Histopathology:

- No treatment-related effects in the animals that survived to the end of the study
- Findings in the animals that died (from 150 mg/kg Formulation B)
  - Male #3004: inflammatory cell infiltration in intestinal mucosa, intestinal mucosal ulceration with neutrophil loss and blood in lumen, neutrophilic inflammation with bacteria throughout body suggesting septicemia secondary to intestinal ulceration (observed within background frequency in marmosets and observed at with lower severity in controls)
  - Male #3003: thymic lymphoid depletion

Toxicokinetics:

- Tmax
  - 48-72 hours on Day 1
  - 7-24 hours on Day 85
  - Similar in M and F
- Exposure ($AUC_{0\text{-}72h}$ and Cmax) values similar for Formulations A vs. Formulation B
- Increased exposure ($AUC_{0\text{-}72h}$ and Cmax) from Day 1 to Day 85 observations, suggesting accumulation for both Formulations
- Results of the TK analyses are presented in the following tables (from the original BLA submission; Note tables mis-labeled by Applicant; dosing was 2X weekly, and not daily):

Table 4-1 Mean concentrations and toxicokinetic parameters of ACZ885 in marmoset serum on week 1

	Group 2				Group 3			
Formulation	Formulation A (150mg/kg/day)				Formulation B(150mg/kg/day)			
TK parameters on Day 1	M*	CV%	F*	CV%	M*	CV%	F*	CV%
t_{max}	60	23.1	48	40.8	54	22.2	48	40.8
C_{max}	1200.25	28.6	1190	14.8	1355	38.7	1208	17.6
C_{max}/Dose	8.00175	28.6	7.933	14.8	9.0335	38.7	8.05325	17.6
$AUC_{(0\text{-}72h)}$	57487.9	34.2	62102.6	21.4	61656.1	15.8	60075.2	38.4
$AUC_{(0\text{-}72h)}$/Dose	383.253	34.2	414.018	21.4	411.041	15.8	400.502	38.4

M: male, F: female, *: n=4,

CV%: coefficient of variation expressed in %

Units : t_{max} [h]. C_{max} [µg/mL]. C_{max}/dose [(µg/mL)/(mg/kg/day)]. $AUC_{(0\text{-}72h)}$ [h·µg/mL]. $AUC_{(0\text{-}72h)}$/dose [(h·µg/mL)/(mg/kg/day)].

Table 4-2 **Mean concentrations and toxicokinetic parameters of ACZ885 in marmoset serum on week 13**

	Group 2				Group 3			
Formulation	Formulation A(150mg/kg/day)				Formulation B(150mg/kg/day)			
TK parameters on Day 85	M*	CV%	F*	CV%	M**	CV%	F*	CV%
t_{max}	19.75	43	13.75	88.6	24	-	19.75	108.2
C_{max}	2785	20.5	3050	11.3	2255	-	2717.5	13.9
C_{max} /Dose	18.5665	20.5	20.3333	11.3	15.033	-	18.1168	13.9
$AUC_{(0-72h)}$	172815	18.4	179980	21.9	143097	-	174530	16.9
$AUC_{(0-72h)}$/Dose	1152.1	18.4	1199.87	21.9	953.977	-	1163.53	16.9

M: male, F: female, *: n=4, **:n=2

CV%: coefficient of variation expressed in %

Units : t_{max} [h]. C_{max} [µg/mL]. C_{max}/dose [(µg/mL)/(mg/kg/day)]. $AUC_{(0-72h)}$ [h·µg/mL]. $AUC_{(0-72h)}$/dose [(h·µg/mL)/(mg/kg/day)].

Other: Anti-ACZ885 antibody determination: No evidence of anti-ACZ885 antibody response

2.6.6.4 Genetic toxicology

No genetic toxicology studies on ACZ885 were conducted.

2.6.6.5 Carcinogenicity

No carcinogenicity studies on ACZ885 were conducted.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: ***ACZ885 Surrogate (01BSUR): A Once Weekly Subcutaneous Injection Fertility Study in the Mouse***

Key study findings:

- No evidence of adverse effects on male and female fertility and early embryonic development in mice administered 01BSUR at doses of 0, 15, 50 and 150 mg/kg/once weekly by SC injection from 4 weeks before mating through 3 weeks

after the end of mating in the male mice, and from 2 weeks before mating through Gestation Days (GD) 3 or 4 in female mice

- NOAEL for parental toxicity and for treatment-related effects on fertility in M and F mice = 150 mg/kg/once weekly
- NOAEL for treatment-related effects on early embryonic development in mice = 150 mg/kg/once weekly

Study no.: Novartis Study # 0680149, ——— Study # 901096
Conducting laboratory and location: ——— b(4)
———

Date of study initiation: September 5, 2006
GLP compliance: Main study methodology - Yes; test article stability/characterization performed according to Good Manufacturing Regulations (21 CFR Part 21), 01BSUR serum level determination conducted according to Swiss Ordinance GLP based on OECD principles of GLP
QA reports: yes (x) no ( )
Drug ACZ885 Surrogate (01BSUR) 14.2 mg/ml, **lot #** (Batch) 7318, **and % purity**: 98.7%

Methods

Doses: 0 (vehicle control), 15 (LD), 50 (MD), and 150 (HD) mg/kg/once weekly SC; doses selected based on multiples of the anticipated MRHD, on results of prior toxicology studies in mice and on feasibility limitations of the test article (e.g., solubility, injection volume, etc)

Species/strain: Crl:CD1(ICR) Mouse *Mus musculus* ——— b(4)
Ages: 9 weeks Males (M), 8 weeks Females (F)
Weights: 33.1-39.1 g M, 23.2-28.8 g F
Number/sex/group:

- 22/sex/dose group
- Animal allocation is presented in the following table (from the original BLA submission):

Group no. identification	Dose level (mg/kg/day)	Dose dose volume (mL/kg/day)	Animal numbers	
			Males	Females
5/ Vehicle control	0	10	5001-5022	5501-5522
6/ 01BSUR	15	1	6001-6022	6501-6522
7/ 01BSUR	50	3.33	7001-7022	7501-7522
8/ 01BSUR	150	10	8001-8022	8501-8522

Route, formulation, volume, and infusion rate: 01BSUR dissolved in 180 mM sucrose dissolved in Sterile Water for Injection, USP, 10 mM L-histidine, 1.0 ml 2% (m/v) Tween 20 aqueous solution, pH adjusted to 5.5 using 1N HCl, volume adjusted using Sterile Water for Injection, USP; Administered by SC injection in dorsal thoracic

region rotating injection site among 6 sites, once weekly at 10, 1, 3.333, and 10 ml/kg/injection (control, LD, MD, and HD, respectively)

Satellite groups used for toxicokinetics: None

Study design:

- Mice housed individually in stainless steel cages in temperature (22 ± 3 degC) and humidity (50% ± 20%) controlled facility, with 12 hour light/dark cycle, and were provided standard PMI Certified Rodent Chow 5002 and tap water *ad libitum*
- M dosed weekly from 4 weeks before mating through 3 weeks after end of mating
- F dosed weekly from 2 weeks before mating through Gestation Day (GD) 3 or 4
- Same-dose M and F were paired for up to 15 days in same cage, with daily examinations for vaginal copulatory plug performed to confirm pregnancy
- Day of vaginal plug identification designated Gestation Day 1

Parameters and endpoints evaluated:

In-life: All F_0 (parental) M and F

Mortality: Twice daily

Clinical signs: Weekly from Baseline through Treatment period

Body weight: Twice weekly from Baseline through Treatment period

Food consumption: Twice weekly from Baseline through Treatment period

Estrous cycles: For 14 days before mating, and during mating until day of pregnancy determination

Serum 01BSUR levels: Planned at study termination (at necropsy in M, and GD 13 in F), blood samples from 10 mice/sex/dose group planned, but due to sampling constraints, full TK evaluation was not possible

Necropsy: All F_0 M and F; 3 weeks after end of mating period in M and on GD 13 in F

Gross pathology: Immediately after euthanization

Organ weights: The following organs were weighed (paired organs weighed together): organ weight ratios relative to body weight (BW) and brain weight also determined): brain, epididymides (right), ovaries, prostate, seminal vesicles, and testes

Histopathology:

- The following organs and tissues were examined microscopically: abnormalities, brain, epididymides, injection sites, mammary glands (cervical and inguinal), ovaries, prostate, seminal vesicles, skin, testes, uterus (horns, body and cervix), and vagina
- Additional abnormal tissues examined in the decedent (#8504): duodenum, jejunum, spleen, stomach
- Peer review conducted by second pathologist

Male reproductive assessments:

- Sperm motility, spermatozoa counts, and spermatozoa morphology
- Histopathology of right testis

Parental mating performance:

- **Mating index** (%) (#M mating/#M placed for mating X 100)
- **Fertility index** (%) (#M producing pregnant F/#males placed for mating X 100)
- **Conception rate** (%) (#F/#mated F X 100)

Uterine data:

Preimplantation loss (%) (#corpora lutea - #implants/#corpora lutea X 100)
Post implantation loss (%) (#implants - # live embryos/#implants X 100)

Results

Mortality: 1HDF (150 mg/kg/week, #8504) found dead on Mating Day 2 (Study Day 17 was second mating day) in absence of clinical signs; necropsy findings: stomach and duodenal hemorrhage; relationship of death to treatment considered unlikely

Clinical signs: No treatment-related effects

Body weight: No treatment-related effects

Food consumption: No treatment-related effects

Estrous cycles: No treatment-related effects on # days in estrus, # cycles and mean cycle length. See Applicant's table, below:

Table 1.12 Summary of estrous cycle data

Group 5 - Vehicle Control
Group 6 - 01BSUR 15 mg/kg/day
Group 7 - 01BSUR 30 mg/kg/day
Group 8 - 01BSUR 150 mg/kg/day

Group	Summary Information	Number of Days in Estrus*	Number of Cycles Seen**
5	Mean	5.9	3.4
	SD	1.1	0.5
	N	20	20
6	Mean	6.0	3.3
	SD	1.3	0.5
	N	22	22
7	Mean	5.9	3.2
	SD	1.7	0.8
	N	22	22
8	Mean	5.9	3.2
	SD	2.1	0.8
	N	22	22

* Includes only the days in E1 and E2
** Includes actual cycles seen (E1/E2)

Fertility parameters/Parental Mating performance: No treatment-related effects on mean days to mating, mating and fertility indices, and conception rate. See Applicant's table below:

Table 1.13 Summary of parental performance data

Group 5 - Vehicle Control
Group 6 - 01BSUR 15 mg/kg/day
Group 7 - 01BSUR 50 mg/kg/day
Group 8 - 01BSUR 150 mg/kg/day

Group	Number Placed for Mating: Males	Number Placed for Mating: Females	Number Mating Males/Females	Mean (SD) Day to Mating	Number Females Pregnant	Mating Index (%)	Fertility Index (%)	Conception Rate (%)
5	22	22	22	2.2 1.22 (N = 21)	18	100.0	81.8	81.8
6	22	22	22	1.8 0.85 (N = 22)	22	100.0	100.0	100.0
7	22	22	22	2.2 1.08 (N = 21)	21	100.0	95.5	95.5
8	22	22	21@	2.0 0.97 (N = 21)	21	95.5	95.5	100.0

@ Excluding animal 8504, found dead first day of mating

Significantly different from control group (group 5) value: a - $P \le 0.05$ b - $P \le 0.01$ c - $P \le 0.001$ (Wilcoxon - day to mating only)
Significantly different from control group (group 5) value: * - $P \le 0.05$ ** - $P \le 0.01$ *** - $P \le 0.001$ (Fisher's)

Uterine examination: No treatment-related effects on # corpora lutea, implantation sites, live and dead fetuses, resorptions, and pre- and post-implantation losses. See Applicant's table below:

Table 1.14 Summary of uterine finding data

Group 5 - Vehicle Control
Group 6 - 01BSUR 15 mg/kg/day
Group 7 - 01BSUR 50 mg/kg/day
Group 8 - 01BSUR 150 mg/kg/day

Group	Summary Information	Total Number of Corpora Lutea	Total Number of Implantation Sites	Number of Live Embryos	Number of Dead Embryos
5	Mean	13.4	12.2	11.8	0.00
	SD	2.7	3.2	3.1	0.00
	N	17	17	17	17
6	Mean	13.7	12.5	11.9	0.05
	SD	2.1	1.7	2.0	0.21
	N	22	22	22	22
7	Mean	14.3	13.1	11.9	0.00
	SD	1.8	1.7	2.1	0.00
	N	20	20	20	20
8	Mean	14.1	13.2	12.1	0.05
	SD	1.8	1.7	1.9	0.22
	N	21	21	21	21

Significantly different from control group (group 5) value: a - $P \le 0.05$ b - $P \le 0.01$ c - $P \le 0.001$ (Wilcoxon)

Necropsy:

- No treatment-related effects in the standard necroscopic evaluation (absolute and relative organ weights, gross pathology, and histopathology)
- Possible vehicle effect on spleen enlargement observed in F in all groups including controls
    - 6/22, 9/22, 5/22, and 9/22 females at 0, 15, 50, and 150 mg/kg, respectively

- Observed in 1/22 M at 50 mg/kg, only
- Not considered to be treatment-related, in agreement with the Applicant

Male reproductive assessments (sperm motility, spermatozoa counts, spermatozoa morphology): No treatment-related effects. See Applicant's table below:

Table 1.15 Summary of epididymal sperm evaluation data

Group 5 - Vehicle Control
Group 6 - 01BSUR 15 mg/kg/day
Group 7 - 01BSUR 50 mg/kg/day
Group 8 - 01BSUR 150 mg/kg/day

Group	Summary Information	Cauda Epididymis Weight (g)	Spermatozoa Count Per Gram (Millions)	Percent Motility
5	Mean	0.0234	688.790	29.2
	SD	0.0028	208.323	12.7
	N	22	22	21
6	Mean	0.0238	635.327	31.4
	SD	0.0057	182.289	11.8
	N	22	22	22
7	Mean	0.0228	725.345	34.1
	SD	0.0020	278.884	12.4
	N	22	22	22
8	Mean	0.0232	741.122	38.4
	SD	0.0025	283.982	15.4
	N	22	22	22

Significantly different from control group (group 1) value: A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)
D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Toxicokinetics: Serum 01BSUR levels: The results of the limited serum drug sampling are presented in the following table (from the original BLA submission):

Concentrations of ACZ885 surrogate (01BSUR) in male and mouse serum

Study/Gestation day	Dose (mg/kg/day)		
	15	50	150
Males - Study Day 64/65	480	1570	3470
Females – Gestation Day 13	30.3	126	4190

Embryofetal development

Study title: ACZ885 Surrogate (01BSUR): A Weekly Subcutaneous Injection Embryo-Fetal Development Study in the Mouse

Key study findings:

- 01BSUR administered to pregnant mice at doses of 0, 15, 50 and 150 mg/kg by subcutaneous injection on Gestation Days (GD) 6, 11, and 17
- No treatment-related maternal toxicity
- Developmental delay noted in the fetuses at 50 and 150 mg/kg:
  - **Increased incomplete ossification** of **parietal bones** at 50 and 150 mg/kg
  - **Incomplete ossification** of **frontal bones** at 150 mg/kg

- No treatment-related findings in any other skeletal sites
- No treatment-related embryo-fetal major malformations: negative for teratogenicity
- NOAEL for maternal toxicity and teratogenicity by 01BSUR= 150 mg/kg SC
- NOAEL for embryofetal toxicity 15 mg/kg SC 01BSUR, due to slight developmental delay (incomplete ossification of parietal bones and/or frontal bones) at 50 and 150 mg/kg

Study no.: ——— Study 901097, Novartis Study 0680148
Conducting laboratory and location: ——— b(4)

Date of study initiation: 24 January 2007
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug 01BSUR solution for injection (14.2 mg/ml), **lot #** (Batch) 7318, **and % purity:** 98.7%

Methods:

Doses: 0 (control vehicle), 15, 50, and 150 mg/kg; dose selection based on multiples of the anticipated MRHD, toxicity data from previous toxicity studies in mice, and feasibility (solubility, and injection volume)

Species/strain: Female (F) *Mus musculus* Crl:CD1(ICR) mice ——— b(4)

Ages: 6 weeks
Weights: 24.2-36.0 g
Number/sex/group: 22F/dose

Group number identification	Dose level (mg/kg/day)	Dose volume (mL/kg/day)	Animal number - Females	
			Main	TK
1/ Vehicle control	0	10	1501-1522	1523-1528
2/ 01BSUR	15	1	2501-2522	2523-2564
3/ 01BSUR	50	3.33	3501-3522	3523-3564
4/ 01BSUR	150	10	4501-4522	4523-4564

Route, formulation, volume, and infusion rate: 01BSUR diluted in vehicle control article (180 mM N.F. sucrose, 10 mM L-histidine, and 1.0 ml 2% (m/v) Tween 20 N.F., pH adjusted to 5.5 using 1N HCl) administered to pregnant mice by subcutaneous (SC) injection at 10, 1, 3.33 and 10 ml/kg/injection at 0, 15, 50, and 150 mg/kg, respectively, on Gestation Days (GD) 6, 11 and 17.
Satellite groups used for toxicokinetics: 3 dams/group, fetuses from the 3 satellite dams/group
Study design:

- Mice were housed individually, except for during the mating phase, in stainless steel cages in a temperature (22 ± 3 degC) and humidity (50% ±

20%) controlled facility, with 12 hour light/dark cycle, and were provided standard PMI Certified Rodent Chow 5002 and tap water *ad libitum*
- F mice were mated prior to drug administration, with pregnancies confirmed by presence of vaginal copulatory plug
- Pregnant F were given SC test article injections on GD 6, 11, and 17 (total 3 injections)
- Standard embryo-fetal toxicity parameters evaluated

Parameters and endpoints evaluated:

In-life examinations:

Mortality: Twice daily
Clinical signs: Baseline, pre-dose and for 3 hours after each dose
Body weight: On GD 0, 3, 6, 9, 11, 14, 17, and 18
Food consumption: On GD 3-6, 6-9, 9-11, 11-14, 14-17, and 17-18

Pathology: Dams euthanized on GD 18

Maternal examination: The following parameters were evaluated:

Body weights (BW), **body weight gains** (BWG), corrected BW (cBW, body weight – gravid uterus weight), corrected BWG (cBWG, BWG on Gestation Days 6-18 – gravid uterus weight)
Complete gross pathology examination of carcass, internal and external
Pregnancy status
Corpora lutea
Uterus weight
Uterus contents, including placentas, number and position of live and dead fetuses, and early, middle and late resorptions, and implantation sites

Fetal examination:

Body Weights
External examination
Sex
Detailed internal examination using dissecting microscope (1/2 fetuses)
Skeletal examination (remaining 1/2 fetuses)
Classification of major malformations, minor external visceral or skeletal variations, common skeletal variations (calculated for group, litter, and individual fetuses)

Calculations:

Pregnancy rate (%): (#pregnant mice/#mated mice) X 100
Pre-implantation loss (%): (#corpora lutea - # live fetuses/# implants) X 100
Post-implantation loss (%): (#implants - # live fetuses/# implants) X 100
Sex ratio: % of M (#F/#M x 100)

Toxicokinetics:

- **Dams:** GD 6 (1 & 6 h post-dose 1), 7 (24 h post-dose 1), 9 (72 h post-dose 1), 11 (pre-dose 2/120 h post-dose 1, and 1 h & 8 h post-dose 2), 12 (24 h post-dose 2), 14 (72 h post-dose 2), 16 (120 h post-dose 2), 17 (pre-dose 3 / 144 h post-dose 2, 1 & 8 h post-dose 3, and 18 (24 h post-dose 3)

- **Fetuses** from the satellite dams: GD 17, blood samples collected pre-dose (144 h post-dose 2) for verification of exposure; however TK analyses not conducted at the 1 h and 8 h post-dose timepoints on GD 18 due to collection errors

Results:

Mortality (dams): No treatment-related mortality

Clinical signs (dams): No treatment-related effects

Body weight (dams): No treatment-related effects on BW, BWG, cBW and cBWG

Food consumption (dams): No treatment-related effects

Uterine findings: No treatment-related effects on pregnancy rates (means of 82%, 96%, 91% and 86% at dose levels of 0, 15, 50, and 150 mg/kg, respectively), corpora lutea, implantation sites, live fetuses, dead fetuses, sex ratio, resorptions, pre-implantation loss, and post-implantation loss. See Applicant's tables below:

Table 1.6 Summary of uterine findings data

Group 1 - Vehicle Control
Group 2 - 01BSUR 15 mg/kg/day
Group 3 - 01BSUR 50 mg/kg/day
Group 4 - 01BSUR 150 mg/kg/day

Group	Summary Information	Total Number of Corpora Lutea	Total Implantation Sites	Male Fetuses	Female Fetuses	Sex Ratio (% Males)
1	Mean	15.29	13.53	6.82	6.00	53.89
	SD	2.23	1.70	1.85	2.37	14.74
	N	17	17	17	17	17
2	Mean	13.76	12.52	5.90	6.05	49.23
	SD	2.14	2.16	2.28	1.99	15.59
	N	21	21	21	21	21
3	Mean	13.55	12.90	6.80	5.50	54.85
	SD	1.57	1.77	2.24	1.82	14.04
	N	20	20	20	20	20
4	Mean	13.83	11.94	5.94	5.50	54.11
	SD	2.26	3.26	2.55	2.77	20.84
	N	18	18	18	18	18

Group	Summary Information	Live Fetuses	Dead Fetuses	Early Resorptions	Middle Resorptions	Late Resorptions
1	Mean	12.82	0.00	0.65	0.00	0.06
	SD	1.94	0.00	0.86	0.00	0.24
	N	17	17	17	17	17
2	Mean	11.95	0.00	0.52	0.05	0.00
	SD	2.22	0.00	0.68	0.22	0.00
	N	21	21	21	21	21
3	Mean	12.30	0.00	0.50	0.05	0.05
	SD	1.89	0.00	0.89	0.22	0.22
	N	20	20	20	20	20
4	Mean	11.44	0.00	0.28	0.00	0.22
	SD	3.11	0.00	0.67	0.00	0.55
	N	18	18	18	18	18

Group	Summary Information	Sum of Resorptions	Pre-Implantation Loss (%)	Post Implantation Loss (%)	Gravid Uterus Weight (g)
1	Mean	0.71	11.04	5.30	21.32
	SD	0.85	7.15	8.27	3.02
	N	17	17	17	14
2	Mean	0.57	9.02	4.67	21.04
	SD	0.68	7.29	5.54	3.53
	N	21	21	21	18
3	Mean	0.60	4.93	4.73	20.82
	SD	0.75	6.03	8.17	2.85
	N	20	20	20	18
4	Mean	0.50	14.58	3.74	21.44
	SD	0.79	19.29	5.94	2.40
	N	18	18	18	14

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Wilcoxon)

Pathology (maternal): No treatment-related effects in the gross pathology examination

Fetal examination:

- No treatment-related effects on fetal weights
- No treatment-related effects on major external, visceral or skeletal malformations
- No treatment-related effects on external and visceral variations/anomalies
- Developmental delay noted at MD and HD:
  - **Increased incomplete ossification** of **parietal bones** at 50 (11.3% of fetuses at that dose) and 150 (24%) mg/kg vs. concurrent controls (2.8%) and vs. historical controls (0%-4.2%)
  - **Incomplete ossification** of **frontal bones** at 150 mg/kg (18.3% vs. concurrent controls (5.7%) and vs. historical controls (0%-4.2%))
  - No other treatment-related skeletal findings
  - The incidence summary of incomplete ossification of parietal and frontal bones is presented in the following table (from the original BLA submission):

