-induced rat liver microsomal enzymes (S-9)	No. of cells analyzed/culture: ≥ 0.3 x 10^9 per mL	GLP compliance: Yes
Vehicles for test article: 10 mmol/L citrate, 140 mmol/L NaCl, 0.5 mmol/L EDTA, 0.1% Tween; pH 6.5	For positive controls: 2-aminoanthracene (1 or 10 µg/plate), 2-nitrofluorene (1 µg/plate), sodium azide (1 µg/plate), 9-aminoacridine (75 µg/plate), methyl methanesulfonate (1000 µg/plate)	
Treatment: Plate incorporation; anakinra (Lot no. [redacted]) at 5 dose levels (33, 100, 333, 1000, and 5000 µg/plate or 12.5 -1887 µg/mL) for 48 hours		Date of treatment: [redacted] to [redacted], 19[redacted]
Cytotoxic effects: None		
Genotoxic effects: Judged to be none after 2 retests of the TA1538 strain failed to reproduce the initial positive result observed in the absence of microsomal enzymes. No other strains exhibited positive results.		

Metabolic activation	Test article	Concentration or dose level (mg/mL)	Cytotoxicity	Back mutations at selected loci					
				TA98	TA100	TA1535	TA1537	TA1538	WP2 *uvrA*
Without S-9 activation	Vehicle	0	–	–	–	–	–	–	–
	Anakinra	200	–	–	–	–	–	–	–
With S-9 activation	Vehicle	0	–	–	–	–	–	–	–
	Anakinra	200	–	–	–	–	–	–	–

Abbreviations: "-", Not reported, no data; eCTD, Electronic Common Technical Document; EDTA, Ethylenediamine tetraacetic acid; GLP, Good Laboratory Practice; IL-1Ra, Interleukin-1 receptor antagonist; NaCl, Sodium chloride; no., Number.

2.6.7.8.2 哺乳類細胞を用いる染色体異常試験

In vitro mammalian cytogenetics test – interleukin-1 receptor antagonist (IL-1Ra)				Test article: Anakinra	
Test for induction of: Clastogenic potential		No. of independent assays: 2 for initial assay, 2 for repeat assay		Study no.: SY05-T92-15	
Cell line: Chinese Hamster Ovary (CHO-K1)		No. of replicate cultures: N/A		Location in eCTD: 4.2.3.3.1	
Metabolizing system: Aroclor 1254-induced rat liver S-9		No. of cells analyzed/culture: 100 for initial assay, 100 for repeat assay		GLP compliance: Yes	
Vehicles for test article: 10 mmol/L citrate, 140 mmol/L NaCl, 0.1 % polysorbate 80, and 0.5 mmol/L EDTA, pH 6.5, (CSEP buffer) Lot # [redacted]		For positive controls: Triethylenemelamine (0.5 µg/mL final concentration); cyclophosphamide (25 [repeat] or 50 µg/mL [initial])			
Treatment: Anakinra (Lot no. [redacted]				Date of treatment: [redacted] 19[redacted]	
Cytotoxic effects: None in the initial assay. Repeat assays: In absence of activation, mitotic indices were reduced by 23 %, 13 %, and 32 % relative to the solvent control after a 4-hour anakinra treatment at dose levels of 500, 1000, and 2000 µg/mL, respectively; by 7.5 % after a 20-hour anakinra treatment at the 500 and 1000 µg/mL dose levels; and by 20 % and 3.9 % after a 44-hour anakinra treatment at the 1000 and 2000 µg/mL dose levels, respectively. After activation, mitotic indices were reduced by 11 %, 37 %, 45 %, and 20 % relative to solvent control after a 20-hour anakinra treatment period at dose levels of 250, 500, 1000, and 2000 µg/mL, respectively; and by 26 %, 5 %, 27 %, and 13 % relative to solvent control after a 44-hour anakinra treatment period at dose levels of 250, 500, 1000, and 2000 µg/mL, respectively.					
Genotoxic effects: None in either initial or repeat assays (positive controls were positive).					
Metabolic activation	Test article	Treatment period and recovery time (hours)	Concentration or dose level (µg/mL)	Cytotoxicity (mitotic index relative to solvent control in % inhibition)	Genotoxicity
Initial assay without S-9 activation	Vehicle	20 hours without recovery time	0	–	–
	Anakinra	20 hours without recovery time	0.2, 0.6, 2, 6, 20, 60, 200, 600, and 2000	–	–
Initial assay with S-9 activation	Vehicle	4-hour treatment with 16-hour recovery time	0	–	–
	Anakinra	4-hour treatment with 16-hour recovery time	0.2, 0.6, 2, 6, 20, 60, 200, 600, and 2000	–	–

In vitro mammalian cytogenetics test - interleukin-1 receptor antagonist (IL-1Ra)					Test article: Anakinra
Test for induction of: Clastogenic potential			No. of independent assays: 2 for initial assay, 2 for repeat assay		Study no.: SY05-T92-15
			Concentration or dose level (μg/mL)	Mitotic Index	Genotoxicity
Repeat assay without S-9 activation	Vehicle	4-hour treatment with 14-hour recovery time	0	–	–
		20-hour treatment without recovery time	0	–	–
		44-hour treatment without recovery time	0	–	–
	Anakinra	4-hour treatment with 4-hour recovery time	250	–	–
			500	23 % D	–
			1000	13 % D	–
			2000	32 % D	–
		20-hour treatment without recovery time	250	–	–
			500	7.5 % D	–
			1000	7.5 % D	–
			2000	–	–
		44-hour treatment without recovery time	250	–	–
			500	–	–
			1000	20 % D	–
			2000	3.9 % D	–