Group 1 - Vehicle Control
Group 2 - 01BSUR 15 mg/kg/day
Group 3 - 01BSUR 50 mg/kg/day
Group 4 - 01BSUR 150 mg/kg/day

	Group 1		2		3		4	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
External (EXT)	17	218	21	251	20	246	18	206
Visceral (VIS)	17	113	21	125	20	123	18	102
Skeletal (SKE)	17	106	21	126	20	124	18	104
Technique of Wilson (WT)	17	113	21	127	20	123	18	102
Major Malformations (Cont'd)	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
Minor Skeletal Anomalies (Total)	16	76	20	91	20	93	17	71
Skull								
Parietal Bone(s): Incomplete Ossification	1	3	5	8	9 *	14 *	8 *	25 ***
Frontal Bone(s): Incomplete Ossification	3	6	6	10	9	16	9	19 **
Supraoccipital Bone: Incomplete Ossification	6	18	11	23	15	34	7	24
Supraoccipital Bone: Bipartite	0	0	0	0	1	1	0	0

L/E = Litters examined L/A = Litters affected
F/E = Fetuses examined F/A = Fetuses affected
Significantly different from control group (group 1) value: * - $P \leq 0.05$ ** - $P \leq 0.01$ *** - $P \leq 0.001$ (Fisher's)

Historical Incidence for Incomplete Ossification of Parietal and Frontal Bones in CD-1 Mice _____ (from the original BLA submission): b(4)

b(4)

SUMMARY OF FETAL SKELETAL ALTERATIONS
Crl:CD1(ICR) MICE

PERIOD	JUNE 2003 - JUNE 2007
# STUDIES	17
# LITTERS EXAMINED	365
# FETUSES EXAMINED	2300

ALTERATION		N	%	RANGE / STUDY N	RANGE / STUDY %
SKULL					
Frontals: Interfrontals present	L	129	35.34	3-12	(13.6-52.2)
	F	231	10.04	3-26	(2.1-20.5)
: Incompletely ossified	L	2	0.55	0-1	(0-4.2)
	F	2	0.09	0-1	(0-0.7)
: Suture large	L	1	0.27	0-1	(0-4.3)
	F	2	0.09	0-2	(0-1.3)
Parietals: Not ossified	L	0	—	—	—
	F	0	—	—	—
: Incompletely ossified	L	2	0.55	0-1	(0-4.2)
	F	2	0.09	0-1	(0-0.7)

Toxicokinetics:

- **Dams:** The results of the serum 01BSUR concentration measurements in the dams are presented in the following table (from the original BLA submission):

Table 4.1 Concentrations of ACZ885 surrogate (01BSUR) in dam mouse serum

Day/hour	Dose (mg/kg/day)			Day/hour	Dose (mg/kg/day)		
	15	50	150		15	50	150
6/1	4.15 (2)*	10.7 [40.6] (3)	16.6 [51.1] (3)	12/24	91.3 [8.3] (3)	358 (1)	504 [65.1] (3)
6/8	87.7 [30.7] (3)	273 (2)	715 [22.0] (3)	14/72	29.9 [11.7] (3)	157 [64.9] (3)	160 [61.0] (3)
7/24	79.7 (2)	282 [8.4] (3)	1300 [16.8] (3)	16/120	3.48 [55.7] (3)	8.13 [34.2] (3)	46.7 [31.9] (3)
9/72	41.6 [76.0] (3)	229 [11.7] (3)	776 [34.8] (3)	17/0	2.29 [20.1] (3)	2.56 [45.3] (3)	15.4 [95.5] (2)
11/0	36.0 [10.3] (3)	135 (1)	352 [4.8] (2)	17/1	6.43 [35.5] (3)	18.4 (2)	44.0 [39.5] (3)
11/1	45.7 (1)	104 [14.0] (3)	421 [28.3] (3)	17/8	37.6 [68.6] (3)	177 [39.8] (3)	321 (1)
11/8	101 [13.5] (3)	379 [18.5] (3)	1290 [13.4] (3)	18/24	62.7 [13.0] (3)	118 [86.4] (3)	481 [24.3] (3)

* Mean [coefficient of variation %, if applicable] (n)

- **Fetuses:** The results of the serum 01BSUR concentration measurements in the fetuses on Day 17 are presented in the following table (from the original BLA submission):

Table 4.2 Concentrations of ACZ885 surrogate (01BSUR) in fetal mouse serum predose on gestation day 17

Dose (mg/kg/day)		
15	50	150
24.9 [32.6] (3)*	67.3 [3.2] (3)	136 [45.0] (2)

* Mean [coefficient of variation %, if applicable] (n)

The toxicokinetic parameters were estimated using non-compartmental methods due to the limited number of data points available for analyses, and are presented in the following table (from the original BLA submission; note that AUC is calculated as $AUC_{0\text{-last}}$ with different "last" data-points):

2 **Post-text tables**

Table 2-1 **TK parameters of ACZ885 surrogate (01BSUR) in female mice dosed s.c. at Days 6, 11 and 17 of gestation [0680148]**

PK Parameter (n=1-3)	Dose: 15 mg/kg			Dose: 50 mg/kg			Dose: 150 mg/kg		
	Day 6	Day 11	Day 17	Day 6	Day 11	Day 17	Day 6	Day 11	Day 17
tmax [h]	8	8	24	24	8	8	24	8	24
Cmax [µg/mL]	87.7	101	62.7	282	379	177	1300	1290	481
Cmax/dose [(µg/mL)/(mg/kg)]	5.85	6.73	4.18	5.64	7.58	3.54	8.67	8.6	3.21
AUC(0-tlast) [h*(µg/mL)]	6436	5872	961	26438	24157	3054	95585	42369	7723
AUC(0-tlast)/dose [h*(µg/mL)/(mg/kg)][a]	429	391	64.1	529	483	61.1	637	282	51.5

[a] last data point 120 h (Day 6), 144 h (Day 11), 24 h (Day 17)

Study title: ***A Subcutaneous Embryo-Fetal Development Study in the Marmoset Monkey***

Key study findings:

- No major treatment-related fetal external, visceral, or skeletal malformations in marmosets administered ACZ885 by SC injection at doses of 15 (LD), 50 (MD), and 150 (HD) mg/kg/twice weekly from Gestation Days 25-109
- Slight treatment-related effects noted in uterine examination, with **decreased mean placental weights** (-22% vs. ..... concurrent controls) and **reduced numbers of fetuses per litter** (-24% vs. controls) at the highest dose administered (150 mg/kg) (historical control data not available for the performing laboratory)
    - Ultrasonography demonstrated no decrease in number of fetuses vs. number of embryos present at Day 50; therefore likely cannot be attributed to late resorptions
    - Early resorptions could not be assessed due to absence of uterine scar formation in the marmoset upon early abortion
    - Reduced # fetuses probably due to fewer triplets and more singlets and twin pregnancies at the HD
- Slight increase in numbers of fetuses with **bent** (1 at the HD [4%] vs. 0 in the controls) **and/or kinked** (3 HD [13.6%] vs. 1 control [4%]) **tail** end at HD
- Slight increase in zygostyle (incomplete terminal caudal **vertebral ossification)** (4, 10, and 2 fetuses at the LD, MD, and HD, respectively, representing 13%, 31%, and 8% the number of fetuses examined in the LD, MD, and HD groups, respectively) compared to controls 1 (4% fetuses examined)
- Misaligned and/or bipartite (5, 17, 16, and 12 in the control, LD, MD, and HD groups respectively, representing 18%, 56%, 50%, and 46% the numbers of fetuses examined in each group, respectively) in all treated groups in the skeletal examination

- Adequate ACZ885 exposure demonstrated in the dams, fetuses and amniotic fluid assessments
- No evidence of anti-ACZ885 antibody formation
- Slight reduction in number of fetuses per litter and mean placental weights at the highest dose tested (150 mg/kg/twice weekly)
- NOAEL for minor embryotoxicity not determined due to bent or kinked tail at the HD and increased incidence of incomplete vertebral ossification in all treated groups
- NOAEL for maternal toxicity and major malformations/teratogenicity was 150 mg/kg/twice weekly

Study no.: Study # 1939-005, Novartis Study # 0480152
Conducting laboratory and location: b(4)

Date of study initiation: December 10, 2004
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug ACZ885, **lot #** (Batches) 6199 and 6305, **and % purity:** 99.5% (6199) and 99.4% (6305)

Methods

Doses: 0 (placebo vehicle), 15 (LD), 50 (MD), and 150 (HD) mg/kg/twice weekly; doses selected based on 1.5X, 5X and 15X estimated MRHD, and the results of the 13-week SC toxicity study in marmosets (#1470033)

Species/strain: Female (F) Common Marmoset, *Callithrix jacchus*
Ages: 2-8 years
Weights: 291-476 g females (F)
Number/sex/group:
The animal allocation is presented in the following table (from the original BLA submission):

Group number	Group description	Color code	Number of pregnant females*	Dose level (mg/kg/twice weekly)	Application concentration (mg/mL)
1	Control	White	17	0	0
2	Low	Blue	18	15	50
3	Intermediate	Green	18	50	50
4	High	Red	18	150	50

* pregnancy losses prior to improvement of animal housing were replaced; pregnancies lost prior to start of treatment are not included into this table

Route, formulation, volume, and infusion rate: ACZ885 (obtained in solution, with no further formlation adjustment performed) or placebo vehicle (30 mM L-histidine, 270 mM 9.2% (m/V) D-sucrose, 0.06% (m/V) Polysorbate 80)

administered twice weekly (Gestation Days [GD] 1 and 4 each Treatment Week) by subcutaneous (SC) injection in posterior region of back, on alternating sides at 50 mg/ml, 3 ml/kg (controls and HD), and 0.3 ml/kg LD and MD

Satellite groups used for toxicokinetics: None; all parameters were evaluated in all animals in each group

Study design:

- Marmosets housed in mating pairs (1 F and 1 male [M] in each cage), in a temperature (22 - 28 degC) and humidity (30% - 70%) controlled facility, with 12 hour light/dark cycle, and were provided fresh food twice daily and tap water *ad libitum*
- Environment enriched with wooden wool nest material, wooden bars
- Pregnancy confirmed using ultrasonography to detect dark gap in uterus lumen
- The day before progesterone peak was designated Gestation Day (GD) 0
- Pregnant F marmosets were given SC test article injections twice weekly on GD 25-109.
- Nearly all pregnancies were lost after the first mating, attributed to stress due to environmental disruptions, such as noise levels, removal of males immediately after mating, frequency of blood collections (twice weekly) for TK analyses and stress caused by frequent handling during collection of data (e.g., body weight measurements, clinical evaluations, etc.).
- Methodology modified to reduce stress-inducing factors: females housed with M until cesarean section

Parameters and endpoints evaluated:

In-Life (maternal):

Mortality: Twice daily from Dose 1 through Cesarean section at pre-dose and 2 hours after dosing

Clinical signs: Twice daily from Dose 1 through Cesarean section at pre-dose and 2 hours after dosing

Confirmation of Pregnancy: Day 20/24, Weekly thereafter through GD 109

Body weights: Twice weekly on GD 20-109

Food consumption: Twice daily from Dose 1 through study termination, estimated for each individual due to paired housing

Terminal Procedures: Cesarean section performed on GD 112 to 114

Necropsy of dams: Not conducted, because no dams were found moribund or dead

Cesarean Section:

Fetal Examination:

Placental and fetal weights

Dead fetuses: external examination and sex

Live fetuses:

Sex

Measurements of distances from coccyx to cranium, tip of nose to os occipitale, from os frontale to os occipitale, width of head, and distance between eyes

Macroscopic and microscopic examinations (external) of all fetuses

External defect examinations: symmetry of head, facial form, formation of lower jaw, eyebrows, eyes and eyelids, hair on head, nipple formation, arms, fingers, toes, nails of fingers and toes, ears, tail, upper and lower extremities, external genitals, palpation of vertebral column, umbilical cord, and palate

Full internal necropsy on all fetuses

Gross examination of stomach, cecum, small and large intestine, testes, epididymides, vas deferens, ovaries, uterus, ureters, kidneys, adrenals, urinary bladder, liver, spleen, gallbladder, diaphragm, thymus, thyroid, heart, aortic arch, cardiac septum and cardiac auricles, lungs, esophagus, trachea, eyes, and brain

Organ weights:

Brain, heart, kidneys, liver, gallbladder, spleen, and thymus

Microscopic examination (internal):

Adrenals, brain, eyes, epididymides, vas deferens, heart, kidneys, liver, gallbladder, lungs, ovaries, spleen, small and large intestine, stomach, testes, thymus, and uterus

Skeletal defect examinations: ossified skeleton stained for examination

Toxicokinetics:

- Blood (0.5 ml from the brachial vein) collected before every second weekly dose and at 24 and 48 hours after first dose of last Treatment week and day of Caesarean section
- Amniotic fluid (2 ml) collected at time of Cesearean section
- Fetal blood (0.5 ml) collected from umbilical cord or fetus at Caesarean section

Anti-ACZ885 antibody formation: Blood collected for analyses at baseline and on day of Cesarean section

Results

Mortality (dams): No deaths observed during the study

Clinical signs (dams): No treatment-related effects

Body weight (dams): No treatment-related effects

Food consumption (dams): No treatment-related effects

Terminal and necroscopic evaluations:

- Slight **decrease (-22% vs. controls) in mean placental weights** at 150 mg/kg, probably related to lower litter size at this dose level
- The results of the placental weight measurements are presented in the following table (from the original BLA submission):

Parameter		Group 1 0 mg/kg/ twice weekly	Group 2 15 mg/kg/ twice weekly	Group 3 50 mg/kg/ twice weekly	Group 4 150 mg/kg/ twice weekly
Weight of placenta (g)	Mean	8.6	7.3	8.1	6.7
	SD	1.3	1.5	2.3	1.7
	N	11	14	14	14

- Slight **reduction in number of fetuses per litter** at 150 mg/kg
  - Not due to late resorptions, as ultrasonography demonstrated no decrease in number of fetuses vs. number of embryos present at GD 50
  - Not possible to detect early resorptions because no uterine scars are formed in the marmoset upon early abortion
  - Reduced # fetuses at HD related to fewer triplets and more singlets and twin pregnancies at the HD
  - The results of the group fetal findings are presented in the following table (from the original BLA submission):

Parameter	Group 1 0 mg/kg/ twice weekly	Group 2 15 mg/kg/ twice weekly	Group 3 50 mg/kg/ twice weekly	Group 4 150 mg/kg/ twice weekly
Number of fetuses examined	27	30	32	26
Number of females with live fetus(es)	11	14	14	14
Total number of fetuses	27	30	32	26
Mean litter size	2.45	2.14	2.29	1.86
Number of fetuses where sex determination not possible	5	4	7	2
Number of males	12	17	7	16
Number of females	10	9	18	8

- No treatment-related effects on fetal weights
- No treatment-related effects on fetal measurement data
- No treatment-related effects on fetal organ weights

Offspring (malformations, variations, etc.):

- Slight increase in numbers of fetuses observed with **kinked and/or bent tail end** in the external examination, presented in the following table (from the original BLA submission):

Parameter	Group 1 0 mg/kg/ twice weekly	Group 2 15 mg/kg/ twice weekly	Group 3 50 mg/kg/ twice weekly	Group 4 150 mg/kg/ twice weekly
Number of fetuses examined	27	30	32	28
External Findings				
Number of fetuses without findings	26	30	29	22
Number of fetuses with findings	1	0	3	4
Number of litters affected	1	0	3	4
Tail end - kinked	1	0	2	3
Tail - slightly bent tail end	0	0	1	1

- No treatment-related visceral variations or malformations
- Slight increase in zygostyle, **incomplete vertebral ossification**, misaligned and/or bipartite in all treated groups in the skeletal examination, presented in the following table (from the original BLA submission):

Parameter	Group 1 0 mg/kg/ twice weekly	Group 2 15 mg/kg/ twice weekly	Group 3 50 mg/kg/ twice weekly	Group 4 150 mg/kg/ twice weekly
Skeletal Findings				
Number of fetuses with findings	27	30	32	28
Number of litters affected	11	14	14	14
Vertebrae				
Additional ossification site prior to normal first	28	29	32	28
Vertebra(e), incomplete ossification, misaligned and/or unossified	1	4	10	2
Vertebra(e) centrum/a, unossified, bipartite, misaligned and/or incomplete ossification	0	2	1	2
Zygostyle, incomplete ossification, misaligned and/or bipartite	5	17	18	12
Gap between zygostyle and last but one vertebra	0	1	0	0
Ribs				
Supernumerary, lumbar or cervical vertebra unilateral/bilateral	12	13	11	11
Supernumerary, lumbar or cervical vertebra shortened, unilateral/bilateral	7	12	15	8
Supernumerary, lumbar or cervical vertebra vestigial and/or isolated	4	1	5	4
Fused and/or branched	0	0	0	1
rib(s) bilateral shortened	0	0	0	1

- No treatment-related effects in the fetal histopathology examination of organs
- Minimal-moderate hepatic necrosis observed in several fetuses from all groups including controls, probably related to vehicle administration

Toxicokinetics:

Maternal

- Dose-proportional increase in exposure (AUC_{0-48h} and Cmax) in the maternal assessment on GD 109 across doses tested
- Increasing plasma levels with increased treatment duration reached plateau from GD 39 to GD 109
- The results of the maternal TK analyses are presented in the following table (from the original BLA submission):

Table 2-3 Mean toxicokinetic parameters of ACZ885 in marmoset maternal serum on Day 109

Dose	15 mg/kg			50 mg/kg			150 mg/kg		
TK parameters	mean	CV%	n	mean	CV%	n	mean	CV%	n
T_{max}	25.7	44.3	14	27.4	31.8	14	30.9	36.5	14
C_{max}	322	19.0	14	1220	21.5	14	3560	32.6	14
C_{max}/Dose	21.5	19.1	14	24.5	21.5	14	23.7	32.5	14
$AUC_{(0-48h)}$	13900	20.8	14	52200	22.5	14	141000	26.9	14
$AUC_{(0-48h)}$/Dose	929	20.8	14	1040	22.4	14	939	26.8	14

Units :t_{max} [h], C_{max} [µg/mL], C_{max}/dose [(µg/mL)/(mg/kg)], $AUC_{(0-48h)}$ [h·µg/mL], $AUC_{(0-48h)}$/dose [(h·µg/mL)/(mg/kg)].

n = number of the corresponding TK parameter

Fetal

- Exposure to ACZ885 detected in all fetuses of treated dams
- Dose-proportional increase in fetal serum and amniotic fluid concentrations of ACZ885 across dose groups tested
- Mean fetal serum ACZ885 concentrations 7.0%-8.7% of the maternal serum concentrations across dose groups
- Mean amniotic fluid concentrations 1.8%-2.0% of the mean maternal serum concentrations across dose groups
- The results of the fetal and amniotic fluid ACZ885 measurements are presented in the following table (from the original BLA submission):

Table 2-2 Mean ACZ885 concentrations in marmoset amniotic fluid and fetal serum

Dose	0 mg/kg			15 mg/kg			50 mg/kg			150 mg/kg		
	mean	CV%	n	mean	CV%	n	mean	CV%	n	mean	CV%	n
Fetal serum	0.000	-	20	29.1	38.8	22	110	30.0	23	246	36.4	18
Fetal amniotic fluid	0.000	-	11	6.14	57.0	14	27.7	31.2	14	62.6	34.3	14

Mean concentration expressed in µg/mL

n = number of determinations

Anti-ACZ885 antibody formation: No anti-ACZ885 antibody formation detected in the maternal and fetal marmosets

Prenatal and postnatal development

Study title: ***ACZ885 Surrogate (01BSUR): A Weekly Subcutaneous Injection Pre and Postnatal Study in the Mouse***

Key study findings:

- F_0 generation
  - 01BSUR negative for adverse prenatal and postnatal development effects in CD-1 mice at the dose range administered (15 (LD), 50 (MD), and 150 (HD) mg/kg/twice weekly SC)
  - **2/25 HD females (F) found dead** on Post Partum Days (PPD) 13 and 17, with possible relationship to treatment
  - Gross and Histopathologic examination in the F_0 dams found dead: **splenic enlargement** (both dams), **pale discoloration of spleen** (1 dam), **liver enlargement** (1 dam), **lymphoid hyperplasia** (both dams), and increased **extramedullary hematopoiesis** (1 dam)
- F_1 generation
  - **Slight increase in incidence of histiocytosis in mandibular and mesenteric lymph nodes** in MD (3/9 and 2/10, respectively, vs. 0/10 in the controls) and HD (4/9 and 3/10, respectively, vs. 2/10 in the controls) F_1 adult males
- F_2 generation
  - No effects noted
- NOAEL for adverse 01BSUR effects on pre and postnatal development in the mouse = 150 mg/kg/once weekly SC administered from GD 6 through PPD 21

Study no.: — Study # 901098, Novartis Study # 0680150 b(4)
Conducting laboratory and location: ——

Date of study initiation: September 1, 2006 (In-Life Observations), September 12, 2006 (Dose administration)
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug ACZ885 Surrogate (01BSUR) obtained in solution at 14.2 mg/ml, **lot #** (Batch) 7318, **and % purity:** 98.7%

Methods

Doses: 0 (vehicle control), 15 (LD), 50 (MD), and 150 (HD) mg/kg/once weekly, selected based on multiples of the anticipated MRHD, toxicity data, and feasibility (e.g., test article solubility, dose volume limitations)

Species/strain: Crl:CD1(ICR) mouse, *Mus cusculus*

Ages: 8-9 weeks
Weights: 26.3-34.3 g
Number/sex/group: Dams: 25/dose group; pups for F_1 pup evaluation: 8/sex/litter/group; F_1 adult evaluation 25/sex/group; F_1 Immunogenicity: 10/sex/group; F_1 Immunology: 10/sex/group

Animal allocation is presented in the following table (from the original BLA submission):

Group number identification	Dose level (mg/kg/day)	Dose dose volume (mL/kg/day)	Animal numbers	
			Main study	Satellite[1]
1/ Vehicle control	0	10	151-174	175-184
2/ 01BSUR	15	1	251-274	275-284
3/ 01BSUR	50	3.33	351-374	375-384
4/ 01BSUR	150	10	451-474	475-484

1 10 dams per dose level were treated until day 2 post partum for blood collection on day 7 *post partum* for determination of blood drug levels in dams and their respective litters.

Route, formulation, volume, and infusion rate: 01BSUR obtained in vials at 14.2 mg/ml; Vehicle: 180 mM N.F. Sucrose, 10 mM, L-histidine, and 1.00 mL of 2% (m/V) Tween 20 NF in Sterile Water for Injection USP, pH adjusted to 5.5 with 1N HCl, administered by subcutaneous (SC) injection at 10, 1, 3.33 and 10 ml/kg (at 0, 15, 50, and 150 mg/kg, respectively) once weekly (see dosing schedule under Study Design, below)
Satellite groups used for toxicokinetics (TK): 10/sex/group TK
Study design: After 2-3 week acclimation period the dams were mated and pregnancies confirmed by presence of vaginal copulatory plug (designated Gestation Day (GD) 0). The dams were administered 01BUSR by SC injection once weekly from GD 6 (GD 6 and 13) through Post Partum (PPD) Day 21 (PPD 2, 9, and 16). Pups were culled to 8/group on PPD 4 (4/sex/group) The evaluation included treatment-related effects on gestation, parturition, and lactation in the dam and the pups, and pup survival, physical development, behavior and reproductive performance.

Parameters and endpoints evaluated:

F_0 in-life:

Mortality: Twice daily
Clinical signs: Twice daily
Body weights: GD 0, 3, 6, 9, 12, 15, and 18, and PPD 0, 4, 7, 10, 14, 17, and 21
Food consumption: GD 3-6, 6-9, 9-12, and 12-15
Reproductive parameters: Observations 3 times daily from GD 17 onward to parturition; the following parameters were recorded or calculated:
- Gestation index
- Length of gestation
- Duration of parturition
- Number of live, dead and malformed pups

Number of implantation scars
Live birth index
Calculation of pregnancy rate (%) (#pregnant F/# mated F X 100), mating index (%) (# M mating/#M placed for mating X 100), fertility index (%) (# M producing pregnant F/# mated M X 100), conception rate (%) (# pregnant F/# mated F X 100), live birth index (%) (# live pups at birth/# implantation site scars X 100), gestation index (%) (# mice with live litters/# pregnant mice X 100)

F_0 necropsy:
Gross pathology: PPD 7

F_1 physical development:
Pups
Viability: Daily throughout lactation, viability index (%) calculated (# live pups PPD 4/# live pups PPD 0 X 100), survival index (%) (# live pups PPD 7 or PPD 14/# live pups PPD 4 X 100), lactation index (%) (# live pups on PPD 21/# live pups PPD 4 X 100)
Survival: Daily throughout lactation
Clinical observations: Standard
Examination for malformations
Body weights: PPD 0, 4, 7, 14, and 21
Physical development
Pinna unfolding; PPD 1-4
Eye opening: PPD 10
Reflex development
Righting reflex: PPD 2-4
Auricular startle response: PPD 10 until positive response
Gross pathology: Standard
Adults (beginning on PPD 21)
Survival: Twice daily
Clinical observations: Twice daily
Body weights: Weekly except mated F (on GD 0, 3, 6, 9, 12, 15, and 18, and PPD 0 and 4)
Physical development
Vaginal opening: PPD 22 until development in F
Preputial separation: PPD 22 until development
Visual placing: PPD 21
Papillary closure: PPD 21
Organ weights: Spleen, thymus
Gross pathology: Standard
Histopathology (peer-reviewed): abnormalities, bone and marrow (sternum), spididymides, injection sites, lymph nodes (mandibular and mesenteric), mammary glands (thoracic and inguinal), ovaries, prostate, seminal vesicles, spleen, testes, thymus, uterus (horns, body and cervix), and vagina

F_1 behavioral evaluation:

Motor Activity: Total motor activity counts, and performance in Figure 8 mazes on PPD 35 and 60 for 1 h each session (6 10-min intervals)
Startle habituation: PPD 55 using 4-min acclimation followed by 50 identical trials at 120 dBA and 8-sec inter-trial intervals
Passive avoidance: PPD 64, using time elapse to crossing between 2-compartment shuttle to avoid foot shock, with appropriate conditions trials

F_1 reproduction: F_1 F mated at age 78 days, for 14 days; pregnancy status confirmed by presence of copulatory plug on day designated as GD 0

Estrous cycle: from 14 days before mating through identification of mating
Mean day to mating
Length of gestation
Duration of parturition
Sex ratio
Number of live and dead pups
Number of malformed pups at birth
Number of implant scars
Calculation of pregnancy rate (%) (#pregnant F/# mated F X 100), mating index (%) (# M mating/#M placed for mating X 100), fertility index (%) (# M producing pregnant F/# mated M X 100), conception rate (%) (# pregnant F/# mated F X 100), live birth index (%) (# live pups at birth/# implantation site scars X 100), gestation index (%) (# mice with live litters/# pregnant mice X 100)

F_1 Immunology Assessment

Blood, thymus, spleen weights: At euthanasia, PPD 79
Blood, spleen, thymus immunophenotyping data: At scheduled euthanasia (PPD 79) in all immunology subset mice

Blood: Samples from abdominal aorta, analysis and determination of total, absolute and percent differential counts; T lymphocytes ($CD3d^+$), Helper T lymphocytes ($CD3e^+/CD4$), Cytotoxic T lymphocytes ($CD3e^+/CD8a^+$), B lymphocytes ($CD19^+$), and Natural Killer (NK) lymphocytes ($CD3e^-/NK1.1^+$)
Spleen: 0.5 ml samples of spleen cell suspension examined for total absolute and percent differential white blood cell counts, and reported as relative proportions and absolute number of Total T lymphocytes ($CD3d^+$), Helper T lymphocytes ($CD3e^+/CD4$), Cytoxic T lymphocytes ($CD3e^+/CD8a^+$), B lymphocytes ($CD19^+$), and Natural Killer (NK) lymphocytes ($CD3e^-/NK1.1^+$)
Thymus: 0.5 ml thymus cell suspension examined for total and percent differential white blood cell counts, and reported as relative proportions and absolute number of double and positive T lymphocytes ($CD3e^+/CD4/CD8a^+$), Helper T lymphocytes ($CD3e^+/CD4^+/CD8a^+$), Cytoxic T lymphocytes ($CD3e^+/CD4^-/CD8a^+$), double negative T lymphocytes ($CD3e^+/CD4/CD8a$), and Total T lymphocytes ($CD3e^+$)

F_1 Maternal blood drug level

Anti-ACZ885 surrogate (01BSUR) antibodies

F_0: Blood collected from abdominal aorta on PPD 7
F_1: Blood collected from abdominal aorta on PPD7 (age 7 days)

F_2 findings:

Body weights: PPD 0 and 4
Sex
Numbers live and dead
Viability
Clinical observations: daily throughout lactation
External and internal examinations, malformations: PPD 0

Results

F_0 in-life:

Mortality

- **2/25 HD (150 mg/kg/wk) F found dead** on PPD 13 (Dam # 451) and 17 (Dam # 463)
- No clinical signs in the dams found dead
- Histopathology findings in the dams found dead: **splenic enlargement** (both dams), **pale discoloration of spleen** (#451), **liver enlargement** (#463), **lymphoid hyperplasia** in both dams, and increased **extramedullary hematopoiesis** in 1 dam

Clinical signs: No treatment-related effects
Body weights: No treatment-related effects
Food consumption: No treatment-related effects
Reproductive parameters:

Gestation index: No treatment-related effects
Length of gestation: No treatment-related effects
Duration of parturition: No treatment-related effects
Number of live, dead and malformed pups: No treatment-related effects
Number of implantation scars: No treatment-related effects
Live birth index: No treatment-related effects

F_0 necropsy:

Gross pathology: No treatment-related effects

F_1 physical development:

F_1 Pups

Viability: No treatment-related effects
Survival: No treatment-related effects
Lactation index: No treatment-related effects
Clinical observations: No treatment-related effects
Body weights: No treatment-related effects
Physical development: No treatment-related effects on day of pinna unfolding and eye opening

Gross pathology: No treatment-related effects

F_1 Adults

Survival: No treatment-related effects

Clinical observations: No treatment-related effects

Body weights: No treatment-related effects on body weights and body weight gain

Physical development:

Vaginal opening: No treatment-related effects on day of opening

Preputial separation: No treatment-related effects on day of separation

Visual placing: No treatment-related effects

Papillary closure: No treatment-related effects

Organ weights: No treatment-related effects

Gross pathology: No treatment-related effects

Histopathology (peer-reviewed): Histiocytosis in mandibular and mesenteric lymph nodes in MD (50 mg/kg/wk) and HD (150 mg/kg/wk) M; the results of the microscopic examination are presented in the following table (from the original BLA submission):

		MALE			
DOSE GROUP		1	2	3	4
NUMBER OF ANIMALS EXAMINED		10	9	9	10
BONE MARROW	EXAMIN:	10	9	9	10
BONE-STERNUM	EXAMIN:	10	9	9	10
L. NODE MANDIBULAR	EXAMIN:	10	9	9	10
- Histiocytosis		-	-	3	2
L.NODE MESENTERIC	EXAMIN:	10	9	9	10
- Histiocytosis		2	3	4	3
SPLEEN	EXAMIN:	10	9	9	10
- Hematopoiesis/extramedullary: increased		4	3	4	4
THYMUS	EXAMIN:	10	9	9	10

		FEMALE			
DOSE GROUP		1	2	3	4
NUMBER OF ANIMALS EXAMINED		10	10	10	10
BONE MARROW	EXAMIN:	10	10	10	10
BONE-STERNUM	EXAMIN:	10	10	10	10
L. NODE MANDIBULAR	EXAMIN:	10	10	10	10
L.NODE MESENTERIC	EXAMIN:	10	10	10	10
- Histiocytosis		3	2	2	2
- Prominent high endothelial venules		-	1	-	-
SPLEEN	EXAMIN:	10	10	10	10
- Hematopoiesis/extramedullary: increased		4	5	5	2
THYMUS	EXAMIN:	10	10	10	10

F_1 behavioral evaluation:

Motor Activity: No treatment-related effects on mean day of righting reflex development
Startle habituation: No treatment-related effects on day of development
Passive avoidance: No treatment-related effects

F_1 reproduction:

Mating index: No treatment-related effects
Fertility index: No treatment-related effects
Conception rate: No treatment-related effects
Mean day to mating: No treatment-related effects
Pregnancy rate: No treatment-related effects
Gestation index: No treatment-related effects
Length of gestation: No treatment-related effects
Duration of parturition: No treatment-related effects
Sex ratio: No treatment-related effects
Number of live and dead pups: No treatment-related effects
Number of malformed pups at birth: No treatment-related effects
Number of implant scars: No treatment-related effects
Live birth index: No treatment-related effects

F_1 Immunology Assessment

Blood, thymus, spleen weights: No treatment-related effects
Blood, spleen, thymus immunophenotyping data: No treatment-related effects
Lymphocyte subsets: No treatment-related effects
Macroscopic examination: No treatment-related effects

Microscopic examination: Treatment-related **increase in histiocytosis in mesenteric lymph nodes** in M at 50 and 150 mg/kg/week, without lesions (see under histopathology results, above)

F_1 blood drug level: 01BSUR detected in F_1 pups at levels 6X higher in the than in the F_0 generation serum, and also detected in the F_1 adults at lower levels

Anti-ACZ885 surrogate (01BSUR) antibodies: None found in the F_1 generation adults

F_2 findings:

Viability: No treatment-related effects
Body weights: No treatment-related effects
Clinical observations: No treatment-related effects
External and internal examinations: No treatment-related effects

2.6.6.7 Local tolerance

Study title: ***Single Dose Intra-Articular Administration Study in the Marmoset***

Key study findings:

- No local toxicity found
- Slight decrease in lymphocytes (41 % vs. 64% at baseline), increase in neutrophils (mean 58% vs. 35% at baseline) with single intra-articular dose given (10 mg/kg)
  - Relationship to treatment is possible but unlikely, due to the small magnitude of the differences from control values, the possible relationship to stress response, and absence of similar findings in the other toxicology studies using more frequent dosing and for longer durations of exposure.
- Hindlimb ataxia in 1/3 marmosets, not considered to be treatment-related
- Mottled and enlarged adrenals and kidneys with histological evidence of cortical hypertrophy and inflammatory cell foci with fibrosis and atrophic tubuli in 1/3 marmosets
  - Also observed in historical controls, and therefore considered to be unlikely related to treatment-related
- Swollen finger with subcutaneous abscesses, ileal invagination and discolored urinary bladder with acute inflammation and ulceration in 1/3 marmosets
  - Also observed in historical controls, and therefore considered to be unlikely related to treatment-related
- Mean serum concentrations 99.84 mcg/ml at 24 h and 86.90 mcg/ml at 48 h after dosing, confirming systemic exposure after intra-articular injection in the knee

Study no.: — ; Study #1939-018, Novartis Study # 0670425 b(4)

Conducting laboratory and location: ——— b(4)

Date of study initiation: October 17, 2006
GLP compliance: No (Not required)
QA report: yes (x) no ( )
Drug ACZ885, **lot #** (Batch Y043 0504), **and % purity:** 101.7% by Size Exclusion Chromatography), 92% by ——— , 99.9% by By- and degradation products by Size Exclusion Chromatography, 94% of reference by Reported Gene Assay with ——— cells (Relative biological activity compared to the reference substance) b(4)

Methods

Doses: 0 (control placebo) or ACZ885 10 mg/kg
Species/strain: Marmoset (*Callithrix jacchus*)
Number/sex/group or time point (main study): 3 females (designated 42136F, 42116F, and 42065F)
Route, formulation, volume, and infusion rate: ACZ88, lyophilisate; 150 mg/vial) in diluent (Water for injection: Ampuva®, single dose by intra-articular injection in the right knee joint at 57.6 mg/ml/0.2 ml/kg (maximum feasible dose), or control item in the left knee at 0.2 ml/kg
Satellite groups used for toxicokinetics or recovery: None
Age: 2-3 years
Weight: 365-418 g
Additional methodology: The animals were housed individually in a temperature (22-28 degC) and humidity (40%-70%) controlled facility, with 12 hour light/dark cycle and environmental enrichment with wood wool nesting material and wood bars. The Marmosets were fed a variety of food twice daily, with rewards (e.g., marshmallows, cereal); tap water provided *ad libitum*. The marmosets received a single injection of ACZ885 in the right knee and control vehicle in the left knee on Day 1, and were sacrificed on Day 3 after dose administration.

Observations

Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Baseline
Food consumption: Not determined due to group housing
Ophthalmoscopy: Not done
EKG: Not done
Hematology:

- Blood samples (1.5 ml from saphenous, cubital or femoral vein/artery) at baseline and on Day 3 after dosing
- Standard parameters and differential blood cell count from blood smears by microscope (juvenile neutrophils, band neutrophils, segmented neutrophils,

basophils, eosinophils, lymphocytes, monocytes, blast cells and nucleated red blood cells)

Clinical chemistry: Not done

Urinalysis: Not done

Gross pathology:

- At end of observation period, 3 days after dosing
- The following tissues were examined: gross lesions, knee joint with surrounding tissues (both knees), and popliteal and inguinal lymph nodes (both sides)

Organ weights:

- At end of observation period, 3 days after dosing

Histopathology: Adequate Battery: yes (x), no ( ) for local toxicity evaluation
Peer review: yes ( ), no (x)

- The following tissues were examined microscopically: gross lesions, knee joint with surrounding tissues (both knees), popliteal and inguinal lymph nodes (both sides)
- The following tissues were examined in animal 42116F, which showed gross findings: full histopathological evaluation on adrenals, aorta (arch and anterior abdominal), bone marrow smear (humerus), brain (cerebral cortex, thalamus, midbrain, medulla, cerebellum), cecum, colon, duodenum, esophagus, eyes and optic nerves, femur with bone marrow and articular surface, gall bladder, glands (mammary), gross lesions, heart, ileum with Peyer's patch, injection site, jejunum, kidneys, knee joint with surrounding tissues (both knees), lacrimal glands, liver, lungs (with mainstem bronchi), mandibular lymph nodes, mesenteric lymph nodes, ovaries, pancreas, pituitary, popliteal and inguinal lymph nodes (both sides), rectum, salivary glands, mandibular, sciatic nerve, skeletal muscle, skin/animal identification, spinal cord cervical spleen, sternum with bone marrow, stomach, thymus, thyroid and parathyroids, tongue, trachea, urinary bladder, uterus/cervix, and vagina.

Toxicokinetics: Blood samples (2 ml) on Day 1 at 24 and 48 hours after drug administration

Results

Mortality: No unscheduled deaths

Clinical signs: Ataxia in hindlimbs (animal 42136F);

Hematology:

- Slight **decrease in lymphocytes** (Mean 41 % vs. 64% at baseline)
- Slight **increase in neutrophils** (SNEU mean 58% vs. 35% at baseline)
- Low WBC, anemia, increased reticulocytes in animal 42116F, also observed at predose; not considered to be test article-related

Gross pathology:

- Animal 42116F: enlarged/mottled adrenals and kidneys

- Animal 42065F: Swollen right finger with subcutaneous abscesses, ileal invagination, red discolored urinary bladder

Organ weights: No treatment-related effects.

Histopathology:
- Animal 42116F: Moderate cortical hypertrophy in adrenals, moderate inflammatory cell foci and slight fibrosis with slight atrophic tubuli in kidneys
- Animal 42065F: Marked acute inflammation and ulceration of urinary bladder
- No treatment-related effects in knee joints, popliteal lymph nodes, inguinal lymph nodes
- Observed effects within historical background range

Toxicokinetics: Toxicokinetic sampling at 24 and 48 hours after dosing confirmed systemic exposure to intra-articular injection of ACZ885 in the marmoset knee joint. The results of the toxicokinetic measurements are presented in the following table (from the original BLA submission):

Table 4-1 Concentrations of ACZ885 in marmoset serum on day 1

Time(h)	Female animal's tattoo number			Mean concentrations
Day 1	42136 F	42116 F	42065 F	
24 h	/	/	/	99.84
48 h				86.90

Units : ACZ885 concentrations [µg/mL].

b(4)

2.6.6.8 Special toxicology studies

Study title: ***ACZ885 Surrogate (01BSUR): A Weekly Subcutaneous Injection Juvenile Toxicology Study in the Mouse***

Key study findings:
- Negative for adverse behavioral, developmental, learning and memory, and reproductive effects in the juvenile mouse treated by subcutaneous injection once weekly for 9 weeks from post-partum days (PPD) 7-70, with doses from 15-150 mg/kg/week
- Slight, but statistically significant adverse effects compared to concurrent controls were observed at the high dose (HD) but not of great concern toxicologically for the reasons specified:
  - Sporadic **reduced food consumption** without body weight effects at 150 mg/kg/wk

- Slight **delay in day of vaginal opening** at 150 mg/kg/wk (HD)
  - Values within historical control range for the laboratory (see under Historical Data, below)
  - No other treatment-related effects on fertility observed in this study
- Slight but significant **increase in mean day of development of auricular startle** at 50 (MD) mg/kg/wk in males (M) and HD in Mand females (F) compared to concurrent controls during pre-weaning period
  - Values similar to controls in Study # 901098;
  - Values within historical control range of the performing laboratory
  - No treatment-related effects seen in the post-weaning evaluation of auditory startle habituation (startle at start, maximum startle, time of maximum startle and average startle)
- **Increase in pre-implantation loss** at HD (20.38%) compared to controls (4.26%), due predominantly to loss in 1 dam (71.4%); mean number of live embryos (12.8) at HD; within historical range of 11.2-13.8 (See under Historical Data, below)
- **Minimal inflammation at injection site** in M and F, reversed during recovery period
- Immune response was detected in 1 MDF on Gestation Day (GD) 13 only
- NOAEL for juvenile 01BSUR toxicity in the mouse = 150 mg/kg/once weekly SC

Study no.: ——— Study # 901383, Novartis Study # 0770274 b(4)
Conducting laboratory and location: ———

Date of study initiation: In-life September 25, 2007, Mating October 5, 2007, Dosing October 29, 2007
GLP compliance: Yes
QA report: yes (x) no ( )
Drug 01BSUR provided in 14.5 mg/ml vials, **lot #** (Batch) 7318, **and % purity:** 98.7%

Methods

Doses: 0 (vehicle control), 0 (untreated control for immunophenotyping evaluation), 15 (LD), 50 (MD), and150 (HD) mg/kg/week, based on multiples of the anticipated MRHD, on the results of previous toxicology studies in CD-1 mice, and feasibility (solubility, dose volume limitation)

Species/strain: — .CD1(ICR) mouse ——— (Mus b(4)
musculus)

Number/sex/group or time point (main study): 20/sex/group for the F1 dosing phase toxicology evaluation

The animal allocation is presented in the following tables (from the original BLA submission):

Table 1-1 Study design - test article doses

Group no. identification	Dose level (mg/kg/day)	Dose volume (mL/kg/day)	Minimum number of litters	Number of Animals	
				Males	Females
1/ Vehicle control	0	10	23	72	72
2/ 01BSUR	15	1	35	120	120
3/ 01BSUR	50	3.33	35	120	120
4 01BSUR	150	10	35	120	120
5/ Untreated[1]	0	0	10	10	10

1 for immunogenicity testing only (baseline data)

Animals from each group were allocated into subgroups as described in Table 1-2.

Table 1-2 Study design - animal allocation

Subgroup identification	Duration of dosing	Recovery period	Number of animals per group	
			Males	Females
A/ Toxicology - Main study	9 weeks	-	20	20
B/ Toxicology – Recovery	9 weeks	4 weeks[2]	20	20
C/ Toxicokinetic	1 day	-	12 control + 60/treated group	12 control + 60/treated group
D/ Immunology – Main study	3 weeks	-	10	10
E/ Immunology - Recovery	9 weeks	4 weeks	10	10

2 extended beyond 4 weeks for animals from reproductive subset

Route, formulation, volume, and infusion rate: 01BSUR administered to juvenile mice by subcutaneous (SC) injection in the dorsal thoracic region at 10, 1, 3.33, 10, and 0 ml/kg (vehicle control, LD, MD, HD, and untreated controls, respectively) once weekly from Post Partum Days (PPD) 7-70 (total 9 injections). The vehicle control article was 180 mM Sucrose, 10 mM L-histidine, and 0.02% (m/V) Tween 20 aqueous solution, in Sterile Water for Injection USP, adjusted to pH 5.5.

Satellite groups used for toxicokinetics (TK) or recovery: 20/sex/group 9-week TK (main study and recovery), 12 control/sex/group + 60 treated/sex/group TK, 10/sex/group Immunology (each, main study and recovery)

Age: F_0 females (F) for mating: 49 days; F_1 Pups at start of dosing: 7 days

Weight: 2.8-6.3 g (toxicology and immunology) and 2.9-6.8 g TK subgroup

Additional Study methodology: The F_0 mice were housed individually and F_1 Pups housed 2-3 per cage from birth to PPD 28, then individually until termination, in a temperature- (22 ± 3 degC) and humidity (50 ± 20%) controlled facility with 12-hour light/dark cycle; standard certified pelleted commercial diet (PMI Certified Rodent Chow 5002) and tap water provided *ad libitum*. The F_0 F were mated and delivered, with the day of parturition designated PPD 0. The F_1 litters were culled to 4 M and 4 F per litter on PPD 4 (23 control and 35/dose treatment litters). The remaining F_1 pups were administered test article or control on PPD 7-70 once weekly, and evaluated as described below.