In vitro mammalian cytogenetics test - interleukin-1 receptor antagonist (IL-1Ra)					Test article: Anakinra
Test for induction of: Clastogenic potential			No. of independent assays: 2 for initial assay, 2 for repeat assay		Study no.: SY05-T92-15
			Concentration or dose level (µg/mL)	Mitotic Index	Genotoxicity
Repeat assay with S-9 activation	Vehicle	4-hour treatment with 4-hour recovery time	0	–	–
		20-hour treatment without recovery time	0	–	–
		44-hour treatment without recovery time	0	–	–
	Anakinra	4-hour treatment with 14-hour recovery time	250	–	–
			500	–	–
			1000	–	–
			2000	–	–
		20-hour treatment without recovery time	250	11 % D	–
			500	37 % D	–
			1000	45 % D	–
			2000	20 % D	–
		44-hour treatment without recovery time	250	26 % D	–
			500	5 % D	–
			1000	27 % D	–
			2000	13 % D	–

Abbreviations: “-”, Not reported, no data; CSEP, Citrate-buffered saline with EDTA and polysorbate; eCTD, Electronic Common Technical Document; D, Decrease; EDTA, Ethylenediamine tetraacetic acid; GLP, Good Laboratory Practice; IL-1Ra, Interleukin-1 receptor antagonist; NaCl, sodium chloride; no., Number.

2.6.7.8.3 マウスリンフォーマ TK 試験

L5178Y thymidine kinase +/- mouse lymphoma mutagenesis assay with a confirmatory assay - interleukin-1 receptor antagonist (IL-1Ra)		Test article: Anakinra
Test for induction of: Specific locus mutations at the thymidine kinase locus of cultured L5178Y thymidine kinase$^{+/-}$ cells	No. of independent assays: 2	Study no.: SY05-T92-14
Strains: L5178Y thymidine kinase +/- mouse lymphoma cells	No. of replicate cultures: 1 for dosing; 3 for expression cloning	Location in eCTD: 4.2.3.3.1
Metabolizing system: Aroclor-induced rat liver microsomal enzymes (S-9)	No. of cells analyzed/culture: 3 x 10^6 cells	GLP compliance: Yes
Vehicle for test article: 10 mmol/L citrate, 140 mmol/L NaCl, 0.1 % polysorbate 80, 0.5 mmol/L EDTA; pH 6.5	For positive controls: Ethyl methanesulfonate (0.25 and 0.5 µg/mL) and 7,12-dimethylbenz(a)anthracene (5.0 and 2.5 µg/mL) in dimethyl sulfoxide	
Treatment: Anakinra (Lot # [redacted]; > 95 % purity) at 375, 501, 667, 890, 1187, 1582, 2109, 2813, 3750, and 5000 µg/mL for 4 hours		Date of treatment: [redacted], 19[redacted]
Cytotoxic effects: None; similar growth rates were observed in all conditions tested.		
Genotoxic effects: Negative in both initial and confirmatory assay (positive controls were positive); none of the non-activated or S-9 activated cultures that were cloned exhibited a mutant frequency that was at least twice the mean mutant frequency of the vehicle controls.		

Metabolic activation	Test article	Concentration or dose level (µg/mL)	Cytotoxicity	Genotoxicity
Without S-9 activation	Vehicle	0	–	–
	Anakinra	375, 501, 667, 890, 1187, 1582, 2109, 2813, 3750, 5000	–	–
With S-9 activation	Vehicle	0	–	–
	Anakinra	375, 501, 667, 890, 1187, 1582, 2109, 2813, 3750, 5000	–	–

Abbreviations: "-", Not reported, no data; eCTD, Electronic Common Technical Document; EDTA, Ethylenediamine tetraacetic acid; GLP, Good Laboratory Practice; IL-1Ra, interleukin-1 receptor antagonist; NaCl, Sodium chloride; no., Number.

2.6.7.9 In Vivo 遺伝毒性試験

Micronucleus cytogenetics assay in mice - interleukin-1 receptor antagonist (IL-1Ra)		Test article: Anakinra
Test for induction of: Micronucleated polychromatic erythrocytes	Treatment schedule: Single injection on Day 1	Study no.: SY05-T92-16
Species/strain: Mouse ICR	Sampling time: 24, 48, and 72 hours after treatment (24 hours for positive control)	Location in eCTD: 4.2.3.3.2
Age: 6 to 8 weeks old	Method of administration: Subcutaneous injection (10 mL/kg)	
Cells evaluated: Bone marrow cells	Vehicle/formulation: 10 mmol/L citrate, 140 mmol/L NaCl, 0.1 % polysorbate 80, 0.5 mmol/L EDTA; pH 6.5	GLP compliance: Yes
No. of cells analyzed/animal: 1000		Date of dosing: [redacted], 19[redacted]
Special features: N/A	Toxic/cytotoxic effects: None	

Genotoxic effects: None.
In positive control group animals treated with intraperitoneal cyclophosphamide (Lot no. [redacted]) at 40 mg/kg, there was a significant increase in micronucleated polychromatic erythrocytes in both male and female mice relative to the vehicle control.

Evidence of exposure:

Test article	Dose (mg/kg)	Sampling time after treatment	No. of animals	Toxic/cytotoxic effects	Genotoxicity (micronucleated polychromatic erythrocytes number per 1000 PCEs scored [mean ± SD])
Vehicle	0	24	5 males; 5 females	–	–
		48	5 males; 5 females	–	–
		72	5 males; 5 females	–	–
Anakinra	500	24	5 males; 5 females	–	–
		48	5 males; 5 females	–	–
		72	5 males; 5 females	–	–
	1000	24	5 males; 5 females	–	–
		48	5 males; 5 females	–	–
		72	5 males; 5 females	–	–
	2000	24	5 males; 5 females	–	–
		48	5 males; 5 females	–	–
		72	5 males; 5 females	–	–
Cyclophosphamide	40	24	5 males; 5 females	–	13.2 ± 2.39; 12.0 ± 5.52

Abbreviations: "-", Not reported, no data; eCTD, Electronic Common Technical Document; GLP, Good Laboratory Practice; IL-1Ra, interleukin-1 receptor antagonist; no., Number; SD, Standard deviation.
Statistical analyses: Kastenbaum-Bowman tables based on binomial distribution (all $p > 0.05$).

2.6.7.10　がん原性試験

がん原性試験は実施していない。

2.6.7.11　生殖発生毒性試験：　重要な試験以外の試験

重要でない生殖発生毒性試験は実施していない。

2.6.7.12 生殖発生毒性試験：受胎能及び着床までの初期胚発生に関する試験

2.6.7.12.1 ラット受胎能、胚・胎児発生、並びに出生前及び出生後の発生に関する試験

Fertility and general reproduction (Segment I) study of IL-1Ra administered by subcutaneous injection to rats				Test article: Anakinra
Design similar to ICH 4.1.1: Not reported	Duration of dosing: Males: 64 days prior to mating to study day 85, Females: 15 days prior to mating, during gestation and lactation until the day prior to euthanasia			Study no.: SY05-T93-01
Species/strain: Rat/Sprague-Dawley Crl:CD BR VAF/Plus	Day of mating: Sperm in vaginal smear = Day 0 of gestation			Location in eCTD: 4.2.3.5.3
Initial age: 8 to 9 weeks	Method of administration: s.c. injection, b.i.d.			
Date of first dose: [redacted], 19[redacted]	Vehicle/formulation: 10 mmol/L sodium citrate, 140 mmol/L NaCl, 0.1 % polysorbate 80; pH 6.5			GLP compliance: Yes
Special features: N/A	Litters culled/not culled: Culled			
No observed adverse effect level: Subcutaneous injection of anakinra at doses up to 200 mg/kg/day b.i.d. did not produce any evidence of parental toxicity or impaired reproductive capabilities in the F_0 generation, or impact survival or growth of the F_1 generation.				
Daily dose (mg/kg/day)	0 (Control)	12.5	50.0	200.0
F_0 females:	40	40	40	40
No. of females sperm-positive	40/40	39/40	39/40	37/40
No. pregnant	34/40	36/39	32/39	32/37
No. of animals examined postbreeding Day 25[a]	5	2	2	4
No. of animals examined postbreeding Period Day 25	0	1	1	3
No. aborted or with total resorption of litter	0	0	0	2
Clinical observations	–	–	–	–
Necropsy observations	–	–	-	-
Gestation body weight (%)	–	–	–	–
Lactation body weight (%)	–	–	–	–
Gestation food consumption (%)	–	–	–	–
Lactation food consumption (%)	–	–	–	–

Fertility and general reproduction (Segment I) study of IL-1Ra administered by subcutaneous injection to rats								Test article: Anakinra
Design similar to ICH 4.1.1: Not reported	Duration of dosing: Males: 64 days prior to mating to study day 85, Females: 15 days prior to mating, during gestation and lactation until the day prior to euthanasia							Study no.: SY05-T93-01
Females with delivery	15		17		17		13	
Mean duration of gestation (days)	22.1		21.9		22.0		22.0	
Abnormal parturition	–		–		–		–	
F_1 litters (preweaning):	–		–		–		–	
No. litters evaluated	19		19		15		17	
Mean no. of implantations	16		17		17		17	
Mean no. pups/litter	–		–		–		–	
Mean no. liveborn pups/litter	14.9		15.5		15.3		14.5	
No. of litters with stillborn pups	0.1		0.4		0.2		0.2	
Postnatal survival to day 4	14.3		15.3		15.1		14.5	
Postnatal survival to weaning	7.7		8		8		8	
Mean weight at birth (g)	7.2		6.7		7.1		7.0	
Mean weight at weaning (g)	57.0		54.4		56.2		57.6	
No. of total litter losses	–		–		–		–	
Change in pup body weights (g)[a]	–		–		–		–	
Pup sex ratios (male/female)	55	45	46	54	54	46	52	48
Pup clinical signs	–		–		–		–	
Pup necropsy observations	–		–		–		–	

Abbreviations: "-", Not reported, no data; b.i.d., Twice daily; eCTD, Electronic Common Technical Document; GLP, Good Laboratory Practice; ICH, International Council for Harmonisation; IL-1Ra, interleukin-1 receptor antagonist; N/A, Not applicable; no., Number; s.c., Subcutaneous.

[a] From birth to weaning.