Observations:

Dams (F_0 Generation):

Mortality: Twice daily from pre-mating through lactation
Clinical signs: Twice daily from pre-mating through lactation, with detailed clinical examination weekly during mating and gestation, and on PPD 0, 7, 14, and 21
Body weights: Baseline (premating), GD 0, 7, and 14, and PPD 0, 7, 14, and 21
Partuition: Twice daily from GD 17-21 for parturition, designated PPD 0

Pups (F_1 Generation):

Mortality: Twice daily during pre-weaning, once weekly thereafter
Clinical signs: Twice daily during pre-weaning, once weekly thereafter
Body weights: At birth and twice weekly (e.g., Days 4, 7, 10, 14, 17, 21, 24, 28, 31, 35, etc., until termination)
Food consumption: PPD 21, 24, 28, 31, 35, 38, and 42; then twice weekly until termination; and in the fertility phase F food consumption measured on GD0. During co-housing, consumption for individual animals estimated based on total per cage.
Physical Development Pre-weaning: PPD 10 until development
Eye opening
Auricular startle
Physical Development Post-weaning: PPD 21 until development
Vaginal opening
Preputial separation
Sensory Development (visual function): PPD 35
Pupillary reflex
Behavioral performance:
Motor activity: PP week 13, for 1 h (6 10-min intervals) using figure 8 maze in main and recovery animals; sound level constant at 70 dBA, room illumination 600-800 Lux
Auditory startle habituation: PPD 55, using 4-minute acclimation followed by 50 identical trials at 120 dBZ with 8-sec inter-trial intervals
Passive avoidance: Recovery animals: PP week 14, using two-compartment rodent shuttle cage with evaluation of time to crossing to dark side and receiving foot shock; time remaining in light side to avoid shock measured for up to 2 minutes
Fertility assessment:
Mating: Recovery animals ages 14-15 weeks: F placed with same-dose M for 14 days, and examined for positive pregnancy (presence of copulatory plug)
Gestation Day 13 uterine examination
Calculations made:
Group mean corpora lutea count, #s of implants, live/dead embryos, resorptions
Preimplantation loss (%) = (#corpora lutea - # implants/#corpora lutea) X 100
Post implantation loss (%) = (#implants - # live embryos/# implants) X 100
Hematology:
- Blood collected from abdominal aorta in main and recovery mice

- The following parameters were measured: blood cell morphology, erythrocyte indices (MCV, MCH, MCHC, RDW), hematocrit, hemoglobin, mean platelet volume, platelet count, red blood cell count (erythrocyte count), reticulocyte count, and white blood cell count (total, absolute and percent differential)

Clinical chemistry:

- Blood collected from abdominal aorta in main and recovery animals
- The following parameters were measured: alanine aminotransferase, albumin (A), alkaline phosphatase, aspartate aminotransferase, blood urea nitrogen, calcium, chloride, cholesterol, creatine kinase, creatinine, globulin (calculated) (G), glucose, inorganic phosphorus, potassium, sodium, total bilirubin, total protein, triglycerides, and A/G ratio (calculated); if blood volume insufficient, the following parameters were prioritized: alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, alkaline phosphatase, total bilirubin, total protein, albumin, sodium, potassium, chloride, glucose, calcium, inorganic phosphorus, triglycerides, cholesterol, and creatine kinase

Urinalysis: Not done

Gross pathology: Main and recovery animals

Organ weights: Main and recovery animals: the following organs were weighed: adrenals, brain, heart (including aorta section), kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid (and parathyroids), and uterus

Histopathology: Adequate Battery: yes (x), no ( )

Peer review: yes (X: — , DVM, MVSc; — , DVM, DipPath, MSc), no ( ) b(4)

The following organs and tissues from the vehicle control and HD groups were examined: abnormalities, adrenals, aorta (thoracic), bone and marrow (sternum), brain (forebrain, midbrain, cerebellum and medulla), cecum, colon, duodenum, epididymides, esophagus, eyes, gallbladder, femero-tibial joint, Harderian glands, heart (including aorta section), ileum, injection sites, jejunum, kidneys, lacrimal glands, larynx (1 level), liver (2 lobes), lungs (2 lobes), lymph nodes (mandibular, unilateral, mesenteric), mammary gland (inguinal), optic nerves, ovaries, pancreas, pituitary, prostate, rectum, salivary gland (mandibular, unilateral), sciatic nerve, seminal vesicles, skeletal muscle, skin (inguinal), spinal cord (cervical), spleen*, stomach, testes, thymus*, thyroid lobes (and parathyroids), tongue, trachea, ureters, urinary bladder, uterus (horns, body, cervix), and vagina

*Spleen and thymus also collected from immunology subgroups for phenotyping of lymphocyte subsets

In the LD and MD groups, only tissues with treatment-related findings (e.g., injection sites) and gross lesions were examined

Toxicokinetics: PPD 7 and at end of dosing period: blood collected from 4 (PPD7, cardiac puncture) or 2 (end of dosing period, abdominal aorta) pups/sex/group/timepoint at 24 h (controls), and at 0, 8, 24, 48, and 72 hours after dosing; blood collected from 10 untreated control mice/sex on PPD 21, 10 F/group in the treated groups on GD13, and 10 M/group in the treated groups at termination

Immunogenicity testing: At predose and termination, blood samples collected from 10 mice/sex in the untreated controls on PPD 21, 10 F/treated group on GD13, and 10 M/treated group at termination

Immunology assessment (immunophenotyping)

Blood: blood collected from abdominal aorta at scheduled euthanasia, all main and recovery mice; Results reported as relative proportion and absolute numbers (cell/mcl) of Total T cells ($CD3e^+$), Helper T cells ($CD3e^+/CD4^+$), Cytotoxic T cells ($CD3e^+/CD8a^+$), B cells ($CD3e^-/CD19^+$) and Natural Killer (NK) cells ($CD3e^-/NK1.1^+$).

Spleen: Spleen cell suspensions measured for relative percentages and absolute numbers (cell/whole organ and cells/grams of organ) of Total T cells ($CD3e^+$), Helper T cells ($CD3e^+/CD4^+$), Cycotoxic T cells ($CD3e^+/CD8a^+$), B cells ($CD3e^-/CD19^+$), and natural Killer (NK) cells ($CD3e^-/NK1.1^+$).

Thymus: Thymus cell suspension analyzed for white blood cell counts (total absolute and percent differentials); Immunophenotyping reported as relative proportions and absolute numbers (cells/whole organ and cells/per grams of organ) of Total T cells ($CD3e^+$), Double negative T cells ($CD3e^+/CD4^+/CD8^-$), Double positive T cells ($CD3e^+/CD4^+/CD8^+$), Helper T cells ($CD3e^+/CD4^+$ /CD8), Cytotoxic T cells ($CD3e^+/CD4^-/CD8^+$).

Results

Mortality: No treatment-related effects

Clinical signs: No treatment-related effects

Body weights: No treatment-related effects

Food consumption:

- Significantly reduced in the HDM from PPD 21-24 and in the MD and HD F from PPD 24-28
- In absence of treatment-related effects at any other time-points and any body weight effects, considered to be incidental

Physical Development Pre-weaning:

Eye opening: No treatment-related effects

Auricular startle: Slight **increase in mean day of development of auricular startle** at MD in M, and HD in M and F compared to concurrent controls, however:

- Values similar to controls in Study # 901098 (13.05 d in M, 13.11 d in F)
- Within historical control range for the laboratory 12.5-12.8 d
- No treatment-related effects seen in the post-weaning evaluation of auditory startle habituation (startle at start, maximum startle, time of maximum startle and average startle)

- Therefore, finding in this study of low concern toxicologically, but should be noted as potentially suggesting very slight treatment-related delay in auditory sensory or reflex development

Physical Development Post-weaning:

Vaginal opening: Significantly **delayed mean day to development of vaginal opening** at the HD (28.5 days) vs. concurrent controls (26.28 days), however:

- Within historical control range of 25.7-29.7 days in the performing laboratory (see under Historical Control, below)
- No treatment-related effects on mean day of preputial separation
- Therefore of low concern toxicologically

Sensory Development (visual function): No treatment-related effects

Behavioral performance:

Motor activity: No treatment-related effects
Auditory startle habituation: No treatment-related effects
Passive avoidance (learning and memory): No treatment-related effects

Fertility assessment:

Mating: No treatment-related effects on mean day to mating, mating and fertility indices, and conception rate

Gestation Day 13 uterine examination: **Significantly increased pre-implantation loss at HD** (20.38%) compared to controls (4.26%), however:

- Due predominantly to loss in 1 dam (71.4%)
- Group mean number of live embryos (12.8) at the HD within historical range of 11.6-13.0
- Therefore not of great concern toxicologically

Hematology: No treatment-related effects

Clinical chemistry: No treatment-related effects

Gross pathology: No treatment-related effects

Organ weights: No treatment-related effects

Histopathology: Treatment-related inflammation at injection site in treated M and F, reversed during recovery period

Toxicokinetics

- Tmax 24 h on PPD 7 and 24-72 h on PPD 63 (48, 72, and 24 hours at 15, 50, and 150 mg/kg/wk, respectively)
- Dose-proportional increase in exposure ($AUC_{0.083\text{-}168h}$ and Cmax) on PPD 7 and 63

- Exposure ($AUC_{0.083-168h}$ and Cmax) increased 6X from PPD 7 to PPD 63, suggesting accumulation
- The following TK parameters were calculated for the F1 generation (from the original BLA submission):

Table 4-5 Mean toxicokinetic parameters of 01BSUR in pooled male and female mouse serum on day 7 post-partum

	Dose (mg/kg/day)		
	15	50	150
T_{max}	24.0	24.0	24.0
C_{max}	54.8	162	462
C_{max}/Dose	3.65	3.24	3.08
$AUC_{(0-72h)}$	2520	7770	28300
$AUC_{(0-72h)}$/Dose	168	156	188

Mean concentration expressed in µg/mL
Units :tmax [h]. Cmax [µg/mL]. Cmax/dose [(µg/mL)/(mg/kg/day)]. AUC(0.083-168h) [h·µg/mL]. AUC(0.083-168h)/dose [(h·µg/mL)/(mg/kg/day)

Table 4-6 Mean concentrations of 01BSUR in male and female mouse serum on day 63 post-partum

	Dose (mg/kg/day)		
	15	50	150
T_{max}	48.0	72.0	24.0
C_{max}	262	924	2270
C_{max}/Dose	17.5	18.5	15.1
$AUC_{(0-72h)}$	17100	59100	154000
$AUC_{(0-72h)}$/Dose	1140	1180	1030

Mean concentration expressed in µg/mL
Units :t_{max} [h]. C_{max} [µg/mL]. C_{max}/dose [(µg/mL)/(mg/kg/day)]. $AUC_{(3.083-153h)}$ [h·µg/mL]. $AUC_{(0.083-153h)}$/dose [(h·µg/mL)/(mg/kg/day)

Immunogenicity testing: Weak immune response (increased anti-01BSUR antibody) in 1 MDF on GD13, only

Immunology assessment (Blood, spleen, thymus immunophenotyping): No treatment-related effects on lymphocyte counts and lymphocyte subset counts in blood, spleen and thymus

Historical Control Data for the CD-1 Mouse b(4)

HISTORICAL CONTROL DATA
MOUSE – CD-1 (Crl:CD1[ICR]), 2005-2008

b(4)

HCD 2.1	PRE AND POSTNATAL STUDIES	
	GROUP MATERNAL PERFORMANCE*	
Parameter	Minimum	Maximum
Gestation Index (%)	100.0	100.0
Length of Gestation (Days)	18.7	18.8
Sex Ratio (% Males)	48.9	57.5
No. of Live Pups at Birth	10.8	12.1
No. of Dead Pups at Birth	0.0	0.2
No. of Implant Scars	12.5	12.8
Live Birth Index (%)	96.7	100.0

HCD 2.2	PRE AND POSTNATAL STUDIES	
	VIABILITY* AND PUP BODY WEIGHTS (G)	
Parameter	Minimum	Maximum
Viability Index (%) - Day 4 Post Partum	90.0	100.0
Survival Index (%) - Day 7 Post Partum	98.0	100.0
Survival Index (%) - Day 14 Post Partum	98.0	100.0
Lactation Index (%) - Day 21 Post Partum	97.4	100.0
BODY WEIGHTS:		
Day 0 Post Partum		
Males	1.61	1.64
Females	1.54	1.57
Total	1.58	1.60
Day 4 Post Partum (post cull)		
Males	2.93	2.98
Females	2.83	2.88
Total	2.88	2.94
Day 7 Post Partum		
Males	4.82	5.01
Females	4.69	4.92
Total	4.75	4.97
Day 14 Post Partum		
Males	7.87	8.77
Females	7.67	8.68
Total	7.77	8.72
Day 21 Post Partum		
Males	12.43	13.44
Females	11.71	12.79
Total	12.07	13.07

* Includes data from non-GLP studies

HISTORICAL CONTROL DATA
MOUSE – CD-1 (Crl:CD1[ICR]), 2003-2008

b(4)

HCD 3.1	EMBRYO-FETAL DEVELOPMENT STUDIES		
GROUP MATERNAL, OVARIAN AND UTERINE FINDINGS			
Parameter	Total	Minimum	Maximum
No. of Mice Mated	122	22	25
No. of Mice Pregnant	103	17	23
No. of Mice Pregnant by Ammonium Sulfide Staining	2	0	1
No. of Mice Dying or Unscheduled Euthanasia on Study	0	0	0
No. of Mice with Total Resorption	2	0	1
No. of Mice Littering Prior to Scheduled Cesarean	4	0	2
Pregnancy Rate (%)	-	68.0	92.0
Total Corpora Lutea / Mouse	-	13.5	16.0
Total Implantation Sites / Litter	-	12.2	14.7
Sex Ratio (% Males)	-	46.7	58.0
Total Live Fetuses / Litter	-	10.9	13.8
Dead Fetuses / Litter	-	0.0	0.1
Early Resorptions / Litter	-	0.2	1.1
Middle Resorptions / Litter	-	0.0	0.1
Late Resorptions / Litter	-	0.0	0.3
Total Resorptions / Litter	-	0.3	1.3
Pre-Implantation Loss (%) / Litter	-	7.0	12.8
Post-Implantation Loss (%) / Litter	-	3.3	9.8

APPEARS THIS WAY ON ORIGINAL

HISTORICAL CONTROL DATA
MOUSE – CD-1 (Crl:CD1[ICR]), 2003-2008

b(4)

HCD 3.2	EMBRYO-FETAL DEVELOPMENT STUDIES	
	FETAL FINDINGS - SUMMARY DATA	
Parameter	Minimum	Maximum
Fetal Weight (G) - Males	1.32	1.44
Fetal Weight (G) - Females	1.28	1.38
Fetal Weight (G) - Total	1.31	1.41
Major Malformations - Litters Affected (%)	0.0	9.5
Major Malformations - Fetuses Affected (%)	0.0	1.9
Minor External and Visceral Anomalies - Litters Affected (%)	0.0	6.7
Minor External and Visceral Anomalies - Fetuses Affected (%)	0.0	1.9
Minor Skeletal Anomalies - Litters Affected (%)	65.2	94.1
Minor Skeletal Anomalies - Fetuses Affected (%)	20.3	78.0
Sternebrae 1 to 4 (unossified/incomplete/ semi-bipartite/bipartite)	0.7	2.9
Sternebrae 5 and Xiphisternum (unossified/incomplete/ semi-bipartite/bipartite)	16.4	28.4
Sternebrae 1 to 6 (unossified/incomplete/ semi-bipartite/bipartite)*	16.9	27.6
Ribs - Total 14th/extra/rudimentary/contralateral (unilateral and bilateral)	30.1	42.1

* Alternate tabulation for sternebral variants

APPEARS THIS WAY ON ORIGINAL

b(4)

HISTORICAL CONTROL DATA
MOUSE – CD-1 (Crl:CD1[ICR]), 2003-2008

HCD 3.3	EMBRYO-FETAL DEVELOPMENT STUDIES			
GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
	Litters examined			
EXTERNAL (EXT)	143	Total no. of studies used		
VISCERAL (VIS)	98	7		
SKELETAL (SKE)	98			
TECHNIQUE OF WILSON (WT)	98			
	Litters affected			
	SUM	AVERAGE %	MIN %	MAX %
MAJOR MALFORMATIONS (TOTAL)	6	4.20	0.00	9.52
Head: Encephalocele (EXT)	1	0.70	0.00	4.35
Head: Cleft palate (EXT)	4	2.80	0.00	9.52
Cranium: Exencephaly (EXT,SKE)	1	0.70	0.00	4.35
Eye(s): Exophthalmia (EXT,WT)	1	0.70	0.00	5.88
Eye(s): Open (EXT,WT)	3	2.10	0.00	5.88
Face: Mandibular micrognathia (EXT)	1	0.70	0.00	5.88
Limb(s): Brachydactyly (EXT)	1	0.70	0.00	5.88
Forelimb(s): Bent radius (SKE)	1	1.02	0.00	5.88
Forelimb(s): Wavy radius (SKE)	1	1.02	0.00	5.88
Hindlimb(s): Bent (EXT)	1	1.02	0.00	5.88
Hindlimb(s): Bent tibia (SKE)	1	1.02	0.00	5.88
Hindlimb(s): Bent fibula (SKE)	1	1.02	0.00	5.88
Tibula\Fibula short (SKE)	1	1.02	0.00	5.88
MINOR VISCERAL AND EXTERNAL ANOMALIES (TOTAL)	132	7.22	0.00	33.33
Eye(s): Subcutaneous edema (EXT)	1	0.70	0.00	6.67
Hindpaw(s): Malpositioned digit(s) (EXT)	1	0.70	0.00	5.88

HISTORICAL CONTROL DATA
MOUSE – CD-1 (Crl:CD1[ICR]), 2003-2008

b(4)

HCD 3.3	EMBRYO-FETAL DEVELOPMENT STUDIES			
GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
SKELETAL (SKE)	Litters examined 98		Total no. of studies used 5	
	Litters affected			
MINOR SKELETAL ANOMALIES (TOTAL)	SUM 74	AVERAGE % 75.51	MIN % 65.22	MAX % 94.12
SKULL				
Frontal bone(s): Incomplete ossification	16	16.33	0.00	94.12
Parietal bone(s): Incomplete ossification	5	5.10	0.00	29.41
Supraoccipital bone: Incomplete ossification	17	17.35	4.55	41.18
Interparietal bone: Incomplete ossification	1	1.02	0.00	5.88
Hyoid bone: Incomplete ossification	2	2.04	0.00	9.52
Hyoid bone: Unossified	1	1.02	0.00	5.88
Nasal bone(s): Unossified	1	1.02	0.00	5.88
Premaxilla: Incomplete ossification	1	1.02	0.00	5.88
Maxilla: Incomplete ossification	1	1.02	0.00	5.88
Mandible: Incomplete ossification	1	1.02	0.00	5.88
Zygomatic bone(s): Incomplete ossification	1	1.02	0.00	5.88
VERTEBRAL COLUMN				
25 pre-sacral vertebrae	1	1.02	0.00	5.88
Ossification center on 1st lumbar vertebra or 14th thoracic vertebra	24	24.49	0.00	45.45
Sacral vertebral centrum: Semi-bipartite	1	0.02	0.00	4.76
Caudal vertebra(e): Reduced no.	42	42.86	26.09	63.64
STERNEBRAL COLUMN				
Extra	5	5.10	0.00	17.65
RIBS				
Extra 14th rib	1	1.02	0.00	5.88
Ossification center(s) on 7th cervical vertebra(e)	12	12.24	0.00	30.43
Rib(s) on 7th cervical vertebra(e)	5	5.10	0.00	29.41
Rib(s): Incomplete ossification	1	1.02	0.00	5.88
PELVIC GIRDLE				
Pubic bone(s): Incomplete ossification	1	1.02	0.00	5.88
LIMBS				
Reduced no. of phalange(s) in forepaw(s)	12	12.24	6.67	17.65
Reduced no. of phalange(s) in hindpaw(s)	8	8.16	0.00	13.33

HISTORICAL CONTROL DATA
MOUSE – CD-1 (Crl:CD1[ICR]), 2003-2008

b(4)

HCD 3.3	EMBRYO-FETAL DEVELOPMENT STUDIES			
GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
SKELETAL (SKE)	Fetuses examined 584		Total no. of studies used 5	
	Fetuses affected			
MINOR SKELETAL ANOMALIES (TOTAL)	SUM 197	AVERAGE % 33.73	MIN % 20.33	MAX % 77.98
SKULL				
Frontal bone(s): Incomplete ossification	71	12.6	0.00	64.14
Parietal bone(s): Incomplete ossification	10	1.71	0.00	9.17
Supraoccipital bone: Incomplete ossification	31	5.31	0.78	11.93
Interparietal bone: Incomplete ossification	1	0.17	0.00	0.92
Hyoid bone: Incomplete ossification	2	0.34	0.00	1.63
Hyoid bone: Unossified	1	0.17	0.00	0.92
Nasal bone(s): Unossified	2	0.34	0.00	1.83
Premaxilla: Incomplete ossification	2	0.34	0.00	1.83
Maxilla: Incomplete ossification	2	0.34	0.00	1.83
Mandible: Incomplete ossification	2	0.34	0.00	1.83
Zygomatic bone(s): Incomplete ossification	2	0.34	0.00	1.83
VERTEBRAL COLUMN				
25 pre-sacral vertebrae	1	0.17	0.00	0.92
Ossification center on 1st lumbar vertebra or 14th thoracic vertebra	25	4.28	1.83	8.59
Sacral vertebral centrum: Semi-bipartite	1	0.17	0.00	0.81
Caudal vertebra(e): Reduced no.	76	13.01	10.37	18.75
STERNEBRAL COLUMN				
Extra	6	1.03	0.00	3.67
RIBS				
Extra 14th rib	1	0.17	0.00	0.92
Ossification center(s) on 7th cervical vertebra(e)	13	2.23	0.00	5.93
Rib(s) on 7th cervical vertebra(e)	8	1.37	0.00	7.34
Rib(s): Incomplete ossification	1	0.17	0.00	0.92
PELVIC GIRDLE				
Pubic bone(s): Incomplete ossification	1	0.17	0.00	0.92
LIMBS				
Reduced no. of phalange(s) in forepaw(s)	17	2.91	1.12	5.50
Reduced no. of phalange(s) in hindpaw(s)	10	1.71	0.00	3.67

HISTORICAL CONTROL DATA
MOUSE – CD-1 (Crl:CD1[ICR]), 2003-2008

b(4)

HCD 3.3	EMBRYO-FETAL DEVELOPMENT STUDIES			
GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
	Fetuses examined			
EXTERNAL (EXT)	1779	Total no. of studies used		
VISCERAL (VIS)	580	7		
SKELETAL (SKE)	584			
TECHNIQUE OF WILSON (WT)	581			
	Fetuses affected			
	SUM	AVERAGE %	MIN %	MAX %
MAJOR MALFORMATIONS (TOTAL)	9	0.51	0.00	1.87
Head: Encephalocele (EXT)	1	0.06	0.00	0.37
Head: Cleft palate (EXT)	7	0.39	0.00	1.87
Cranium: Exencephaly (EXT,SKE)	1	0.06	0.00	0.31
Eye(s): Exophthalmia (EXT,WT)	1	0.06	0.00	0.47
Eye(s): Open (EXT,WT)	3	0.17	0.00	0.47
Face: Mandibular micrognathia (EXT)	4	0.22	0.00	1.87
Limb(s): Brachydactyly (EXT)	3	0.17	0.00	1.40
Forelimb(s): Bent radius (SKE)	1	0.17	0.00	0.92
Forelimb(s): Wavy radius (SKE)	3	0.51	0.00	2.75
Hindlimb(s): Bent (EXT)	4	0.68	0.00	3.67
Hindlimb(s): Bent tibia (SKE)	1	0.17	0.00	0.92
Hindlimb(s): Bent fibula (SKE)	2	0.34	0.00	1.83
Tibula\Fibula short (SKE)	4	0.68	0.00	3.67
MINOR VISCERAL AND EXTERNAL ANOMALIES (TOTAL)	5	0.28	0.00	1.87
Eye(s): Subcutaneous edema (EXT)	1	0.06	0.00	0.57
Hindpaw(s): Malpositioned digit(s) (EXT)	4	0.22	0.00	1.87