2.6.7.12.2 ラット受胎能及び着床までの初期胚発生に関する試験

A study of fertility and early embryonic development to implantation of recombinant-methionyl human interleukin-receptor antagonist (r-metHulL-1Ra) administered subcutaneously in rats		Test article: Anakinra
Design similar to ICH 4.1.1: Not reported	Duration of dosing: Males: from 29 days prior to mating to the day before necropsy Females: from 14 days prior to mating to 7 days after mating	Study no.: 960044
Species/strain: Rat/Sprague-Dawley Crl:CD VAF/Plus	Day of mating: F: Sperm in vaginal smear = Day 0 of gestation	Location in eCTD: 4.2.3.5.1
Initial age: 9 to 10 weeks	Day of C-section: Gestation day 15	
Date of first dose: N/A	Method of administration: s.c. injection	GLP compliance: Yes
Special features: N/A	Vehicle/formulation: 10 mmol/L sodium citrate, 140 mmol/L NaCl, 0.5 mmol/L EDTA, 0.1 % (w/w) polysorbate 80; pH 6.5	

No observed adverse effect level: Based on the results of this study, the NOAEL was considered to be > 200 mg/kg/day for parental toxicity and reproductive performance.

Daily dose (mg/kg)	0 (Control)	10	100	200
No. of males:	25	25	25	25
No. evaluated	25	25	25	25
No. died or sacrificed moribund	0	0	0	0
Clinical observations	–	–	–	–
Necropsy observations	–	–	–	–
Body weight (%)	–	–	–	–
Food consumption (%)	–	–	–	–
Mean no. days prior to mating	–	–	–	–
No. of males with evidence of mating	24	24	23	24
No. of fertile males	24	23	22	24
No. of females:	25	25	25	25
No. evaluated	25	25	25	25
No. died or sacrificed moribund	0	0	0	1
Clinical observations	–	–	–	–
Necropsy observations	–	–	–	–
Premating body weight (%)	–	–	–	–
Gestation body weight (%)	–	–	–	–
Premating food consumption (%)	–	–	–	–

A study of fertility and early embryonic development to implantation of recombinant-methionyl human interleukin-receptor antagonist (r-metHulL-1Ra) administered subcutaneously in rats				Test article: Anakinra
Design similar to ICH 4.1.1: Not reported	Duration of dosing: Males: from 29 days prior to mating to the day before necropsy Females: from 14 days prior to mating to 7 days after mating			Study no.: 960044

Daily dose (mg/kg)	0 (Control)	10	100	200
Gestation food consumption (%)	–	–	–	–
Mean no. estrous cycles/14 days	–	–	–	–
Mean no. days prior to mating	–	–	–	–
No. of females sperm-positive	24/25	25/25	24/25	24/25
No. of pregnant females	25/25	24/25	24/25	24/25
No. aborted or with total resorption of litter	–	–	–	–
Mean no. corpora lutea	17.7	18.2	18.0	17.3
Mean no. implantations	15.5	17.0	15.0	15.8
Mean % preimplantation loss	12.7	6.3*	16.9	8.1
Mean no. live conceptuses	14.8	16.3	13.7	15.0
Mean no. resorptions	0.7	0.7	1.3	0.8
No. dead conceptuses	0	0	0	0
Mean % postimplantation loss	4.7	4.4	8.5	4.9

Abbreviations: “-”, Not reported, no data; eCTD, Electronic Common Technical Document; EDTA, Ethylenediamine tetraacetic acid; GLP, Good Laboratory Practice; ICH, International Council for Harmonisation; N/A, Not applicable; NaCl, sodium chloride; no., Number; NOAEL, No observed adverse effect level; r-metHulL-1Ra, Recombinant-methionyl human interleukin receptor antagonist; s.c., Subcutaneous.
Statistical analyses: Mann-Whitney U-test; * = significantly different from the control group at the 0.01 level.

2.6.7.13 生殖発生毒性試験：胚・胎児発生に関する試験

2.6.7.13.1 ラット胚・胎児発生に関する試験

Developmental toxicity study in rats with interleukin-1 receptor antagonist (IL-1Ra)				Test article: Anakinra
Design similar to ICH 4.1.1: Not reported	Duration of dosing: F_0 Females - GD 6 to 17			Study No.: SY05-T92-03
Species/strain: Rat/Sprague-Dawley Crl:CD BR VAF/Plus	Day of mating: Sperm in vaginal smear = Day 0 of gestation			Location in eCTD: 4.2.3.5.3
Initial age: 13 weeks	Method of administration: s.c. injection, b.i.d.			
Date of first dose: [redacted], 19[redacted]	Vehicle/formulation: 10 mmol/L citrate, 140 mmol/L NaCl, 0.5 mmol/L EDTA, 0.1 % polysorbate 80; pH 6.5			GLP compliance: Yes
Special features: N/A	Litters culled/not culled: Culled			
No observed adverse effect level: Subcutaneous injection of anakinra at doses up to 200 mg/kg/day b.i.d. did not produce any evidence of maternal toxicity in rats through the F_1 generation or developmental toxicity in rats through the F_2 generation.				
Daily dose (mg/kg/day)	0 (Control)	12.5	50.0	200.0
F_0 females:	40	40	40	40
No. of animals euthanized postbreeding Day 25	0	0	0	1
No. of females sperm-positive	21/21	21/21	21/21	21/21
No. pregnant	20/21	21/21	20/21	20/21
Evaluated pregnant females	20	21	20	20
No. aborted or with total resorption of litter	0	0	0	0
Mean no. corpora lutea	15.4	15.3	15.1	15.7
Mean no. implantations	14.2	14.6	14.5	14.6
Mean % preimplantation loss	–	–	–	–
Mean no. live conceptuses	13.3	13.4	13.1	13.3
Mean no. resorptions	1.0	1.1	1.5	1.3
No. dead conceptuses	0	0	0	0
Mean weight of fetuses (g)	3.5	3.5	3.6	3.5
Mean % postimplantation loss	–	–	–	–
Clinical observations	–	–	–	–
Necropsy observations	–	–	–	–
Gestation body weight (%)	–	–	–	–