HCD 2.3	PRE AND POSTNATAL STUDIES	
PRE-WEANING*#		
Parameter	Minimum	Maximum
Pinna detachment (days) - males	3.6	3.8
Pinna detachment (days) - females	3.7	3.9
Eye opening (days) - males	13.9	14.6
Eye opening (days) - females	14.1	14.4
Righting reflex (days) - males	2.0	2.1
Righting reflex (days) - females	2.1	2.1
Auricular startle ontogeny (days) - males	12.5	12.6
Auricular startle ontogeny (days) - females	12.7	12.8

HCD 2.4	PRE AND POSTNATAL STUDIES
POST WEANING*#	

Parameter	Minimum	Maximum
Vaginal opening (days)	25.7	29.6
Preputial separation (days)	26.0	29.5
Visual function - placing - males (%)	100	100
Visual function - placing - females (%)	100	100
Visual function - pupillary closure - males (%)	100	100
Visual function - pupillary closure - females (%)	100	100
Motor activity (total counts)	624	832
Motor activity (total counts)	657	936
Startle Habituation		
Time of maximum startle (msec) - males	22.4	29.1
Time of maximum startle (msec) - females	25.2	30.4
Maximum startle (mV) - males	175.3	297.1
Maximum startle (mV) - females	117.0	186.1
Average startle (mV) - males	41.6	61.9
Average startle (mV) - females	28.6	38.9
Passive avoidance (25-hour memory) (sec) - males	108.5	109.9
Passive avoidance (25-hour memory) (sec) - females	98.6	106.7

* Includes data from non-GLP studies
\# Includes data from unculled litters

b(4)

SEXUAL MATURATION
HISTORICAL CONTROL DATA
Crl:CD1(ICR) MICE

CODE:
DRAFT FINAL REPORT MAIL DATE:
TYPE OF STUDY:

SUMMARY

STUDIES INCLUDED IN ANALYSIS

SEXUAL MATURATION		AVERAGE	MINIMUM	MAXIMUM	# STUDIES INCLUDED
MALE MICE	N	22.0	12	30	3
Preputial Separation (Avg. day postpartum obs.)	MEAN	31.3	29.9	33.6	3
Body Weight (G) on day of sexual maturity	MEAN	22.2	20.9	22.9	3
FEMALE MICE	N	22.3	12	30	3
Vagina Patent (Avg. day postpartum obs.)	MEAN	28.8	26.0	32.5	3
Body Weight (G) on day of sexual maturity	MEAN	15.7	14.2	16.7	3

Study title: ***ACZ885 Surrogate (01BSUR): A 28-Day (Weekly Dosing) Subcutaneous Injection Immunotoxicity Study in the Albino Mouse with a 28 Day Recovery Period***

Key study findings:

- 01BSUR negative for immunotoxicity in the CD-1 mouse at subcutaneous (SC) doses of up to 150 mg/kg/week (HD) for 4 weeks (total 5 doses)
- (Statistically) significantly lower anti-KLH IgG response in males (M) at 10 (LD) mg/kg/week, and 61% lower at 50 mg/kg/wk (MD) and HD (Day 29); however:
  - Apparent decrease due to excessively high response in 2 control M
  - Increased response at LD, MD, and HD in females (F) due to high variability among animals
  - Therefore, of low concern toxicologically
- Sporadic findings of dendritic cell hyperplasia with adjacent marginal zone lymphoid hyperplasia, without dose-relationship, of low incidence, and also observed in 1 control recovery group female, therefore not considered to be toxicologically relevant
- NOAEL for 01BSUR immunotoxicity in CD-1 mice = 150 mg/kg/once weekly for 4 weeks

Study no.: —— Study #301461, Novartis Study #0670570 b(4)

Conducting laboratory and location: ——— b(4)

Date of study initiation: February 26, 2007
GLP compliance: Yes
QA report: yes (x) no ( )
Drug 01BSUR at 15 mg/ml, 52.5 mg/3.5 ml vial), **lot #** (Batch) 7318, **and % purity:** 98.73% by By- and degradation products by Size Exclusion Chromatography

Methods

Doses: 0, 10 (LD), 50 (MD), 150 (HD) mg/kg/week, doses selected based on the results of 4-week toxicology study in CD-1 mice administered 01BSUR at 10, 50, and 150 mg/kg/week
Species/strain: *Mus musculus* Crl:CD1(ICR) Mouse
Number/sex/group or time point (main study): 20/sex/group; the animal allocation is presented in the following table (from the original BLA submission):

Table 3-1 Study design, animal allocation and test article doses

Group number identification	Dose level (mg/kg/dose)	Dose volume (mL/kg/dose)	Animal number: Immunophenotyping, immunogenicity and toxicokinetics				TDAR/immunogenicity			
			Main study		Recovery study		Main study		Recovery study	
			Males	Females	Males	Females	Males	Females	Males	Females
1/ Vehicle control	0	10	1001 to 1010	1501 to 1510	1011 to 1020	1511 to 1520	1021 to 1030	1521 to 1530	1031 to 1040	1531 to 1540
2/ 01BSUR	10	0.67	2001 to 2010	2501 to 2510	2011 to 2020	2511 to 2520	2021 to 2030	2521 to 2530	2031 to 2040	2531 to 2540
3/ 01BSUR	50	3.33	3001 to 3010	3501 to 3510	3011 to 3020	3511 to 3520	3021 to 3030	3521 to 3530	3031 to 3040	3531 to 3540
4/ 01BSUR	150	10	4001 to 4010	4501 to 4510	4011 to 4020	4511 to 4520	4021 to 4030	4521 to 4530	4031 to 4040	4531 to 4540

TDAR: T-cell Dependant Antibody Response.

Route, formulation, volume, and infusion rate: Control vehicle: 180 mM Sucrose, 10 mM L-histidine/0.02% (m/V) Tween 20 aqueous solution in Sterile Water for Injection USP, pH 5.5; Test article administered as provided by Applicant, by SC injection once weekly on Study Days 1, 8, 15, 21, and 28 at 0.67, 3.33, and 10 ml/kg/dose, respectively in the test article-treated groups, and at 10 ml/kg in the vehicle control group
Satellite groups used for toxicokinetics or recovery: 20/sex/group 28-day recovery group (10/sex/group as TDAR/immunogenicity cohort, 10/sex/group as immunophenotyping/immunogenicity/toxicokientics cohort)
Age: 10 weeks
Weight: 31.1-41.8 g males (M), 23.3-32.3 g females (F)
Unique study design/methodology:

- Mice were housed individually in stainless steel cages, in a temperature (19-25 degC) and humidity (30-70%) controlled facility with 12 hour light/dark cycle; food (PMI Certified Rodent 5002 diet) and tap water provided *ad libitum*

- **Immunizing agent:** Imject Mariculture Keyhole Limpet Hemocyanin (KLH) in aqueous solution at 200 mcg/ml, given by IP injection (0.5 ml/animal) on Days 15 and 22 in the main study animals and Days 42 and 49 in the recovery animals that were assigned to TDAR/immunogenicity evaluation, only

Observations:
Mortality: Twice daily from baseline to day of necropsy, all animals
Clinical signs: Twice daily from baseline to day of necropsy, all animals
Body weights: Weekly from randomization to end of recovery period
Food consumption: Weekly from initiation of treatment through recovery period
Immunophenotyping

Blood:

- Day 29 (main study animals) and Day 57 (recovery animals)
- Blood collected from abdominal aorta from all animals for lymphocyte subset analysis, white blood cell counts (total absolute and percent differential)
- Phenotyping reported as relative and absolute numbers of:
  - Total T lymphocytes ($CD3e^+$)
  - Helper T lymphocytes ($CDe3^+/CD4^+$)
  - Cytotoxic T lymphocytes ($CDe3^+/CD8a^+$)
  - B lymphocytes ($CD19^+$)
  - Natural Killer (NK lymphocytes ($CDe3^-/NK1.1^+$)

Spleen

- Day 29 (main study animals) and Day 57 (recovery animals)
- Spleens isolated at necropsy for lymphocyte subset analysis
- Half spleen used for immunophenotyping as relative proportions and absolute numbers of
  - Total T lymphocytes ($CD3e^+$)
  - Helper T lymphocytes ($CD3e^+/CD4^+$)
  - Cytotoxic T lymphocytes ($CD3e^+/CD8a^+$)
  - B lymphocytes ($CD19^+$)
  - Natural Killer (NK lymphocytes ($CD3e^-/NK1.1^+$)
- Half spleen used for histopathology evaluation, if needed
- Spleen weights and gross examinations performed
- Total absolute and percent differential white blood cell counts measured

Thymus

- Day 29 (main study animals) and Day 57 (recovery animals)
- Thymus isolated at necropsy for lymphocyte subset analysis
- Half thymus used for immunophenotyping as relative proportions and absolute numbers of
  - Total T lymphocytes ($CD3e^+$)
  - Double positive T lymphocytes ($CD3e^+/CD4^+/CD8a^+$)
  - Double negative T lymphocytes ($CD3e^+/CD4^-/CD8a^-$)
  - Helper T lymphocytes ($CD3e^+/CD4^+/CD8a^-$)

- Cytotoxic T lymphocytes ($CD3e^+$/ $CD4^-$/$CD8a^+$)
- Half spleen used for histopathology evaluation, if needed
- Thymus weights and gross examinations performed

Immunogenicity:

- Main TDAR/immunogenicity animals and recovery immunophenotyping, immunogenicity and TK cohorts tested
- Blood samples collected (abdominal aorta) at necropsy, and serum analyzed for anti-01BSUR antibody formation

T-cell Dependent Antibody Response (TDAR):

- Days 15 and 22: IP injection of KLH (0.5 ml in USP Sterile Water) 3h after dosing
- Blood samples collected before and at 7 and 14 days after KLH injection (Days 22, 29), from abdominal aorta, and analyzed for anti-KLH IgM and anti-KLH IgG antibodies using relative quantitative ELISA
- T-cell dependent antigen (KLH) IgM and IgG antibody response also analyzed in recovery animals injected with KLH on Days 14 and 21 (Study Days 42, 49), with blood (0.15 ml) collected from jugular vein on recovery day 14 and from the abdominal aorta on recovery Days 21 and 28 (Study Days 49 and 56)

Gross pathology: External (including gross lesions) and detailed internal examinations on all animals at necropsy and all animals found dead

Organ weights: The following organs were weighed at necropsy, only: brain, spleen, thymus

Histopathology: Adequate Battery: yes (x): selected tissues for evaluation of immunotoxicity, only

Peer review: yes ( ), no (x)

The following organs and tissues from the immunophenotyping, immunogenicity and TK cohorts were examined microscopically:

Main study animals (all control and treated groups): lymph nodes (mandibular, unilateral, inguinal, bilateral, mesenteric), spleen, and thymus

Recovery animals (control and high dose groups only): lymph nodes (mandibular, unilateral, inguinal, bilateral, mesenteric), spleen, and thymus

Animals found dead or sacrificed before scheduled euthanasia: all tissues

Toxicokinetics: Blood samples collected from jugular vein in the TK recovery cohort from 2 mice/sex/dose/timepoint on Days 28-57 at 24 h after dosing(Days 28, 29), 168 h after dosing (Day 35), 336 h after dosing (Day 42), and 696 h after dosing (Day 57)

Results

Mortality:

- No treatment-related deaths

- Death in animals #1017 (vehicle control recovery M, Study Day 44) and #2033 (10 mg/kg/week recovery M, Study Day 41), with histopathology findings of obstructive uropathy, unrelated to treatment
- Animal #4014 (150 mg/kg/week recovery M, Study Day 14) euthanized, with histopathology findings of malignant lymphoma, unrelated to treatment

Clinical signs: No treatment-related effects

Body weights: No treatment-related effects

Food consumption: No treatment-related effects

Immunophenotyping

Blood:

- No treatment-related effects due to high individual variability, absence of findings in both sexes within dose groups or observation day, absence of statistical significance, and findings within the control value ranges
- Differences (≥30%) from controls in total lymphocyte counts observed in treated groups are presented in the following table (from the original BLA submission):

Summary table of statistically significant differences and/or difference ≥ 30% for the total lymphocyte counts in blood when compared to control group 1

Parameter	Group	Difference vs control group	Statistically significant[a]	Test-article related[b]	Comments
Mean total lymphocyte counts					
Study day 29					
Males	3	-36.7%	No	No	c
Study day 57					
Females	2	-43.5%	No	No	c
	3	-31.4%	No	No	c
	4	-42.5%	No	No	c

a Significantly different from control group (group 1) value:

A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)

D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

b Based on interpretation of all available data taking into account individual data variability and/or absence of clear trends.

c Although these changes in mean values are ≥ 30%, they are not considered TA related nor biologically relevant when taking into account individual animal variability and/or range of values obtained in the control group (group 1).

- No statistically significant treatment-related effects on absolute lymphocyte counts in blood; ≥30% differences from controls are presented in the following table (from the original BLA submission):

Summary table of statistically significant differences and/or difference ≥ 30% for mean absolute lymphocyte counts in blood when compared to control group 1

Parameter	Group	Difference vs control group	Statistically significant[a]	Test-article related[b]	Comments
Mean absolute CD3e+ counts					
Study day 57					
Males	4	+42.5%	No	No	c,d
Females	2	-30.5%	No	No	c,d
	3	-30.3%	No	No	c,d
	4	-40.9%	No	No	c,d
Mean absolute CD3e+/CD4+ counts					
Study day 57					
Males	4	+40.3%	No	No	c,d
Females	2	-32.9%	No	No	c,d
	3	-34.1%	No	No	c,d
	4	-42.6%	No	No	c,d
Mean absolute CD3e+/CD8a+ counts					
Study day 29					
Females	2	-41.2%	No	No	c,d
Study day 57					
Males	3	+31.7%	No	No	c,d
	4	+57.5%	No	No	c,d
Females	4	-41.7%	No	No	c,d
Mean absolute CD3e-/NK1.1+ counts					
Study day 29					
Males	3	-50.7%	No	No	c,d
	4	-40.8%	No	No	c,d
Study day 57					
Males	2	-32.0%	No	No	c,d
	4	+40.6%	No	No	c,d
Females	3	-46.6%	No	No	c,d
Mean absolute CD19+ counts					
Study day 29					
Males	3	-47.9%	No	No	c,d
	4	-33.6%	No	No	c,d
Females	3	+34.0%	No	No	c,d
Study day 57					
Females	2	-42.4%	No	No	c,d
	3	-31.7%	No	No	c,d
	4	-44.5%	No	No	c,d

a Significantly different from control group (group 1) value:

A - $P \leq 0.05$ B - $P \leq 0.01$ C - $P \leq 0.001$ (Dunnett)

D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

b Based on interpretation of all available data taking into account individual data variability and/or absence of clear trends.

c Although these changes in mean values are ≥ 30%, they are not considered TA related nor biologically relevant when taking into account individual animal variability and/or range of values obtained in the control group (group 1).

d No difference ≥ 30% was observed in terms of relative percentage of this population.

- No statistically significant treatment-related effects on mean relative percentages of lymphocytes in blood; ≥30% differences from controls are presented in the following table (from the original BLA submission):

Summary table of statistically significant differences and/or difference ≥ 30% for the mean relative percentage of lymphocytes in blood when compared to control group 1

Parameter	Group	Difference vs control group	Statistically significant[a]	Test-article related[b]	Comments
Mean relative percentage CD3e-/NK1.1+ counts					
Study day 57					
Females	4	+34.4%	No	No	c

a Significantly different from control group (group 1) value:
A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)
D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

b Based on interpretation of all available data taking into account individual data variability and/or absence of clear trends.

c Although these changes in mean values are ≥ 30%, they are not considered TA related nor biologically relevant when taking into account individual animal variability and/or range of values obtained in the control group (group 1).

Spleen:

- No treatment-related effects due to high inter-individual variability, absence of findings in both sexes within dose groups or observation day, absence of statistical significance, and findings within the control value ranges
- Differences (≥30%) from controls in total lymphocyte counts observed in treated groups are presented in the following table (from the original BLA submission):

APPEARS THIS WAY ON ORIGINAL

Summary table of statistically significant differences and/or difference ≥ 30% for total lymphocyte counts in spleen when compared to control group 1

Parameter	Group	Difference vs control group	Statistically significant[a]	Test-article related[b]	Comments
Mean total lymphocyte counts					
Study day 57					
Males	2	-33.3%	No	No	c
Mean total lymphocyte counts per whole spleen					
Study day 57					
Males	2	-33.3%	No	No	c

a Significantly different from control group (group 1) value:

A - $P \leq 0.05$ B - $P \leq 0.01$ C - $P \leq 0.001$ (Dunnett)

D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

b Based on interpretation of all available data taking into account individual data variability and/or absence of clear trends.

c Although these changes in mean values are ≥ 30%, they are not considered TA related nor biologically relevant when taking into account individual animal variability and/or range of values obtained in the control group (group 1).

Summary table of statistically significant differences and/or difference ≥ 30% for absolute lymphocyte counts in spleen when compared to control group 1

Parameter	Group	Difference vs control group	Statistically significant[a]	Test-article related[b]	Comments
Mean absolute CD3e+ counts per gram of spleen					
Study day 57					
Males	2	-36.9%	No	No	c,d,e
Mean absolute CD3e+ counts per whole spleen					
Study day 57					
Males	2	-33.6%	No	No	c,d,e
Mean absolute CD3e+/CD4+ counts per gram of spleen					
Study day 57					
Males	2	-37.6%	No	No	c,d,e
Mean absolute CD3e+/CD4+ counts per whole spleen					
Study day 57					
Males	2	-35.2%	No	No	c,d,e
Mean absolute CD3e+/CD8a+ counts per gram of spleen					
Study day 57					
Males	2	-30.0%	No	No	c,d,e
	4	+41.9%	No	No	c,d
Mean absolute CD3e+/CD8a+ counts per whole spleen					
Study day 57					
Males	4	+39.9%	No	No	c
Mean absolute CD3e-/NK1.1+ counts per gram of spleen					
Study day 29					
Males	2	+43.1%	No	No	c,d
Study day 57					
Males	2	-37.0%	No	No	c,d,e
Mean absolute CD3e-/NK1.1+ counts per whole spleen					
Study day 29					
Females	2	+34.5%	No	No	c,d
Study day 57					
Males	2	-31.4%	No	No	c,d,e
Mean absolute CD19+ counts per gram of spleen					
Study day 57					
Males	2	-34.8%	No	No	c,d,e
Mean absolute CD19+ counts per whole spleen					
Study day 29					
Females	4	+33.3%	No	No	c,d
Study day 57					
Males	2	-33.0%	No	No	c,d,e

a Significantly different from control group (group 1) value:

A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)

D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

b Based on interpretation of all available data taking into account individual data variability and/or absence of clear trends.

c Although these changes in mean values are ≥ 30%, they are not considered TA related nor biologically relevant when taking into account individual animal variability and/or range of values obtained in the control group (group 1).

d No difference ≥ 30% was observed in terms of relative percentage of this population.

e Percentage differences are mainly due to decrease of mean absolute lymphocyte counts and mean absolute lymphocyte counts per whole spleen observed for group 2 males at day 57.

- No statistically significant treatment-related effects on mean relative percentages of lymphocytes in spleen; ≥30% differences from controls are presented in the following table (from the original BLA submission):

Summary table of statistically significant differences and/or difference ≥ 30% for the mean relative percentages of lymphocytes in spleen when compared to control group 1

Parameter	Group	Difference vs control group	Statistically significant[a]	Test-article related[b]	Comments
Mean relative percentage CD3e+/CD8a+					
Study day 57					
Males	3	+32.9%	No	No	c

a Significantly different from control group (group 1) value:
A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)
D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

b Based on interpretation of all available data taking into account individual data variability and/or absence of clear trends.

c Although these changes in mean values are ≥ 30%, they are not considered TA related nor biologically relevant when taking into account individual animal variability and/or range of values obtained in the control group (group 1).

Thymus:

- No treatment-related effects due to high inter-individual variability, absence of findings in both sexes within dose groups or observation day, absence of statistical significance, and findings within the control value ranges
- Differences (≥30%) from controls in total lymphocyte counts observed in treated groups are presented in the following table (from the original BLA submission):

APPEARS THIS WAY ON ORIGIN

Summary table of statistically significant differences and/or difference ≥ 30% for absolute lymphocyte counts in thymus when compared to control group 1

Parameter	Group	Difference vs control group	Statistically significant[a]	Test-article related[b]	Comments
Mean total lymphocyte counts					
Study day 57					
Males	2	+38.0%	No	No	c
	3	+53.8%	No	No	c
Mean total lymphocyte counts per whole thymus					
Study day 57					
Males	2	+38.0%	No	No	c
	3	+53.9%	No	No	c

a Significantly different from control group (group 1) value:
A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)
D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

b Based on interpretation of all available data taking into account individual data variability and/or absence of clear trends.

c Although these changes in mean values are ≥ 30%, they are not considered TA related nor biologically relevant when taking into account individual animal variability and/or range of values obtained in the control group (group 1).

- The following statistically significant decreases in absolute lymphocyte counts in thymus in F
  - $CD3e^+/CD4^+/CD8a^+$ at LD and MD
  - $CD3e^+/CD4^+/CD8a^-$ at LD
    - The observed differences from controls are presented in the following table (from the original BLA submission):

APPEARS THIS WAY ON ORIGINAL

Summary table of statistically significant differences and/or difference ≥ 30% for the absolute lymphocyte counts in thymus when compared to control group 1

Parameter	Group	Difference vs control group	Statistically significant[a]	Test-article related[b]	Comments
Mean absolute CD3e+ counts per gram of thymus					
Study day 29					
Females	2	-34.7%	No	No	c,d
	3	-35.9%	No	No	c,d
Mean absolute CD3e+ counts per whole thymus					
Study day 29					
Females	2	-33.4% **A**	Yes	No	c,d,f
Study day 57					
Males	3	+36.2%	No	No	c,d,e
Females	2	-35.1%	No	No	c,d
Mean absolute CD3e+/CD4+/CD8a+ counts per gram of thymus					
Study day 29					
Females	2	-46.1% **A**	Yes	No	c,f
	3	-42.4% **A**	Yes	No	c,d,f
	4	-39.6%	No	No	c,f
Mean absolute CD3e+/CD4+/CD8a+ counts per whole thymus					
Study day 29					
Females	2	-46.0% **A**	No	No	c,d,f
Study day 57					
Males	2	35.9%	No	No	c,d,e
	3	50.7%	No	No	c,d,e
Mean absolute CD3e+/CD4-/CD8a- counts per gram of thymus					
Study day 29					
Females	3	-43.2%	No	No	c,d
	4	-34.3%	No	No	c,d
Study day 57					
Females	2	-30.8%	No	No	c,d

Parameter	Group	Difference vs control group	Statistically significant[a]	Test-article related[b]	Comments
Mean absolute CD3e+/CD4-/CD8a- counts per whole thymus					
Study day 57					
Males	3	+48.4%	No	No	c,e
Females	2	-37.2%	No	No	c,d
	4	-33.2%	No	No	c,d
Mean absolute CD3e+/CD4+/CD8a- counts per gram of thymus					
Study day 29					
Females	2	-48.1%	No	No	c,d
	3	-39.0%	No	No	c,d
	4	-36.3%	No	No	c,d
Mean absolute CD3e+/CD4+/CD8a- counts per whole thymus					
Study day 29					
Females	2	-46.4% D	Yes	No	c,d,f
Study day 57					
Males	3	37.7%	No	No	c,d,e
Females	2	-42.4%	No	No	c,d
	3	-30.4%	No	No	c,d
Mean absolute CD3e+/CD4-/CD8a+ counts per gram of thymus					
Study day 29					
Females	2	-33.4%	No	No	c,d
	3	-30.4%	No	No	c,d

Parameter	Group	Difference vs control group	Statistically significant[a]	Test-article related[b]	Comments
Mean absolute CD3e+/CD4-/CD8a+ counts per whole thymus					
Study day 29					
Females	2	-31.6%	No	No	c,d
Study day 57					
Males	3	+31.4%	No	No	c,d,e
Females	2	-37.6%	No	No	c,d
	3	-35.6%	No	No	c,d

a Significantly different from control group (group 1) value:

A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)

D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

b Based on interpretation of all available data taking into account individual data variability and/or absence of clear trends.

c Although these changes in mean values are ≥ 30%, they are not considered TA related nor biologically relevant when taking into account individual animal variability and/or range of values obtained in the control group (Group 1).

d No difference ≥30% was observed in terms of relative percentage of this population.

e Percentage differences are mainly due to decrease of mean absolute lymphocyte counts per gram and per whole spleen observed for groups 2 and 3 males at day 57.

f Although these changes in mean values are considered statistically significant, they are not considered to be related to 01BSUR administration

- No statistically significant treatment-related effects on mean relative percentages of lymphocytes in thymus; ≥30% differences from controls are presented in the following table (from the original BLA submission):

Summary table of statistically significant differences and/or difference ≥ 30% for the mean relative percentages of lymphocytes in thymus when compared to control group 1

Parameter	Group	Difference vs control group	Statistically significant[a]	Test-article related[b]	Comments
Mean relative percentage CD3e+/CD4+/CD8a+					
Study day 29					
Females	2	-30.1%	No	No	c,d
	4	-34.1%	No	No	c,d

a Significantly different from control group (group 1) value:

A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)

D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

b Based on interpretation of all available data taking into account individual data variability and/or absence of clear trends.

c Although these changes in mean values are ≥ 30%, they are not considered TA related nor biologically relevant when taking into account individual animal variability and/or range of values obtained in the control group (group 1).

Immunogenicity:

- No anti-drug antibody found
- False negative results could be possible due to high 01BSUR plasma levels which could interfere with detection of anti-drug antibody; however confirmatory assays did not repeat any positive results

T-cell Dependent Antibody Response (TDAR):

- Statistically significant inhibition of anti-KLH IgG response at LD (M) due to unusually high response in 2 control M
- Statistically significant, 61% inhibition of anti-KLH IgG response at MD and HD (M, Day 29), due to unusually high response in 2 control M
- Increased response at LD, MD, and HD in F due to unusually high variability among treated animals
- No treatment-related changes in anti-KLH IgG response in the recovery animals
- No treatment-related changes in anti-KLH IgM response

Gross pathology: No treatment-related effects

Organ weights:

- Slight increase in absolute and relative thymus weights in F in the main study phase at MD
    - Not observed in recovery animals
    - Without macroscopic and histopathology correlates
    - Not statistically significant
    - Relationship to treatment unlikely

Histopathology:

- No treatment-related findings in the mandibular, mesenteric and inguinal lymph nodes, spleen and thymus
- Sporadic findings of dendritic cell hyperplasia with adjacent marginal zone lymphoid hyperplasia, without dose-relationship, of low incidence, and observed in 1 recovery control F; therefore not considered treatment-related

Toxicokinetics:

- Dose-related increase in exposure
- Low serum clearance (3.66 ml/kg/d)
- Low V_d (89.4 ml/kg), reflecting little extra-circulatory distribution
- Long terminal $t_{1/2}$ (16.9 d), probably due to protection from intracellular catabolism by FcRn binding and recycling back into circulation
- No differences in TK parameters between males and females

The results of the TK analyses are presented in the following table (from the original BLA submission):

Dose (mg/kg)	Gender	T_{max}(h)	C_{max} (µg/mL)	C_{max}/D (µg/mL)/(mg/kg)	$AUC_{428-457}$ (µg.hr/mL)	$AUC_{428-457}$/D (µg.hr/mL)/(mg/kg)
10	Female	168	329	33	184164	18416
	Male	24	408	41	205956	20596
50	Female	24	1810	36	591588	11832
	Male	24	1990	40	696816	13936
150	Female	24	3880	26	1304400	8696
	Male	24	4610	31	1508640	10058

01BSUR compartmental pharmacokinetic parameters in albino mice after repeat subcutaneous injection of ACZ885 surrogate (01BSUR)

Model	Parameter	Value
1 compartment	CL (mL/kg/day)	3.66
	V (mL/kg)	89.4
	Ka (1/day)	0.976 (fixed)

2.6.6.9 DISCUSSION AND CONCLUSIONS

The Applicant conducted the appropriate nonclinical studies to support the clinical safety of an indication for subcutaneous canakinumab treatment of CAPS in patients ages ≥ 4 years old at the proposed dose of 150 mg in patients weighing more than 40 kg and 2 mg/kg SC in patients weighing 40 kg or less, once every 8 weeks. The nonclinical studies were of sufficient duration and included adequate recovery period evaluations, used the same, subcutaneous route of administration intended for clinical use for the general toxicology studies, as well as the intravenous route for chronic investigation to support the safety of chronic clinical treatment. Immunogenicity assessments were included in all toxicology studies and in the batch comparison studies during product development in support of the interpretation of study results. The nonclinical study program was conducted in accordance with Agency recommendations during product development and in agreement with ICH guidelines (Refer to the Guidance for Industry – Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals; ICH S6 (Jul 1997), Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (Feb 1997); Leader B., et al. 2008. Protein therapeutics: A Summary and Pharmacological Classification. Drug Discovery 7:21-39; Chapman K., et al. 2007. Preclinical Safety Testing of Monoclonal Antibodies: The Significance of Species Relevance. Nature Reviews – Drug Discovery 6:120-126; and Woodcock J., et

al. 2007. The FDA's Assessment of Follow-on Protein Products: A Historical Perspective. Nature Reviews – Drug Discovery 6:437-442).

Chronic and sub-chronic toxicology was investigated in marmosets administered ACZ885 by subcutaneous injection (SC) for 3 months, and by intravenous injection (IV) for 6 months. The 6-month chronic toxicology study in marmosets, the species selected to be most appropriate for use in the toxicology studies, is acceptable to support chronic clinical ACZ885 treatment in accordance with the Guidance for Industry – Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals; ICH S6 (Jul 1997) due to the in the absence of concerns raised with respect to the mechanism of action and absence of findings in the toxicology studies of shorter duration that could indicate a signal for a treatment duration-related increase in risk of toxicity. Additionally, the Applicant received prior concurrence from the Agency to conduct the chronic nonclinical toxicology study, in one relevant species with a 6-month duration. Studies on Safety Pharmacology, Genetic Toxicology and Carcinogenicity are generally considered to be of little use for the registration of most biological drug products in the absence of such concerns, and therefore were not recommended by the Agency for submission of the canakinumab BLA for marketing, nor were conducted by the Applicant.

No effects on QT interval were found in the ECG analyses conducted in the general toxicology studies on ACZ885 in marmosets, and ACZ885 binding in heart tissue was weak in the tissue cross reactivity studies in human and marmoset tissues. Due to highly selective and exclusive binding to the IL-1β receptor, little or no interaction with hERG channels is expected. Renal toxicity by canakinumab is unlikely due to absence of glomerular filtration of the large protein molecule. Regarding carcinogenic potential, no canakinuamb interactions with DNA and chromosomal material are expected. The large canakinumab protein requires active transport by FcRn for cellular uptake, a mechanism that is not available at the nuclear membrane. No direct DNA binding is anticipated, canakinumab is not electophilic, and has no active metabolites. However, the hypothetical potential for increased incidence of malignancy due to immunosuppression with increased duration of treatmnet, observed for several other immunosuppressive drugs, should be noted.

The Applicant provided appropriate support for the selection of marmoset for the toxicology studies with results from comparability and bridging studies, cross-reactivity in normal human and marmoset tissues, and comparative pharmacokinetics in humans and marmosets. Comparative PK/PD evaluation was performed for drug substance produced by different cell-lines as a result of manufacturing changes during the course of drug development. As recommended in the ICH Guidance, the Applicant assessed the relative sensitivity of marmoset, mouse and human to canankinumab *in vitro*, and determined receptor occupancy, affinity and pharmacological effects for species selection and to support extrapolation of the results of the toxicology studies to findings in the clinical studies. The results of the PK/TK analyses demonstrated comparable pharmacokinetic profiles in marmosets, mice and humans, including absorption characteristics, relative exposures, distribution and target tissues (with IL-1B receptor presence). Canakinuamb undergoes endosomal proteolysis and does not interact with

cytochrome P450 isoenzymes, therefore producing no metabolites. The volume of distribution in marmosets, as in humans was approximately equivalent to blood volume. An appropriately developed homologous agent, mouse anti-mouse IL-1β surrogate 01BSUR was evaluated in the reproductive toxicology, juvenile toxicity and immunogenicity studies in mice, and validated using the results of pharmacokinetic and biologic activity comparison with ACZ885 in separate studies.

The potential for immunotoxicity is a concern for most biotechnology-derived drugs. Although there was little evidence for ACZ885-induced immunomodulation in the nonclinical studies conducted for this submission, an increased risk for immunosuppression possibly leading to increased infection and potential for malignancies should be monitored in the clinical setting. Increased incidences of malignancies and infection, and anti-drug antibody formation have been demonstrated for other immunosuppressive products, IL-1β, monoclonal antibodies and Fc fusion proteins. The evidence for immunosuppression by ACZ885 was somewhat inconsistent across the nonclinical studies and not strongly compelling.

The potential for formation of anti-drug antibodies in the nonclinical studies on ACZ885 was minimized by appropriate species selection, using marmoset which showed identical pharmacokinetics and pharmacodynamic properties to those in humans for evaluation of general toxicology and embryo-fetal development, and by using an appropriate surrogate model with CD-1 mice administered 01BSUR, that shows identical biologic effects in mouse to those in humans.

The results of the 4-week SC tolerability study in female marmosets showed slight hyperplasia (spithelial and Peyer's patch) with marked lymphocytic infiltrate and minimal abscess in one of four high-dose (150 mg/kg/twice weekly) females, slight leukocytic infiltrate in the gall bladder in one of four mid-dose (50 mg/kg/twice weekly) and one (of four) high-dose females, and anemia and inflammatory leukocytosis with increased lymphocytes, monocytes and eosinophils in one mid-dose female, that may reflect ACZ885-related immune suppression. Also, there was a dose-related (15-150 mg/kg/twice weekly) increase in minimal lymphoid hyperplasia of the spleen (large active follicles) in treatment and recovery male marmosets, in the 13-week SC toxicity study with 8-week recovery period, in the absence of treatment-related effects on phenotyping of splenic suspensions and blood samples, changes in anti-drug antibody levels and any effects in the female animals and in the other general toxicology studies, including the longer-duration 6-month IV toxicity study in marmosets. A possible relationship of the findings to a vehicle effect (180 mM sucrose in Sterile Water for Injection, 10 mM L-histidine, 1.0 ml 2% (m/v0 Tween 20 aqueous solution, 1N HCl to adjust pH to 5.5) was suggested by the results of the fertility study (Segment I Reproductive Toxicology) in CD-1 mice administered 01BSUR. In that study, the necropsy examination of the F_0 dams showed spleen enlargement in all groups including controls (6/22, 9/22, 5/22, and 9/22 at 0, 15, 50, nad 150 mg/kg, respectively, and in 1 of 22 F_0 males given 01BSUR at the mid-dose of 50 mg/kg. Although different vehicle components were used in the 13-week toxicity study in marmosets, and consisted of 30 mM L-histidine, 9.2% (m/v, 2780 mM) D-sucrose and 0.06% (m/v) Polysorbate 80, the

results of the fertility study show splenic enlargement related to factors other than test article effect. Furthermore, lymphoid depletion and extramedullary hematopoiesis were found in 1 control marmoset each in the control group in the 13-week toxicity study, and has been observed in the control animals in the historical data for the laboratories. Spleen size was monitored in clinical study CACZ8852201, with no resulting treatment-related findings. Although a possible relationship of the findings in spleen, to treatment-related immune system effects in that study is not compellingly strong, the potential cannot be ruled out. Given that this effect suggested treatment-relationship in the one study in the females, only, and was not found in the other nonclinical studies, and was observed without correlating treatment-related effects on phenotyping of splenic suspensions and blood samples, and any changes in anti-drug antibody levels, the level of concern is lower that there was a direct relationship to treatment with ACZ885. However, it is appropriate that an adequate and clearly stated warning be conveyed in the product label, and adequate monitoring for indices of immunosuppression be conducted during clinical treatment.

One male marmoset given ACZ885 produced by the ——— (used in the original nonclinical studies and in the early clinical trials) at 150 mg/kg twice weekly for 13 weeks showed septicemia with inflammatory cell infiltration in the intestinal mucosa, intestinal mucosal ulceration with neutrophil loss and neutrophilic inflammation with bacteria present throughout the body. This finding was in the absence of any other evidence of immunosuppression in that study. A relationship to dose could not be determined, because only one dose, 150 mg/kg twice weekly was evaluated for each formulation (produced by ———). There were no findings suggesting treatment-related infection in the marmosets given ACZ885 produced using the ——— to-be-marketed formulation) at the same dose, nor in the other 4-13 week subcutaneous and 26 week IV toxicology studies, and in the embryo-fetal toxicity study (Segment II) study in marmosets. In the intra-articular local toxicity study, there was a very slight decrease in lymphocytes (mean 41% vs. 64% at baseline) and increase in neutrophils (mean 58% vs. 35% at baseline), in addition to low WBS, anemia and increased reticulocytes in one of 3 female marmosets given the only dose tested (10 mg/kg). Enlarged adrenals and kidneys with histological evidence of cortical hypertrophy and inflammatory cell foci with fibrosis and atrophic tubuli were found in one of the marmosets, and swollen finger with abscesses and acute inflammation of the bladder in another marmoset in that study. These findings were equivocal, however, for treatment-related toxicity via immunosuppression-induced inflammation and infection through systemic exposure from the intra-articular injection site, because the findings were were within historical background range. No anti-ACZ885 antibody formation was detected in any study in marmosets.

b(4)

b(4)

There were several, though sporadic treatment-related findings in the nonclinical studies that could be secondary to immunosuppression by ACZ885, that are considered to be equivocal. The results of the study on pre- and post-natal development (Segment III) in mice revealed splenic enlargement in 2 of 25 F_0 generation dams, with lymphoid hyperplasia and increased extramedullary hematopoiesis in one dam. There was a slight increase in incidence of histiocytosis in the mandibular (3/9 at 50 mg/kg/week and 4/9 at

140 mg/kg/week) and mesenteric (2/10 at 50 mg/kg/week and 3/10 at 150 mg/kg/week) lymph nodes in the F_1 adult males in that study, but not in the females or in the F_0 dams and F_2 generation pups. No treatment-related effects were uncovered in the F1 immunology assessment of blood, thymus, and spleen (weights, immunophenotyping, lymphocyte subsets) in that study. On the other hand, there were no effects suggestive of immunotoxicity in the juvenile toxicity study in CD-1 administered 01BSUR at doses of up to 150 mg/kg/week from post partum Days 7-70, with a 4-week treatment-free recovery period.

A 28-day SC injection study with 28-day recovery period was conducted in CD-1 mice given 01BSUR to specifically to address the concerns regarding potential ACZ885 immunotoxicity. The methodology in that study included immunophenotyping (blood, spleen, thymus), immunogenicity, and measures of T-cell dependent antibody response (TDAR), lymphocyte subpopulations in the main and recovery animals, anti-KLH IgM and anti-KLH IgG responses in main and recovery animals, toxicokinetic analyses, organ weights, and gross and histopathology examinations of the spleen, thymus and lymph nodes. The study methodology included testing for anti-drug antibody formation. The results showed inhibition of anti-KLH IgG response at 10 and 61% inhibition at 50 and 150 mg/kg/week on Day 29 in the M, that appears to be a result of comparison with an abnormally high response in the control M group. The anti-KLH IgG response was increased in the treated F, but in the context of extremely high variability, and there were no differences from controls in anti-KLH IgG response in the recovery animals. There were no effects in the immunophenotyping assays, lymphocyte counts and mean relative percentages in blood, spleen, and thymus, no treatment-related immunogenicity/anti-drug antibody, and no T-cell dependent anti-KLH IgM response. In the necropsy examinations, there were no changes in gross pathology, organ weights and histophathology findings, particularly in lymph node tissues, that could be conclusively attributed to treatment. Treatment-related effects on IgG response as a result of hypersensitivity reaction and complement activation can neutralize drug activity, but there were no effects on drug exposure in the TK analyses, in this study. Evidence for an immunosuppression-related basis for these findings was equivocal.

The approaches to immunogenicity study methodology are still under development and not yet fully understood or established from a commercial and a regulatory point of view, and the results of nonclinical immunogenicity evaluation do not always predict the occurrence of treatment-related immune responses to biological agents in humans. For example, life threatening cytokine storm was observed in the first human patients given TGN1412 (anti-CD 28 super-agonist mAb), in which full cross-reactivity had been demonstrated in the non-human primate used for toxicologic testing (Suntharalingam, G. et al. Cytokine storm in a phase I trail of the anti-CD28 monoclonal antibody TGN1412. N. Engl. J. Med. 355, 1018-1028 (2006); cited in a review article by Chapman, K. et al. Preclinical safety testing of monoclonal antibodies: the significance of species relevance. Nature Perspectives 6, 120-126 (2007). Patients in the clinical trials on canakinumab were monitored for potential immunosuppression and/or inflammatory reactions in the clinical studies on canakinumab during product development.

No target organs of toxicity were conlusively identified in the general toxicology studies in marmosets. There were deaths in two of four male marmoset cagemates, but not in the female marmosets given ACZ885 produced by the —— . There were no deaths in the group given ACZ885 produced using the —— for the current, to-be-marketed drug substance at the same 150 mg/kg/twice weekly dose for 13 weeks in the Subcutaneous Batch Comparison Study. There were signs indicating probable septicemia in one, and lymphoid depletion in the other decedent. Additional findings in the marmoset with septicemia were mixed inflammatory cell infiltration in the intestinal mucosa and neutrophilic inflammation with bacteria throughout the body. There was a similar finding without mortality in a control female, and according to historical data these effects have been observed in control marmosets in previous studies in the laboratory. In agreement with the Applicant, the death can be attributed to septicemia secondary to intestinal ulceration, and the relationship to treatment is doubtful. There were no other deaths in the general toxicology studies at comparable ACZ885 exposures in marmosets, including the 6-month IV toxicity study. However, the marked thymus lymphoid depletion in the other decedent is possibly treatment-related.

b(4)

In contrast, the results of the SC and IV toxicology studies conducted on Rilonacept (Interleukin-1 Trap [IL-1 Trap], approved for the Treatment of CAPS, BLA 125249, Pharmacology Toxicology Reviewer Mamata De, Ph.D.), conducted in cynomolgus monkeys for durations of up to 6 months, showed treatment-related deaths, clinical signs (e.g., lethargy, emesis), and target organ toxicity in the injection site, heart, kidney, lung, reproductive organs and immune system. Rilonacept administration resulted in histopathologic findings of mono/polymorphonuclear cell tissue infiltration (macrophages, eosinophil, plasmocytes and lymphocytes), arteritis, cellular degeneration, granuloma formation, and mineralization. These findings were attributed by Dr. De to possible proinflammatory reaction due to immune complex deposition or hypersensitivity vasculitis associated with mono and polymorphonuclear cell infiltration (e.g., eosinophil), to increased infection due to immunosuppression, or to exaggerated pharmacology of IL-1 Trap.

As previously discussed above, the CD-1 mouse was used to investigate ACZ885 reproductive and developmental toxicology, using the surrogate antibody 01BSUR. Additionally, a second embryo-fetal toxicity study was conducted in marmosets administered the fully human antibody ACZ885. Potential effects on male reproductive system were also addressed in the 26-week intravenous administration toxicity study with 6-month recovery period in marmosets. A similar role for IL-1 has been shown in mouse and human reproductive systems, with comparable endometrial expression of IL-1$_\alpha$ and IL-1β secretion during implantation. Disruption of IL-1 signalling alters implantation, trophoblast invasion and decidualization in mice. The Applicant provided adequate evidence of the comparability of 01BSUR PK and biological function in mice to that of canakinumab in humans, in studies conducted separately. The results of the reproductive and developmental toxicology studies using 01BSUR in mice and ACZ885 in marmosets showed no evidence of adverse canankinumab effects on male and female fertility (Segment I), major embryo-fetal malformations (Segment II), and pre- and post-natal neurobehavioral development (Segment III). No ACZ885-related effects were found on

male marmoset sperm count, motility, and morphology, and on testicular size, circulating male reproductive hormones and in the microscopic examination of testicular tissue in the 6-month toxicology study.

Maternal toxicity was evident in the Segment III study in CD-1 mice however, with deaths in 2 F_0 dams on post partum days 13 and 17. The histopathology examination in the decedents revealed splenic enlargement and lymphoid hyperplasia, with pale discoloration of spleen in one dam, and liver enlargement with increased extramedullary hematopoiesis in the other dam. There were slight ACZ885-related effects on maternal reproductive function in the Segment II study in marmosets, with slight decreases in mean placental weights (-22% vs. controls) and numbers of fetuses per litter (-24% vs. controls), resulting in a reduction in numbers of offspring at the highest dose tested (140 mg/kg/twice weekly during gestation days 25-109. However, the decreases are considered to be likely due to a reduced number of triplets, and more singlets and twin pregnancies at that dose, because there was no decrease in the number of fetuses vs. the number of embryos present at Day 50 in the ultrasonography assessments and there were no other effects on reproductive behavior or function in the maternal marmosets.

Embryofetal findings in the Segment II studies in both mice and marmosets suggested slight developmental delay. These observations included incomplete ossification of the parietal bones at 50 (MD, 11.3% vs. control of 2.8%) and 150 mg/kg (HD, 24% vs. control incidence) and frontal bones at 150 mg/kg (18.3% vs. control incidence) 01BSUR in the CD-1 mice on gestation days 6, 11, and 17, in the absence of maternal toxicity. The incidences of incomplete ossification in the MD and HD groups were also higher than the historical control ranges of 0%-4.2% for the laboratory. The findings were similar in the Segment II investigation in marmosets; and included slight increases in incomplete vertebral ossification compared to controls in all treated groups in the skeletal examination. In no study were treatment-related effects on fetal growth (weights, measurements, organ weights) or any external, visceral or major skeletal malformations found. Further, no effects of maternal treatment on pup (F_0 and F_2) and adult offspring physical development, behavioral performance (e.g., motor activity, startle habituation, passive avoidance), reproduction, and immunology assessments were detected, with exposures in the mouse pups as high as 6 times those in the treated dams.

In comparison, there were multiple treatment-related findings in the reproductive toxicity studies conducted on Rilonacept (IL-1 Trap, BLA 125249, refer to Pharmacology Toxicology review by Mamata De, Ph.D.) in mice given a surrogate IL-1 Trap molecule (Segments I and III) and cynomogus monkeys administered human IL-1 Trap (Segment II). The results included treatment-related decreased fertility index in male and female mice, early resorptions at all doses, post implantation loss, increased abortion and still born pups, presence of IL-1 Trap antibodies in F_0 males, and in the Segment III study, 8-fold increase in F_1 pup and F_2 litter deaths. Increaased skeletal variation in lumbar vertebrae and late pregnancy spontaneous abortions were noted at all doses in the monkeys. Most of the findings in the reproductive toxicity battery of studies were observed in all dose groups, providing either very low or no safety margins for potential

adverse effects on fertility, embryofetal development and pre- and postnatal development in clinical use.