Developmental toxicity study in rats with interleukin-1 receptor antagonist (IL-1Ra)								Test article: Anakinra	
Design similar to ICH 4.1.1: Not reported	Duration of dosing: F_0 Females - GD 6 to 17							Study No.: SY05-T92-03	
Daily dose (mg/kg/day)	0 (Control)		12.5		50.0		200.0		
Lactation body weight (%)	–		–		–		–		
Gestation food consumption (%)	–		–		–		–		
Lactation food consumption (%)	–		–		–		–		
Females with delivery	19/19		19/19		19/19		18/19		
Females that did not deliver	0/19		0/19		0/19		1/19		
Mean duration of gestation (days)	21.9		22.0		22.0		21.9		
Abnormal parturition	–		–		–		–		
Pup sex ratios (male/female)	54	46	49	51	48	52	47	53	
F_1 litters (preweaning):	19		19		19		18		
No. litters evaluated	–		–		–		–		
Mean no. of implantations	15		16		16		15		
Mean no. pups/litter	–		–		–		–		
Mean no. liveborn pups/litter	13.8		14.2		14.0		13.6		
No. of litters with stillborn pups	0.4		0.4		0.5		0.3		
Postnatal survival to Day 4	13.4		13.9		13.3		13.1		
Postnatal survival to weaning	7.9		8		7.8		7.7		
Mean weight at birth (g)	6.5		6.7		6.5		6.5		
Mean weight at weaning (g)	49.1		47.4		48.7		48.6		
No. of total litter losses	–		–		–		–		
Change in pup body weights (g)[a]	–		–		–		–		
Pup sex ratios (male/female)	52	48	52	48	43	57	49	51	
Pup clinical signs	–		–		–		–		
Pup necropsy observations	–		–		–		–		
F_1 females:	20		20		20		20		
No. of females sperm-positive	19/20		20/20		20/20		20/20		
No. pregnant	16/20		16/20		19/20		20/20		
Evaluated pregnant females	16		16		19		20		

Developmental toxicity study in rats with interleukin-1 receptor antagonist (IL-1Ra)								Test article: Anakinra
Design similar to ICH 4.1.1: Not reported	Duration of dosing: F_0 Females - GD 6 to 17							Study No.: SY05-T92-03
Daily dose (mg/kg/day)	0 (Control)		12.5		50.0		200.0	
No. aborted or with total resorption of litter	0		0		1		0	
Females with delivery	16/20		15/20		19/20		20/20	
Females that did not deliver	4/20		5/20		1/20		0/20	
Mean duration of gestation (days)	21.8		21.5		21.7		21.9	
F_2 litters (preweaning):	16		15		19		20	
Mean no. implantations	15		16		16		15	
Mean no. liveborn pups/litter	14.2		15.0		13.8		13.6	
No. of litters with stillborn pups	0.4		0.1		0.1		0.3	
Postnatal survival to Day 4	13.9		14.7		12.6		13.3	
Postnatal survival to weaning	7.9		8		7.6		7.3	
Mean weight at birth (g)	6.4		6.2		6.5		6.6	
Mean weight at weaning (g)	46.1		48.3		48.7		48.7	
Pup sex ratios (male/female)	46	54	49	51	53	47	45	55

Abbreviations: "-", Not reported, no data; b.i.d., Twice daily; eCTD, Electronic Common Technical Document; GD, Gestation day; GLP, Good Laboratory Practice; ICH, International Council for Harmonisation; IL-1Ra, Interleukin-1 receptor antagonist; N/A, Not applicable; no., Number; s.c., Subcutaneous.

[a] From birth to weaning.

2.6.7.13.2 ウサギ胚・胎児発生に関する試験

Developmental toxicity study in rabbits with interleukin-1 receptor antagonist (IL-1Ra)				Test article: Anakinra
Design similar to ICH 4.1.1: Not reported		Duration of dosing: GD 6 to 18		Study no.: SY05-T92-04
Species/strain: Rabbit/New Zealand White		Day of mating: Insemination = Day 0 of gestation		Location in eCTD: 4.2.3.5.2
Initial age: 6 months		Day of C-section: GD 29		
Date of first dose: [redacted], 19[redacted]		Method of administration: s.c. injection, b.i.d.		GLP compliance: Yes
Special features: N/A		Vehicle/formulation: 10 mmol/L citrate, 140 mmol/L NaCl , 0.5 mmol/L EDTA, and 0.1 % polysorbate 80; pH 6.5		
No observed adverse effect level: Based on the results of this study, the NOAEL was considered to be > 200 mg/kg/day for systemic maternal toxicity or developmental toxicity including teratogenicity when administered s.c. (b.i.d.) daily during days 6 through 18 of gestation in the rabbit.				