Adequate ACZ885 and 01BSUR exposures were supported in all of the reproductive toxicology studies with the results of serum sampling in the F_0 adult males and females, and in the F_1 and F_2 (Segment III study) fetuses and pups. Complete toxicokinetic analyses were conducted in the Segment II studies in mice and marmosets, and used to estimate the AUC exposures for comparison with the proposed clinical doses, in the Segments I and III studies in mice. The results of evaluations for anti-ACZ885 and anti-01BSUR antibody formation were negative in all of the reproductive toxicology studies. The issue of interspecies differences in metabolism, and consequently the potential for qualitatively or quantitatively different exposure to metabolites with potential reproductive toxicity in the species tested (mice and marmosets) and humans is not of concern, because ACZ885 and 01BSUR undergo protolytic degradation without cytochrome P450 isoenzyme interaction or formation of metabolites.

A subcutaneous injection juvenile toxicology study was performed in CD-1 mice using 01BSUR, in support of the proposed subcutaneous canakinumab indication for treatment of CAPS in pediatric patients ages 4 and above. The results uncovered no evidence of serious ACZ885 effects on behavioral, developmental, learning and memory and reproductive effects in juvenile mice treated for 9 weeks at doses of 15-150 mg/kg/weekly for 9 weeks, from post partum days 7-70. There were minor but statistically significant increases in several measures of adverse post-natal developmental effects in the juvenile mice, which are considered not to be toxicologically relevant for several reasons. For example, in the pre-weaning physical development evaluation, a slight increase was observed in the mean day of auricular startle at the mid-dose (50 mg/kg/week) in the male pups and high-dose male and female pups compared to control pups before post-partum day 21, although the results were similar to control values in another study, were within historical range for the laboratory, and there were no treatment-related effects seen in the post-weaning evaluation of auditory startle habituation. Also, the post-weaning physical development assessments found a slight but statistically significant increase in the mean number of days to vaginal opening in the adult high dose (150 mg/kg/week) females compared to concurrent controls; however, the mean day of opening was well-within the historical control range for the performing laboratory. A statistically significant increase in pre-implantation loss (group mean 20.38%) at the high dose compared to concurrent (4.26%) and historical (7%-12.8%) controls, in the fertility assessment of dams that had received treatment as juveniles can be attributed to a 74% loss in one of 20 female mice. Also, the mean number of live embryos in the HD group (12.8) was within the historical range for the laboratory, of 11.6-13.0. Therefore, the results of this study are considered to be equivocal for adverse effects on juvenile development by 01BSUR.

Studies to evaluate genetic toxicology and carcinogenicity are generally not required for biologic drugs unless there are special concerns based on tissue cross-reactivity and mechanism of action study results, and/or findings in the general toxicology studies suggestive of potentially genotoxic or carcinogenic potential. Genetic toxicology and

Carcinogenicity studies were not conducted on ACZ885, in prior agreement with the Agency. Canakinumanb does not cause signal transduction, is not a growth factor or hormone, contains no non-peptide chemical linker molecules, produced no cell toxicity *in vitro*, and is not expected to cause cell proliferation or DNA damage. Canakinumab, a large molecular weight glycoprotein is degraded by lysosomal proteolysis or recycled to the cell surface bound to FcRn without nuclear interaction. No tumors were found in the histopathology examinations in the nonclinical studies of duration up to 6-months in marmosets, and there was no evidence of chronic inflammation. However, an increased risk of tumor proliferation via immunosuppression rather than by direct genotoxic effect in humans cannot be ruled out, and should be clearly conveyed in the product label.

In conclusion, the nonclinical study program was adequate to support clinical safety of canakinumab administration according to the general recommendations in the ICH Guidance to Industry and in agreement with Agency recommendations and concurrence during product development. The methodology used was appropriate, well-validated, and in agreement with the ICH Guidelines and Agency concurrence. Canakinumab does not interact with cytochrome P450 enzymes and thus a risk for drug-drug interactions is low. There were no effects on the QT interval in the electrocardiography assessments in marmosets, the binding affinity in human cardiac tissues was very low in the cross-reactivity studies in normal human tissues, and canakinumab is unlikely to have an effect on hERG channels due to highly specific binding to IL-1β. Canakinumab clearance is not expected to be affected by hepatic or renal impairment, due to clearance by endosomal catabolism and absence of renal glomuerular filtration because of the large size of the protein molecule. There were no effects of age and/or sex suggested by the nonclinical study results. No drug-drug interactions are expected.

The potential for hypersensitivity and/or adverse effects such as increased risk of infection and/or malignancies related to immunosuppression remain a concern, although the evidence for these effects was equivocal in the nonclinical toxicology studies, and there were no findings in the Immunotoxicology Study conducted CD-1 mice. No concerns were raised with respect to mechanism of action. Although there was no evidence of serious, primary target organ toxicity by ACZ885 and surrogate antibody 01BSUR in the nonclinical general toxicology studies, several minor nonclinical safety issues relevant to clinical use were raised. These issues include potential risks related to immuotoxicity, and slight adverse effects on the unborn fetus and developing juvenile, in the absence of serious ACZ885- and 01BSUR-related effects on fertility, major malformations or pre- and post-natal neurobehavioral development. These risks should be adequately discussed in the product label, and appropriate clinical monitoring and preventative measures are warranted. Additionally, a residual solvent that is known to present a serious risk in the newborn was identified in the drug product intended for clinical treatment, but is expected to be eliminated prior to formulating the drug substance. There are no concerns at this time with regard to the excipients in the proposed formulation.

The risk of immunotoxicity, particularly for hypersensitivity reactions by biotechnology-derived pharmaceuticals is a common concern regarding biotechnology-derived drugs,

and has generally been shown to increase with dose, duration and frequency of administration, particularly when given by the subcutaneous route. Evidence for ACZ885-induced suppression of immune function in the nonclinical studies was inconsistent across studies, and without correlative within-study indices of quantitative and qualitative immune cell alterations. Because ACZ885 is a fully human monoclonal IgG/K antibody against IL-1 $_{B}$, a risk of toxicity secondary to immunosuppression, such as infection and/or malignancy may be increased, however, and should not be ruled out based on the results of the nonclinical studies on ACZ885 and 01BSUR. Increased incidence of malignancies and infection, and anti-drug antibody formation have been demonstrated in nonclinical and clinical studies on several other, less selective immunosuppressive products, monoclonal antibodies and Fc fusion proteins.

There were several treatment-related effects in the Embryo-Fetal Development studies in mice given 01BSUR and in marmosets administered ACZ885 that point to a slightly increased risk for delayed fetal development, although there were no major malformations were observed in these studies. Slight developmental delay was suggested by findings of treatment-related increases in incomplete ossification of the parietal and frontal bones compared to concurrent and historical control incidence in mice, and slight treatment-related increases in zygostyle, incomplete vertebral ossification, misaligned and/or bipartite compared to concurrent control findings (historical control data for the laboratory unavailable) in the marmosets.

The results of the juvenile development study in CD-1 mice administered 01BSUR showed no major, adverse treatment-related effects on behavioral, developmental, learning and memory, and reproductive measures. However, there were minor, but statistically significant treatment-related changes in some measures of pre-weaning and post-weaning physical development, and in reproductive performance of adult mice that were administered 01BSUR as juveniles (from age 7-70 days), that should be noted as equivocal. Slight, but statistically significant increases were observed in the mean day of auricular startle in the pre-weaning evaluation, and in the mean number of days to vaginal opening and pre-implantation loss in the adult females given 01BSUR as juveniles. The increase in mean day of auricular startle was similar to controls in another study in CD-1 mice, within historical control range for the laboratory, and without a corresponding effect in the post-weaning evaluation of auditory startle habituation. The mean day of vaginal opening was within historical control range for the performing laboratory. Pre-implantation loss was significantly increased compared to both concurrent and historical controls, although there was a 74% loss in one of the females in the affected group that is considered to have biased the assessment. Therefore, these effects did not provide a strong signal for juvenile toxicity, but do suggest a need for appropriate assessment of risk-benefit and adequate monitoring for signs of delays in physical and/or behavioral development in the treatment of pediatric patients with canakinumab.

Approximate exposure margins for the human antibody ACZ885 relative to the recommended human dose of 150 mg or 2 mg/kg in patients ≤ 40 kg in weight are presented in the following table (by the reviewer):

Exposure Margins in the Nonclinical Studies on ACZ885 and 01BSUR

Species	Study	Doses (all w/ vehicle controls)	Target Organ Findings	Dose at NOAEL	AUC_{0-t} (mcg.h/ml) mean♂+♀	Multiple of Clinical AUC[b]
Marmoset	26-wk IV Toxicity	10, 30, 100 mg/kg ACZ885 2X weekly	None	100 mg/kg	86,978 (0.083-24h, Wk 23) Equiv. AUC over 0-8 wks = 4,872,000[b]	(5X) 287X
Marmoset	13-week SC Toxicity	15, 50, 150 mg/kg ACZ885 2X weekly	-Dose-related spleen hyperplasia ♂s no findings in phenotyping, ♀s or other gen. tox. & clin. studies, probably 2° to possible immunosuppression	-If treatm. related: none -w/o spleen effects: 150 mg/kg	(Wk 14) 402430 (0-1368h)	24X
Mouse	Fertility	15, 50, 150 mg/kg SC 01BSUR 1X weekly[e]	None	150 mg/kg	95585 (0-120h, 1st dose)[f]	NA[g]
Mouse	EFT	15, 50, 150 mg/kg/wk SC 01BSUR (GD 6,11, 17)[e]	-No major malformations. -Developmental delay: incompl. ossification parietal (at 50 & 100 mg/kg) and frontal (100 mg/kg) bones	EF toxicity: 15 mg/kg Teratogenicity: 150 mg/kg	Dev. Delay: 6436: (0-120h) Teratogenicity: 95585 (0-120h)[b]	NA[g]
Marmoset	EFT	15, 50, 150 mg/kg/2X weekly SC ACZ885 (GD25-109)	-No maj. malformations -↑Bent/kinked tail at 150 -↑Developmental delay: incompl. vertebral ossification in all treated	Embryotoxicity: not identified Major malformations: 150 mg/kg	(Day 109) 141,000 (0-48h) Equivalent AUC over 0-8 wks = 3,950,000[b]	Major malformation: 232X
Mouse	PPD	15, 50, 150 mg/kg/wk SC 01BSUR (GD6-PPD 21)	-Slight ↑histiocytosis mandibular,mesenteric lymph nodes in F_1 adult ♂ at 50 and 150, without lesions	For pre- and post-natal development 150 mg/kg	95585 (0-120h, 1st dose)[f]	NA[g]
Juvenile Mouse	SC Juvenile Toxicity	15, 50, 150 mg/kg/wk SC 01BSUR (PPD 7-70)	-Slight delay day of vaginal opening at 150 (w/in historical range) -Slight ↑day auricular startle development at 50&150 (w/in historical range) -↑pre-implantation loss at 150 (due to loss in 1 dam), w/in historical range)	For major behavior, developmental, learning, memory, reprod. effects: 150 mg/kg	(Day 63) 154,000 (0-72h)	NA[g]
Mouse	28-Day SC Immunoto.	10, 50, 150 mg/kg/wk SC 01BSUR	None	150 mg/kg	(Day 25-57) 1,406,520 (D28-57)	NA[g]

[a] For detailed description of the TK analyses, refer to the original study review, above under Section 2.6 Pharmacology Toxicology Review

[b] Relative to approximately equivalent AUC_{0-inf} values of approximately 17,000 mcg/h/ml in adult CAPS patients treated by SC injection at 150 mg/once every 8 weeks, and AUC_{0-inf} value of approximately 14,400 mcg.h/ml in pediatric patients at 2 mg/once every 8 weeks; AUC values for the nonclinical exposures at the NOAEL were extrapolated to reflect approximate values over 0-8 weeks, compared with the values provided for clinical exposure

[c] Based on proposed clinical dose 150 mg in a 70 kg patient and 2 mg/kg in patients ≤ 40 kg in weight

[d] — lyophilisate ACZ885 produced by the — used in toxicology studies and early clinical trials; — lyophilisate ACZ885 produced by — after production changes, to be marketed substance

b(4)

[e] For detailed methodology, refer to the original study review, above, under Section 2.6.6.6 Reproductive and Developmental Toxicology

[f] Based on results of EFT study TK analyses in maternal mice administered 0, 15, 50, and 150 mg/kg 01BSUR on gestation days 6, 11, and 17
[g] NA = not applicable

OVERALL SUMMARY, CONCLUSIONS AND RECOMMENDATIONS:

Background

Canakinumab, also called NVP-ACZ885-NX-1; ACZ885; ACZ885-NXA; and ACZ885 antibody during product development, is a highly specific, high affinity, fully human monoclonal IgG1/κ antibody against interleukin-1 beta (IL-1β). The proposed canakinumab indication is for the treatment of Cryopyrin Associated Periodic Syndromes (CAPS), including Familial Cold-Induced Autoinflammatory Syndrome/Familial Cold-Induced Urticaria (FCAS), Muckle-Wells Syndrome (MWS) —— b(4)

—— by subcutaneous (SC) injection at the doses of 150 mg in patients weighing more than 40 kg or 2 mg/kg SC in patients weighing 40 kg or less, once every 8 weeks. The rationale for development of this drug product to treat CAPS is the potential neutralization of cytokine activity believed to underlie symptoms and progression of CAPs, by inhibiting IL-1β activity which has demonstrated upregulation in systemic autoinflammatory disorders (Arend WP. The balance between IL-1 and IL-1Ra in disease. Cytokine Growth Factor Rev. 13:405, 323-349 (2002); Agostini L. *et al.* NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. Immunity 20(2), 319-325 (2004); Dinarello CA. Blocking IL-1 in systemic inflammation. J. Exp. Med. 201(9), 1355-1359 (2005)). The Applicant provided an argument for the hypothesis that canakinumab may have a lower potential for induction of pro-inflammatory reactions, or serious infections and/or increased incidence of malignancies due to immunosuppression than might be associated with less specific IL-1 receptor blocking agents. This argument is supported by evidence presented in the published literature that IL-1β may advance tumor development via inflammatory activity, while IL-1α may promote anti-tumor activity (Apte RN, *et al.* The involvement of IL-1 in tumorigenesis, tumor invasiveness, metastasis and tumor-host interactions. Cancer Metastasis Rev. 25(3), 387-408 (2006)). Additionally, canakinumab half-life is considerably longer than that of the lower-specificity IL-1 receptor blocking agents currently used in the treatment of CAPS, therefore providing a longer duration of action and requiring less frequent injections compared to currently available treatments.

Manufacturing

Canakinumab, derived using hybridoma technology was developed using —— immunized with recombinant human IL-1β alone or conjugated to keyhole limpet hemocyanine (KLH). The —— carry part of the human IgG repertoire, and the hybridomas derived from the —— express fully human antibodies. ——

b(4)

——. Product A (lyophilisate formulation for the drug substance produced by the ——) was evaluated in the early clinical trials in CAPS patients and in the general toxicology, embryo-fetal development (Segment II Reproductive Toxicology) and tissue cross-reactivity studies in marmosets. Product B (lyophilisate formulation for the drug substance produced by the ——, also referred to as ——

[redacted] was used in the study to investigate single dose, local intra-articular toxicity and pharmacokinetics in marmosets, and in the Phase 3 clinical studies in CAPS patients. Product C [redacted] b(4)
[redacted], and the liquid formulation [redacted], were used in the later clinical trials in CAPS patients and to evaluate comparative tissue cross-reactivity in humans and marmosets. Product D [redacted], is the substance intended for marketing. Products A-D are considered to be comparable by Product reviewer, based on structural characterizations identified by physiochemical and biological testing. Products A-C were shown to be comparable in *in vitro* and *in vivo* bridging pharmacokinetics (PK) studies in marmosets and in the tissue cross-reactivity studies in humans and marmosets, and in the clinical PK and pharmacodynamic (PD) studies conducted during product development. Although no nonclinical bridging studies were conducted specifically using Product D, comparability of Product D with Products A-C was supported based on manufacturing and structural characterizations, biological testing, immunotoxicity and potential formation of anti-drug antibody. The manufacturing changes were conducted in agreement with European regulatory authorities and with FDA Agency recommendations during development, including pre-IND (January 2006) and pre-BLA (October 2008) meeting discussions.

Pharmacology

Canakinumab (ACZ885) binds with high potency and affinity to human and marmoset IL-1β, a cytokine produced by mononuclear phagocytes in response to injury and infection. ACZ885 does not cross-react with any of the other IL-1 antibodies (e.g., IL-1α, IL-1Ra, IL-18, IL-33), nor any of the recombinant IL-1β from other mammalian species tested, including mice, rats, rabbits, and cynomolgus and rhesus monkeys, due to differences in the amino acid position 64 of IL-1β, which consists of glutamic acid in humans and marmosets, and alanine in the other species. Upon binding, the interaction of IL-1β with the IL-1 type I and II receptors is prevented (IC_{50} = 40 pM in humans and approximately 1/2 the potency in marmosets).

A mouse anti-mouse IL-1β surrogate 01BSUR, a murine analog of ACZ885 was developed for the evaluation of reproductive toxicity, juvenile toxicity and immunotoxicity in CD-1 mice, due to the absence of cross-reactivity by ACZ885 with murine IL-1β. The surrogate antibody was produced in mice that were immunized with recombinant mouse IL-1β. In this procedure, the mouse IgG1/κ isotype, which has no interaction with Fcγ receptors was converted to the IgG2a/κ isotype which is functionally equivalent to the human IgG1 isotype with regard to Fcγ receptor binding. 01BSUR binds to IgG1 and IgG2a isotypes of mouse IL-1β. The parent (IgG1) and the isotype IgG2a inhibit mouse IL-1β activity via inhibition of IL-6 production in [redacted] b(4)
fibroblasts.

IL-1β receptor activation leads to signal transduction related to that by the Toll-like receptors in the immune response to infection. Upon receptor activation and signal

transduction to intracellular machinery (including NFkB, Erk1/2, p38, Jnk1/2/3, and pI3 kinase), intracellular adapter molecules and IRAK protein kinases are activated, releasing cytokines, chemokines, and pro-inflammatory mediators. The main signaling pathways are similar in all mammals. According to the Applicant, IL-1β is involved in the pathobiology of autoinflammatory syndromes ——— b(4)

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Three studies were conducted to compare cross-reactivity profiles of ACZ885 produced using the ——— antibody, the ——— e antibody ——, and the —— line antibody ——, in normal and human tissues. The results of these studies showed b(4) qualitatively similar cross reactivity in human and marmoset tissues with regard to cell types, stain intensity, frequency and subcellular localization that are consistent with known distribution of IL-1β expressing cells, although the cross-reactivity profile in the marmoset tissues was quantitatively less than in human tissues. Minor differences observed, included ACZ885 staining n marmoset but not in human follicular and germinal cells in the ovary and in Leydig, Sertoli and gametogenic precursors in the testis. There were several differences noted in distribution of low-grade cytoplasmic staining in epithelial tissues at high concentrations of ACZ885-FITC, with staining observed in human but not marmoset prostate and thyroid, and in marmoset but not human parathyroid and vas deferens. Overall, the results of the cross-reactivity studies support the biocomparability of ACZ885 binding and IL-1β bioactivity by drug substance produced using the ——— , and comparability of marmoset and human b(4) responses supporting the selection marmoset for evaluation in the toxicology studies.

The results of *in vitro* mechanism of action studies demonstrated that upon binding to and inactivation of the IL-1 receptors IL-1RI and IL-1RII, ACZ885 effectively prevented the interaction of IL-1β with these receptors and subsequent production of interleukin 6 (IL-6) in human primary fibroblasts in a dose dependent manner. Comparative evaluation showed similar neutralizing activity of IL-1β by canakinumab in humans and marmosets.

Several nonclinical models of inflammation and neutrophil migration were used to investigate ACZ885 activity related to the proposed indication *in vivo*. Joint inflammation and architecture destruction induced in response to intra-articular injection of —— expressing human IL-1β was attenuated in a dose-dependent manner in mice given intraperitoneal (IP) ACZ885 2 hours before the IL-1β injection. In that b(4 study, proteoglycan synthesis in the cartilage was also normalized by pre-treatment with IP ACZ885. In other models, pre-treatment with ACZ885 reduced —— engineered human IL-1β-induced neutrophil migration in the mouse airpouch, and blocked fever induced by IV injection of human IL-1β in the rat. No enhancement of human IL-1β pharmacodynamic activity by ACZ885 was observed in the rodent models.

Several *in vivo* studies in mouse models of arthritis were used to evaluate the pharmacologic activity of 01BSUR with that of ACZ885. The surrogate antibody, given

by IP injection after induction of paw swelling in response to collagen injection, significantly reduced the swelling. Paw swelling was prevented in that model by prior 01BSUR injection.

No studies were performed to investigate secondary pharmacodynamic effects of ACZ885, in part due to the absence of evidence for non-specific tissue binding in the *in vitro* binding and cross-reactivity studies. No treatment-related effects were observed in the toxicology studies on ACZ885 and 01BSUR to indicate secondary, off-target pharmacodynamic activity. Although ACZ885 demonstrated Fcγ receptor binding *in vitro*, no cell surface-bound antigen binding, required for antibody dependent cellular toxicity was found. ACZ885 did not bind IL-1β producing human CD14+ monocytes, nor was found to recruit human monocytic cells of the C1q component pathway. Based on thses findings no effects on antibody dependent cellular cytotoxicity and complement dependent cytotoxicity would be expected. Furthermore, no immunosuppressive properties by ACZ885 were found in IL-1 receptor deficient mice *in vivo*, although IL-1β is involved in enhancement of immune function of the Th2 T cell subset. No inhibition of human mixed lymphocyte reaction was found and therefore no immunosuppression of T cell function is anticipated.

Safety Pharmacology

Safety pharmacology and pharmacodynamic drug interaction studies were not conducted on ACZ885 by the Applicant, and are generally not required for investigation and registration of biologic drugs. No primary drug effects are expected in the central nervous, cardiovascular, pulmonary, renal, and gastrointestinal systems in the absence of evidence for non-specific tissue binding. No effects on QT interval are expected, as ACZ885 shows a high degree of selectivitiy for the IL-1β receptor. The results of the cross-reactivitiy studies showed very weak binding in human cardiac tissue, and therefore no hERG channel interaction is unlikely as well. However, toxicity secondary to inflammatory changes and/or infection remain a theoretical concern and have been observed in nonclinical studies on other agents that inhibit IL-1β. The parameters evaluated in the 43-day and 13-week subcutaneous (SC) toxicology, and in the 4- and 26-week intravenous (IV) toxicology evaluations in marmoset included those generally employed in the standard Safety Pharmacology studies. There were no treatment-related adverse neurobehavioral effects or changes in cardiovascular, pulmonary, renal and gastrointestinal function and morphology observed using standard toxicology study methodology, such as clinical signs observations, blood pressure measurements, electrocardiography, and histopathology examinations. No adverse pharmacodynamic drug interactions with canakinumab are expected in clinical treatment.

Pharmacokinetics/Toxicokinetics

ACZ885 and 01BSUR pharmacokinetic (PK) and toxicokinetic (TK) parameters were assessed in CD-1 mice and marmosets, using single and repeated dose intravenous (IV) and subcutaneous (SC) administration. Additionally, six exploratory PK studies were conducted to optimally determine and validate the analytical (ELISA) and

immunogenicity (surface plasmon resonance spectroscopy) methodology used in the PK/TK evaluations on ACZ885 and 01BSUR. Comparative PK studies were conducted on the drug substance batches produced by different cell lines with manufacturing changes during product development, to bridge the ACZ885 lyophilisates produced using the ——— ; and in the marmoset toxicology and early clinical studies, to the drug substance produced using the ——— , that is the intended to-be-marketed drug. b(4)
Human and marmoset PK/TK profiles were characterized for comparison as well.

ACZ885 showed approximately dose-proportional increases in exposure in the nonclinical assessments, without gender differences. The primary systemic absorption pathways following extravascular injection of the antibody proteins are generally by convective transport through lymphatic vessels into circulating blood, and to a lesser extent by diffusion across blood vessels near the site of administration. Plasma concentrations increased with repeated administration suggesting accumulation or FcRn receptor saturation in the species studied. In contrast, accumulation with increased duration of administration was not reported consistently in the clinical trials. Exposure values (AUC and C_{max}) were similar in the marmosets for the SC ACZ885 batches that were manufactured using the —— (Product A) and ——(Productes B and C) cell lines in several bridging PK studies, including a 13-Week Batch Comparison Study. Bridging PK evaluation was conducted to investigate potential changes in elimination rates after IV administration of the IgG1 antibodies with ——— as a result of manufacturing changes during product development. No differences were found in the PK parameters with these changes, b(4)
including elimination profiles when comparing products containing the different ——— Overall, SC ACZ885 bioavailability was determined to be approximately 60% in marmoset, comparable to the human bioavailability of approximately 67%.

Steady state volume of distribution (Vss) values were low after IV and SC injection of ACZ885 in the marmosets and 01BSUR in the CD-1 mice, as was observed in the clinical studies on canakinumab. The volume of distribution (Vd) values approximated plasma volumes, suggesting little distribution into other tissues and organs outside of the circulatory system. ACZ885 placental transfer was demonstrated in the Embryo-Fetal Development (Segment II) study TK assessments in marmosets, and in the Segments II and II studies in mice administered the ACZ885 surrogate 01BSUR.

ACZ885 is believed to be metabolized and cleared by proteolytic degradation, consistent with the pathway observed for the immunoglobulins and other large protein molecules. No active ACZ885 metabolites are predicted. Renal clearance is unlikely, due to the large size of the ACZ885 protein molecule preventing glomerular filtration. (Wang W, *et al.* Monoclonal antibody pharmacokinetics and pharmacodynamics. Clin. Pharmacol. Ther. 84(5):548-558, 2008). No increases in clearance were observed with increased duration of treatment, lending support for the absence of immune response to ACZ885 in marmosets.

Toxicology

No studies were conducted specifically to evaluate single dose ACZ885 toxicology; acute treatment-related toxicity was tested using first dose observations in the repeated dose toxicology studies.

The repeated-dose toxicology studies were conducted in marmoset using the IV route for 4 and 26 weeks at doses of up to 100 mg/kg/twice weekly, and by subcutaneous injection for 43 days and 13 weeks at doses of up to 150 mg/kg twice weekly. The marmosets were administered ACZ885 by slow bolus IV injection at doses of 0, 10 (LD), 30 (MD), and 100 (HD) mg/kg twice weekly for 4 weeks for a total of 8 infusions, with a 2-month reversibility period, for dose-selection for the chronic IV toxicology study. The observations included standard toxicology parameters throughout the dosing period, and anti-ACZ885 antibody analyses were conducted using sheep anti-ACZ885 serum as the positive control and pre-treatment serum for the negative control. Lymphocyte (CD3, CD4, CD8, CD14, CD16, CD20, CD56) subpopulations and monocytes were immunophenotyped using flow cytometry. The results showed no treatment-related effects at any dose level, during the dosing and recovery periods. No anti-ACZ885 antibodies were found, and there were no treatment-related changes in monocytes, leucocytes, and lymphocyte subpopulations, at up to the highest dose and NOAEL of 100 mg/kg IV.

No target organ toxicity was identified in the 6-month chronic IV toxicology in marmosets. The marmosets were administered ACZ885 by IV bolus injection at doses of 0, 10 (LD), 30 (MD), and 100 (HD) mg/kg/twice weekly for 6 consecutive months, with a 6-week reversibility period following treatment. Standard toxicology parameters, lymphocyte and monocyte immunophenotyping, and anti-ACZ885 antibody evaluations were performed. The assessments also included measurement of serum testosterone, sperm evaluation (motility, number, and morphology), testicular size, and microscopic examinations of testicular tissue for testicular cell population quantitation in the male marmosets. No treatment-related effects wee found on any of the parameters investigated, and no anti-ACZ885 antibodies were detected.

In the first of 3 SC ACZ885 toxicology studies performed to bridge the results of the 6-month IV study to the SC route intended for marketing, marmosets administered 2 injections at doses of 0 (vehicle control), 5 (LD), 50 (MD), and 150 (HD) mg/kg, 43 days apart showed no treatment-related effects at the HD, although anemia was found in 1 MDF with decreased red blood cells, hemoglobin and hematocrit. Inflammatory leukocytosis with increased lymphocytes, monocytes and eosinophils were observed in another 1 MDF. Sporadic histopathology findings included slight hyperplasia in the jejunum (epithelial and Peyer's patch) with marked lymphocytic infiltrate and minimal abscess in 1 HDF, slight leukocytic infiltrate in 1 MDF and 1 HDF, slight (1 HDF), and minimal to moderate (1 control, 3 LDF, 2 MDF, and 3 HDF) hepatocellular vacuolation. These findings were also not observed in the longer-term SC and IV toxicity studies, and therefore are considered not to be treatment-related.

In the first of two 13-week SC toxicology studies, male (M) and female (F) marmosets were administered ACZ885 at the doses of 0 (vehicle control), 15 (LD), 50 (MD), and

150 (HD) mg/kg/twice weekly for 13 consecutive weeks, with additional marmosets included for an 8-weeks reversibility period following the end of the dosing period. Standard toxicology parameters were evaluated throughout dosing and recovery, and additionally, serum testosterone was analyzed in the blood samples from all marmosets for immunophenotyping of peripheral blood leukocytes (CD20, CD3, CD4, CD8, CD16, CD4:CD8, CD3:CD20) and splenic nucleated cell suspension (CD20, CD3, CD4, CD8, CD4:CD8, CD3:CD20), anti-ACZ885 antibody determination, and gene expression analysis in tissue samples from liver, kidney, spleen, lung, and mesenteric lymph nodes. The results showed minimal leukocytic or mononuclear infiltrates at the injection site, without a relationship to dose. The histopathology examination also revealed a dose-related increased minimal lymphoid hyperplasia of the spleen (0 control, 1 LD [minimal], 2 MD [slight], and 3HD [2 minimal, 1 slight]), in large active follicles in the Treatment and Recovery males but not in the females. A relationship to ACZ885 treatment to the findings in spleen cannot be ruled out, but the effects in spleen were in the absence of any treatment-related effects on phenotyping of splenic suspensions and blood samples. The findings in spleen were not observed in the other toxicology studies in marmosets, including the longer duration 6-month IV toxicity study. The Applicant monitored spleen size in canakinumab-treated patients during product develeopment without detecting a treatment-related effect. Therefore, the findings in spleen in this study are noted but not compelling evidence of target organ toxicity by ACZ885. The NOAEL is considered to be the highest dose evaluated, of 150 mg/kg/twice weekly.

A second 13-week SC bridging toxicology study was conducted in marmosets to compare ACZ885 TK and toxicity profiles of ACZ885 produced by the ——— (intended for marketing) and by the ——— (evaluated in the early nonclinical toxicology studies and clinical trials). In this study, male (M) and female (F) marmosets were administered 0 (vehicle control), 150 mg/kg/twice weekly ACZ885 produced by the ———, or 150 mg/twice weekly ACZ885 produced by the ——— for 13 consecutive weeks. The standard toxicology evaluations, except electrocardiography and urinalyses were performed, and sampling was conducted to detect formation of anti-ACZ885 antibodies. There were 2 deaths in male cagemates given ACZ885 by the ——— on Treatment Days 57 (euthanized) and 72 (found dead). One of the decedents (Day 57) showed mixed inflammatory cell infiltration and ulceration in the intestinal mucosa associated with neutrophil loss and blood in the lumen, and neutrophilic inflammation with bacteria throughout the body, attributed to intestinal ulceration-induced septicemia. The cagemate found dead on Day 72 showed marked thymus lymphoid depletion. Fecal changes of diarrhea and mucoid feces were observed in 75%-100% males given the ——— formulation and 50%-75% F given the ——— formulation. Body weight loss, although observed in all groups including controls, was higher in the males given ACZ885 by the ———than in the other groups. There was no evidence of anti-ACZ885 antibody response in any group, and no differences in exposure between the two Batches in the marmoset TK analysis. The NOAEL in this study can be considered to be 150 mg/kg ACZ885 produced using the ———, but was not determined for the ——— formulation. It should be noted that septicemia secondary to intestinal ulceration has been observed in historical control marmosets.

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Reproductive Toxicology

A full battery of reproductive toxicology studies was conducted by the Applicant, including evaluations of embryo-fetal toxicity (Segment II) in marmosets given SC ACZ885, and fertility (Segment I), embryo-fetal development and pre- and post-natal development in mice administered 01BSUR. No significant treatment-related effects were observed on male and female fertility, major fetal malformations, and post-natal neurobehavioral and reproductive development. Additionally, there were no effects in the immunology assessments in adult (F_1) offspring of treated mice, including blood, thymus and spleen immunophenotyping using determination of total, absolute and percent differential counts for T, Helper T, Cytotoxic T, B, and Natural Killer lymphocytes, and organ weights, and no anti-ACZ885 antibody formation was detected. Minor treatment-related effects suggestive of slight developmental delay were observed in the Segment II studies included incomplete ossification in the marmosets and mice. The marmosets were administered ACZ885 by SC injection at the doses of 0 (vehicle control), 15 (LD), 50 (MD), and 150 (HD) mg/kg twice weekly on gestation days (GD) 25-109, and standard Segment II maternal and fetal examinations, TK analyses and evaluation of anti-ACZ885 antibody formation were performed. In the absence of maternal toxicity by ACZ885 at any dose, there were slight decreases in placental weights (-22%) at the HD compared to control placental weights, probably due decreased litter size (-24% vs. controls) at this dose. However, ultrasonography demonstrated no decreases in the numbers of fetuses vs. the numbers of embryos present at Day 50, and therefore the reduced litter size was probably not a result of resorptions, but was likely due to a lower number of triplets and more singlets and twins at the HD. Early resorptions could not be assessed because uterine scars are not formed in marmosets upon early abortion. There was a slight increase in numbers of fetuses with kinked and/or bent tail end in the external examination. The skeletal examination revealed slight incomplete vertebral ossification in the terminal caudal vertebra in 1/27, 4/30, 10/32, and 2/26 fetuses in the control, LD, MD, and HD groups, respectively. Adequate maternal exposure that increased with duration of treatment was demonstrated in the TK analyses supported validation of this study. Additionally, all fetuses of the treated dams were exposed to ACZ885 in a dose-proportional manner, showing fetal serum and amniotic fluid concentrations of 7.0%-8.7% and 1.8%-2.0%, respectively, the concentrations in maternal serum. No anti-ACZ885 antibody formation was detected in the maternal and fetal marmosets.

A second embryo-fetal study was conducted in CD-1 mice administered 01BSUR at SC doses of 0 (vehicle control), 15 (LD), 50 (MD), and 150 (HD) mg/kg on gestation days (GD) 6, 11, and 17, with additional satellite mice treated for theTK analyses. The results showed no maternal toxicity in the in-life and necroscopic examinations, and no treatment-related effects on reproductive parameters. However, treatment-related developmental delay was suggested by findings of increased incidence of incomplete ossification of the parietal bones at the MD and HD, and of the fronal bones at the HD when compared to the concurrent and historical controls for the laboratory. The results of the TK analyses showed adequate 01BSUR exposure in the fetuses via placental transfer and in the dams, supporting validation of the study.

Juvenile Toxicity

Juvenile toxicology was investigated using the ACZ885 surrogate 01BSUR in CD-1 mice to support of the safety of ACZ885 administration in pediatric patients ages 4 years and above. The juvenile study was performed in agreement with Agency recommendations (see meeting minutes for October 21, 2008 pre-BLA meeting with the Applicant). 01BSUR was administered by SC injection at the doses of 0 (control vehicle and a untreated control group), 15 (LD), 50 (MD), and 150 (HD) mg/kg/once weekly on post partum days (PPD) 7-70, with additional satellite animals treated for evaluation of reversibility, toxicokinetics, and immunology in the dosing and recovery phases. Standard toxicology parameters were measured, and physical development was evaluated throughout pre-weaning (eye opening, auricular startle, PPD10) and post-weaning (vaginal opening, preputial separation, PPD 21 until end of study) periods. The Assessments were made for behavioral performance (motor activity, auditory startle habituation, and passive avoidance), fertility parameters (e.g., mating and uterine examinations), and immunophenotyping (blood, spleen and thymus) for measurement of Total, Helper, Cytotoxic, Double negative, and Double positive T cells, B cells and Natural Killer cells. The results showed a slight increase in the mean day of development of auricular startle in the pre-weaning MD males and HD males and females compared to concurrent controls. However, the values observed were similar to control values in Study # 901098, and there were no treatment-related effects in the post-weaning evaluation of auditory startle habituation. There was a statistically significant delay in the mean day to vaginal opening at the HD compared to controls, but the values were within historical control range for the performing laboratory. A statistically significant increase in pre-implantation loss at the HD compared to controls was noted in the evaluation of fertility, predominantly due to a 71.4% litter loss in 1 dam, although the mean number of live embryos in the HD group was within the historical control range for the laboratory. Treatment-related inflammation observed at the injection site during the dosing period was reversed during the recovery period. There were no treatment-related effects in the evaluation of immunogenicity and in the immunophenotyping in blood, spleen and thymus, on lymphocyte and lymphocyte subset counts. The results of the TK analyses showed a dose-proportional increase in exposure, up to 6-fold from PPD7 to PPD63, suggesting accumulation and/or FcRn receptor saturation. The effects noted in this study are considered to be equivocal and therefore the NOAEL for adverse 01BSUR-related effects on juvenile development is highest dose tested of 150 mg/kg/week.

Studies to evaluate ACZ885 genetic toxicology and carcinogenicity are generally not required for biologic drugs unless concerns are raised based on the mechanism of action or findings in the general toxicology studies, and these studies were not requested by the Agency or conducted by the Applicant.

Immunotoxicity

Potential ACA885 immunotoxicity was addressed in a comprehensive investigation in CD-1 mice administered the ACZ885 surrogate 01BSUR by SC injection at doses of 0

(vehicle control), 10 (LD), 50 (MD), and 150 (HD) mg/kg/once weekly for 28 days. Satellite groups were included to explore reversibility, and for assessment of T-cell Dependent Antibody Response (TDAR) immunogenicity, immunophenotyping, immunogenicity, and toxicokinetics. The immunizing agent, Imject Mariculture Keyhole Limpet Hemocyanin (KLH) was administered by IP injection on Study Days 15 and 22 (main study), and 42 and 49 (recovery). The assessments in the main study and recovery mice included immunophenotyping of blood samples, and spleen and thymus tissue for relative proportions and absolute numbers of Total ($CD3e^+$), Helper ($CDe3^+/CD4^+$), and Cytotoxic ($CDe3^+/CD8a^+$) T lymphocytes, Double positive T lymphocytes (spleen and thymus, $CD3e^+/CD4^+/CD8a^+$), Double negative T lymphocytes (spleen and thymus, $CD3e^+/CD4^-/CD8a^-$), B lymphocytes ($CD19^+$), and Natural Killer lymphocytes ($CDe3^-/NK1.1^+$), anti-01BSUR antibody formation, TDA and T-cell dependent antigen IgM and IgG antibody responses following the IP KLH injections, and standard toxicology parameters, with particular focus microscopic examination of lymph nodes, spleen, and thymus. The results showed no treatment-related effects on immunophenotyping and on the mean absolute and relative percentages of lymphocytes in blood, spleen and thymus, and no formation of anti-drug antibody. There was an apparent treatment-related inhibition of anti-KLH IgG response (up to 61% at the MD and HD, main study but not in the recovery mice), as a result of comparison with controls showing abnormally high response in 2 M. On the other hand, increased anti-KLH IgG response was observed in the treated F, attributed to extremely high variability. There were no treatment-related changes in the anti-KLH IgG response in the recovery animals and no anti-KLH IgM response in any group. Slight increases noted in absolute and relative thymus weights in the main study F at ≥50 mg/kg/wk vs. controls, was without statistical significance, macroscopic and histopathology correlates, or findings in the recovery animals, and therefore unlikely related to treatment. In conclusion, 01BSUR is considered to be negative in the immunotoxicity study in the CD-1 mice under the conditions of this study.

Local (Intra-articular) Tolerance

A single-dose intra-articular local tolerance study was conducted in marmosets given control injection in the left knee joint and 10 mg/kg ACZ885 in the right knee joint. The assessements included a limited battery of standard toxicology parameters, with additional microscopic examinations of the knee joints with surrounding tissues in both knees, as well as in popliteal and inguinal lymph nodes. Ataxia in the hindlimbs was noted in one ACZ885-injected marmoset. There were also singular observations of decreased lymphocytes and increased neutrophils, enlarged/mottled adrenals and kidneys, swollen right finger with subcutaneous abscesses, ileal invagination, and red discolored urinary bladder in other test article-treated marmosets. The animal with enlarged adrenals and kidneys was examined histologically and showed moderate cortical hypertrophy in the adrenals, moderate inflammatory cell foci, and slight fibrosis with slight atrophic tubuli in the kidneys. Discolored urinary bladder was associated with marked acute inflammation and ulceration of the urinary bladder upon microscopic evaluation. There were no local treatment-related effects in the knee joints, or in the popliteal and inguinal lymph nodes in any animal in this study. ACZ885 is considered to be negative for local effects by intra-articular injection in marmosets under the conditions of this study.

Although apparent systemic ACZ885-related effects, that could suggest a relationship to inflammation and/or infection, were observed and TK sampling confirmed systemic exposure using serum sampling, these effects are not considered to be toxicologically relevant because of the small magnitude of the differences from control values, the observations were within historical control value ranges, these findings can be typically found in response to stress, and/or absence of similar findings in the other toxicology studies using more frequent dosing and for longer durations of exposure.

CONCLUSIONS:

The nonclinical study program, conducted according to the general recommendations in the ICH Guidance to Industry and in agreement with Agency recommendations and concurrence during product development provided an adequate overall safety profile for canakinumab treatment of CAPS in patients ages 4 and older. The methodology used was appropriate, well-validated, and in agreement with the ICH Guidelines and Agency concurrence. Canakinumab does not interact with cytochrome P450 enzymes and thus a risk for drug-drug interactions is low. There were no effects on the QT interval in the electrocardiography assessments in marmosets, the binding affinity in human cardiac tissues was very low in the cross-reactivity studies in normal human tissues, and canakinumab is unlikely to have an effect on hERG channels due to highly specific binding to IL-1β. Canakinumab clearance is not expected to be affected by hepatic or renal impairment, due to clearance by endosomal catabolism and absence of renal glomuerular filtration because of the large size of the protein molecule. There were no effects of age or sex suggested by the nonclinical study results. No drug-drug interactions are expected.

The potential for hypersensitivity and/or adverse effects related to immunosuppression such as increased risk of infection and/or malignancies remain a concern, although the evidence for these effects was equivocal in the nonclinical toxicology studies on ACZ885 and 01BSUR, and 01BSUR was negative in the more exhaustive Immunotoxicology Study in mice. No concerns were raised with respect to mechanism of action. Although there was no nonclinical evidence of primary target organ toxicity by ACZ885 and 01BSUR in the general toxicology studies, several minor nonclinical safety issues relevant to clinical use were raised. These issues include potential risks related to immuotoxicity, and slight developmental delay in the unborn fetus, in the absence of ACZ885-related effects on fertility, major malformations or pre- and post-natal neurobehavioral development. These risks should be adequately discussed in the product label, and appropriate clinical monitoring and preventative measures are warranted. Additionally, a residual solvent that is known to present a serious risk in the newborn was identified in the drug product intended for clinical treatment, but is expected to be eliminated prior to formulating the drug substance. There are no concerns at this time with regard to the excipients in the proposed formulation.

UNRESOLVED TOXICOLOGY ISSUES:

There are no unresolved toxicology issues and no further animal studies are needed at this time.

RECOMMENDATIONS:

Canakinumab can be approved for the proposed indication under BLA 125319, from a pharmacology and toxicology perspective.

SUGGESTED LABELING: Revisions to the sections of the label on Pregnancy, Carcinogenesis, Mutagenesis, and Impairment of Fertility will be needed, to include additional information from the nonclinical toxicology findings, and to refine the language. The recommended labeling revisions are summarized in the Executive Summary, above.

Signatures (optional):

Reviewer Signature 5/14/09

Supervisor Signature 5-14-09 Concurrence Yes X No ___

APPENDIX/ATTACHMENTS

None

PHARMACOLOGY/TOXICOLOGY NDA FILEABILITY CHECKLIST

NDA/BLA Number: 125319 **Applicant: Novartis** **Stamp Date: December 17, 2008**

Drug Name: Canakinumab **NDA/BLA Type: 505(b)(2)** **DAARP/OND/CDER/FDA**

On **initial** overview of the NDA application for Refuse to File (RTF):

	Parameters	Yes	No	Comment
1	On its face, is the pharmacology section of the NDA/BLA organized (in accord with 21 CFR 314 and current guidelines for format and content) in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section of the NDA/BLA indexed and paginated in a manner allowing substantive review to begin?	X		
3	On its face, is the pharmacology/toxicology section of the NDA/BLA legible so that substantive review can begin?	X		
4	Are all required (*) and requested BBIND studies (in accord with 505(b1) and (b2) including referenced literature) completed and submitted in this NDA/BLA (carcinogenicity*, mutagenicity*, teratogenicity*, effects on fertility*, juvenile studies, acute and repeat dose adult animal studies*, maximum tolerated dose determination, dermal irritancy, ocular irritancy, photo co-carcinogenicity, animal pharmacokinetic studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies been conducted with the appropriate formulation?	X		

6	Is (are) the excipient(s) appropriately qualified (including interaction between the excipients if applicable)?	X		
7	On its face, does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted a rationale to justify the alternative route?	X		
8	Has the sponsor submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
9	Has the sponsor submitted all special studies/ data requested by the Division during pre-submission discussions with the sponsor?	X		To include comparison of the drug substance produced by different manufacturing methods, immunotoxicity evaluation and studies on juvenile animal toxicity, and the standard battery of reproductive toxicity studies
10	Are the proposed labeling sections relative to pharmacology, reproductive toxicology, and carcinogenicity appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		Revisions to wording to be conveyed to Sponsor at the time of the labeling review. ———— b(4)
11	Has the sponsor submitted any toxicity data to address impurities, new excipients, leachables, etc. issues.			NA
12	Has the sponsor addressed any abuse potential issues in the submission?			NA
13	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			NA

14	From a pharmacology/ toxicology perspective, is the NDA/BLA fileable? If ``no`` please state below why it is not.	X		

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Comments to Sponsor: None at this time.

Reviewing Pharmacologist: Kathleen Young, Ph.D. 1/26/09 2009
Date

Team Leader: Adam Wasserman, Ph.D. 1/27/09
Date