Daily dose (mg/kg)	0 (Control)		12.5		50.0		200.0	
Dams/does:	20		20		20		20	
Toxicokinetics: AUC ([units])	N/A		N/A		N/A		N/A	
No. pregnant	15/20		17/20		18/20		17/20	
No. died or sacrificed moribund	0		0		0		0	
No. aborted or with total resorption of litter	0		0		0		0	
Clinical observations	–		–		–		–	
Body weight (%)	–		–		–		–	
Food consumption (%)	–		–		–		–	
Mean no. corpora lutea	10.7		9.8		9.7		9.8	
Mean no. implantations	7.1		7.6		8.1		7.3	
Mean % preimplantation loss	–		–		–		–	
Litters:	15		17		18		17	
No. litters evaluated	15		17		18		17	
No. live fetuses	6.8		7.1		7.2		7.0	
Mean no. resorptions	0.3		0.5		0.9		0.3	
No. of litters with dead fetuses	–		–		–		–	
Mean % postimplantation loss	–		–		–		–	
Mean fetal body weight (g)	44.0		43.5		43.9		41.1	
Fetal sex ratios (M/F)	49	51	46	54	55	45	60	40

Developmental toxicity study in rabbits with interleukin-1 receptor antagonist (IL-1Ra)			Test article: Anakinra	
Design similar to ICH 4.1.1: Not reported	Duration of dosing: GD 6 to 18		Study no.: SY05-T92-04	
Daily dose (mg/kg)	0 (Control)	12.5	50.0	200.0
Fetal anomalies	–	–	–	–
Gross external	1	1	0	0
Visceral anomalies	1	0	0	0
Skeletal anomalies	3	4	6	2
Total affected fetuses (litters)	5	5	6	2

Abbreviations: “-”, Not reported, no data; b.i.d., Twice daily; eCTD, Electronic Common Technical Document; EDTA, Ethylenediamine tetraacetic acid; GD, Gestation day(s); GLP, Good Laboratory Practice; ICH, International Council for Harmonisation; IL-1Ra, interleukin-1 receptor antagonist; NaCl, sodium chloride;no., Number; NOAEL, No observed adverse effect level; s.c., Subcutaneous.

2.6.7.14 生殖発生毒性試験：出生前及び出生後の発生並びに母体の機能に関する試験

Perinatal and postnatal study of interleukin-1 receptor antagonist (IL-1Ra) administered by subcutaneous injection to rats				Test article: Anakinra
Design similar to ICH 4.1.1: Not reported	Duration of dosing: F_0 females - GD 6 through lactation day 20			Study no.: SY05-T93-02
Species/strain: Rat/Sprague-Dawley Crl:CD BR VAF/Plus	Day of mating: Sperm in vaginal smear = Day 0 of gestation			Location in eCTD: 4.2.3.5.3
Initial age: 96 days	Method of administration: s.c. injection, b.i.d.			
Date of first dose: [redacted], 19[redacted]	Vehicle/formulation: 10 mmol/L sodium citrate, 140 mmol/L NaCl, 0.1 % polysorbate 80; pH 6.5			GLP compliance: Yes
Special features: N/A	Litters culled/not culled: Culled			
No observed adverse effect level: Subcutaneous injection of anakinra at doses up to 200 mg/kg/day b.i.d. did not produce any evidence of maternal toxicity in rats through the F_1 generation or developmental toxicity in rats through the F_2 generation.				
Daily dose (mg/kg/day)	0 (Control)	12.5	50.0	200.0
F_0 Females:	25	25	25	25
No. died or sacrificed moribund	0	1	0	0
No. of females sperm-positive	–	–	–	–
No. pregnant	24	17	23	23
Evaluated pregnant females	–	–	–	–
No. aborted or with total resorption of litter	–	–	–	–
Mean no. corpora lutea	–	–	–	–
Mean no. implantations	–	–	–	–
Mean % preimplantation loss	–	–	–	–
Mean no. live conceptuses	–	–	–	–
Mean no. resorptions	–	–	–	–
No. dead conceptuses	–	–	–	–
Mean weight of fetuses (g)	–	–	–	–
Mean % postimplantation loss	–	–	–	–
Clinical observations	–	–	–	–
Swollen limb(s)	1	1	1	–
Hair loss (limb)	1	4	6	6
Necropsy observations	–	–	–	–
Enlarged spleen	–	1	–	–

Perinatal and postnatal study of interleukin-1 receptor antagonist (IL-1Ra) administered by subcutaneous injection to rats								Test article: Anakinra	
Design similar to ICH 4.1.1: Not reported	Duration of dosing: F_0 females - GD 6 through lactation day 20							Study no.: SY05-T93-02	
Daily dose (mg/kg/day)	0 (Control)		12.5		50.0		200.0		
Gestation body weight (%)[a] - Day 6 to 9	–		–		I[#]		I[#]		
Lactation body weight (%)	–		–		–		–		
Gestation food consumption (%)	–		–		–		–		
Lactation food consumption (%)	–		–		–		–		
Females with delivery	23/25		17/25		23/25		23/25		
Females that did not deliver	2/25		7/25		2/25		2/25		
Mean duration of gestation (days)	22.0		21.9		21.9		22.0		
Abnormal parturition	–		–		–		–		
Pup sex ratios (male/female)	–	–	–	–	D*	–	–	–	
F_1 litters (preweaning):	–		–		–		–		
No. litters evaluated	–		–		–		–		
Mean no. of implantations	15.5		15.8		16.0		15.5		
Mean no. pups/litter	–		–		–		–		
Mean no. liveborn pups/litter	15.5		15.8		16.0		15.5		
No. of litters with stillborn pups	–		–		–		–		
Postnatal survival to Day 4	350/356		263/268		360/369		356/357		
Postnatal survival to weaning	–		–		–		–		
Mean weight at birth (g)	–		–		–		–		
Mean weight at weaning (g)	–		–		–		–		
No. of total litter losses	–		–		–		–		
Change in pup body weights (g)[b]	–		–		–		–		
Pup sex ratios (male/female)	195	161	143	125	173	196*	186	171	
Pup clinical signs	–		–		–		–		
Pup necropsy observations	–		–		–		–		
F_1 Females:	20		20		20		20		
No. of females sperm-positive	–		–		–		–		
No. pregnant	14		13		12		12		
Evaluated pregnant females	–		–		–		–		

Perinatal and postnatal study of interleukin-1 receptor antagonist (IL-1Ra) administered by subcutaneous injection to rats							Test article: Anakinra	
Design similar to ICH 4.1.1: Not reported	Duration of dosing: F_0 females - GD 6 through lactation day 20						Study no.: SY05-T93-02	
Daily dose (mg/kg/day)	0 (Control)		12.5		50.0		200.0	
No. aborted or with total resorption of litter	0		0		1		0	
Females with delivery	14/20		12/20		12/20		12/20	
Females that did not deliver	6/20		8/20		8/20		8/20	
Mean duration of gestation (days)	21.9		21.9		21.9		21.7	
F_2 Litters (Preweaning):	–		–		–		–	
Mean no. implantations	14.6		14.3		15.3		15.1	
Mean no. liveborn pups/litter	14.6		14.3		15.3		15.1	
No. of litters with stillborn pups	–		–		–		–	
Postnatal survival to Day 4	201/204		168/172		161/183**		180/181	
Postnatal survival to weaning	–		–		–		–	
Mean weight at birth (g)	–		–		–		–	
Mean weight at weaning (g)	–		–		–		–	
Pup sex ratios (male/female)	103	101	75	97	81	102	94	87

Abbreviations: "-", Not reported, no data; ANOVA, Analysis of variance; b.i.d., Twice daily; D, Decrease; eCTD, Electronic Common Technical Document; GD, Gestation day; GLP, Good Laboratory Practice; I, Increase; ICH, International Council for Harmonisation; IL-1Ra, Interleukin-1 receptor antagonist; NaCl, sodium chloride;no., Number; s.c., Subcutaneous.

[a] At the end of gestation or lactation. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

[b] From birth to weaning.

Statistical analyses: ANOVA: # = $p < 0.05$; Chi Square Test: * = $p < 0.05$, ** = $p < 0.01$.

2.6.7.15 新生児を用いた試験

2.6.7.15.1 新生児を用いた重要な試験以外の試験

Single and repeat-dose toxicity: nonpivotal studies						Test article: Anakinra	
Species/strain	Method of administration and frequency (vehicle/formulation)	Duration of dosing	Doses (mg/kg)	Sex/ No. per group	NOAEL (mg/kg)	Noteworthy findings	Study no.
Rat, Sprague-Dawley	s.c., q.d. for Group 1 and b.i.d. for Group 2 (citrate, 10 mmol/L; NaCl, 140 nmol/L; EDTA, 0.5 mmol/L; polysorbate 80, 0.1 % (v/v); pH 6.5)	From Day 4 until Day 10 postpartum (Group 1) or until Day 19 postpartum (Group 2)	Group 1: 100 Group 2: 200	Male: 4 Female: 4	100	The q.d. or b.i.d. administration of anakinra to juvenile animals was well tolerated at dose levels of 100 mg/kg.	497888

Abbreviations: “-”, Not reported, no data; b.i.d., Twice daily; EDTA, Ethylenediamine tetraacetic acid; NaCl, sodium chloride;no., Number; NOAEL, No observed adverse effect level; q.d.; Once daily; s.c., Subcutaneous; v/v, Volume per volume.

2.6.7.15.2 新生児を用いた重要な試験

A 6-week neonatal/juvenile study to determine the adverse effects on memory and learning functions in rats after subcutaneous injection followed by a 1-month recovery period							Test article: Anakinra	
Species/strain: Rat/Sprague-Dawley		Duration of dosing: 6 weeks					Study no.: 498001	
Initial age: 7 days postpartum		Duration of postdose: 1 month					Location in eCTD: 4.2.3.5.3	
Date of first dose: [redacted], 20[redacted]		Method of administration: s.c. bolus injection, b.i.d.					GLP compliance: Yes	
Special features: N/A		Vehicle/formulation: 10 mmol/L citrate buffer, 140 mmol/L NaCl, 0.5 mmol/L EDTA and 0.1 % polysorbate 80, pH 6.5						
No observed adverse effect level: 200 mg/kg/day								
Daily dose (mg/kg)		0 (Control)		20		60		200	
No. of animals		M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20
Noteworthy findings									
Body weight (%)		–	–	–	–	–	–	–	–
Clinical observations	Fur staining	–	–	–	–	I	I	I	I
	Wet fur	–	–	–	–	I	I	I	I
Behavior (learning and memory) Multiple Y Water Maze Test		–	–	–	–	–	–	–	–
Organ weights	Brain	–	–	–	–	–	–	–	–
	Liver	–	–	–	–	–	–	–	–
Serum level bioanalysis		Not detected		–	–	–	–	22 µg/mL (range: 15 to 33 µg/mL)	
Cerebrospinal fluid bioanalysis		Not detected		–	–	–	–	0.18 µg/mL (range: 0.13 to 0.22 µg/mL)	

Abbreviations: "-", Not reported, no data; b.i.d., Twice daily; eCTD, Electronic Common Technical Document; EDTA, Ethylenediamine tetraacetic acid; F, Female; GLP, Good Laboratory Practice; I, Increased; IL-1Ra, Interleukin-1 receptor antagonist; M, Male; N/A, Not applicable; NaCl, sodium chloride; no., Number; s.c., Subcutaneous.

2.6.7.16　局所刺激性試験

単独の局所刺激性試験は実施していないが、ICH S6(R1)ガイドラインに準拠して、アナキンラの局所刺激性は反復投与毒性試験（一般状態観察並びに投与部位の肉眼的及び病理組織学的検査）において評価した。

2.6.7.17 その他の毒性試験

Other toxicity studies					Test article: anakinra	
Type of Study	**Species/strain**	**Method of administration**	**Doses (mg/kg)**	**Sex/no. per group**	**Noteworthy findings**	**Study no.**
Immunotoxicity	Rat/Fisher 344	s.c. (anakinra and TNF) i.v. (anti-asialo GM1 antibody) i.p. (cyclophosphamide)	Anakinra – 0, 20, 200 mg/kg/day TNF – 5, 50 mg/kg Anti-asialo GM1 antibody – 24 hours prior to scheduled sacrifice (Phase 1 only) Cyclophosphamide – 25 mg/kg 4 days prior to sacrifice	F12	No animals died or were sacrificed in extremis in any group. Treatment for at least 21 days with anakinra had minimal effects on the structure and function of the immune system in female rats, except for an apparent slight enhancement of NK cell activity. Treatment for at least 21 days with PEG-s TNF-R1 caused an increase in lymphocyte counts, decreased spleen and thymus cellularity, sporadic decreases in lymphocyte proliferation following mitogenic stimulation, decreased spleen weights (in immunized animals), and slight, but not statistically significant, decreased induction of AFC. These changes were generally more apparent at the 50 mg/kg/day treatment. There was no evidence that any of the observed effects associated with PEG-s TNF-R1 treatment were exacerbated by concomitant treatment with anakinra.	100797
Mechanistic study (bacterial clearance and organ colonization)	Rat/Tac: N(SD)fBR	i.v.	0, 173, 346 mg/kg/day (CI)	M10	High-dose anakinra treatment resulted in an increased incidence in the recovery of CFU in the blood, spleen, and liver tissues. The increase was statistically significant only for the spleen. No vehicle or low-dose rats had detectable CFU in the blood. Continuous infusion of anakinra and inoculation with *E. coli* was tolerated without mortality. There was a dose-related increase in the incidence of recovery of *E. coli* from blood and liver tissue of rats challenged with *E. coli*.	SY05-T92-17

Other toxicity studies					Test article: anakinra	
Type of Study	**Species/strain**	**Method of administration**	**Doses (mg/kg)**	**Sex/no. per group**	**Noteworthy findings**	**Study no.**
Mechanistic study (bacterial clearance and organ colonization)	Rat/Tac: N(SD)fBR	i.v.	0, 173, 346 mg/kg/day (CI)	M10	CFU data: All rats (except 1 control) had quantifiable CFU/gm in the spleen and liver, with no statistical differences between groups. 1 control and 1 high-dose rat had quantifiable CFU in the blood, and there were no significant differences between groups. Treatment was well tolerated without mortality. There was no dose-related increase in the incidence of recovery of *S. aureus* from blood, spleen, or liver.	SY05-T92-18
Mechanistic study (bacterial clearance and organ colonization)	Rat/Tac: N(SD)fBR	i.v.	0, 173, 346 mg/kg/day (CI)	M10	Low-dose anakinra decreased CFU/g by 45 % in liver, compared to controls; high-dose anakinra had no effect in liver. In spleen, the low-dose of anakinra decreased CFU/g, while the high-dose increased CFU/g. Treatment was well tolerated without mortality. There was no alteration in the response to the bacterial challenge as measured by mortality or clinical signs. After 3 days, no quantifiable bacteria were seen in the blood of any of the rats, indicating clearance of bacteria from the blood.	SY05-T92-19
Antigenicity	Monkey/rhesus (*Macaca mulatta*)	i.v.	10 mg/kg/day	M4	All in-life and clinical pathology were within normal limits.	SY05-T89-07
A 1-month toxicity study of r-metHuIL-1Ra and PEG r-metHu-sTNF-RI administered subcutaneously in cynomolgus monkeys with a 1-month	Monkey/cynomolgus	s.c.	Anakinra – 0, 100 mg/kg; sTNF-RI – 0, 1, 5, 25 mg/kg	M and F/ 2 or 3	Co-administration of anakinra with sTNF-RI did not result in any significant interactive toxicity. Multiple s.c. doses of PEG-sTNF-RI had no apparent effects on the multiple dose s.c. PK of anakinra.	100484

Other toxicity studies					Test article: anakinra	
Type of Study	**Species/strain**	**Method of administration**	**Doses (mg/kg)**	**Sex/no. per group**	**Noteworthy findings**	**Study no.**
recovery period						

Abbreviations: AFC, Antibody forming cell; CFU, Colony-forming unit; CI, Continuous infusion; *E. coli, Escherichia coli*; F, Female; GM1, Ganglioside-monosialic acid; i.p., Intraperitoneal; i.v., Intravenous; M, Male; NK, Natural killer; no., Number; NOAEL, No observed adverse effect level; PEG-sTNF-RI, Polyethylene glycosylated soluble tumor necrosis factor-receptor type I; PK, Pharmacokinetic(s); *S. aureus, Staphylococcus aureus*; s.c., Subcutaneous; sTNF-RI, soluble tumor necrosis factor-receptor type I; TNF, Tumor necrosis factor;　TNF-RI, Tumor necrosis factor-receptor I.