## Safety Pharmacology

Safety pharmacology studies assessed potential effects of Project J on APD and hERG current in vitro, cardiovascular and respiratory systems in dogs, and central nervous system in rats. All studies were performed in accordance with Good Laboratory Practice (GLP).

A tabulated overview of safety pharmacology studies are found in [End-of-Text Table 1.2].

## In Vitro Effects on hERG Current [Project J-PT-0001]

The effects of Project J on the hERG current were studied in

hERG-transfected human embryonic kidney 293 cells (HEK293 cells) at concentrations of 3, 10, and 30 μmol/L.

The mean compensated suppression rates of Project J at the concentrations of 3, 10, and 30 μmol/L were 9.8, 24.1, and 52.8%, respectively; statistically significant differences were noted at 10 and 30 μmol/L when compared to the rate in the control group. The IC50 value of Project J is estimated to be 27 μmol/L.

These results indicate that Project J suppresses the hERG current in a concentration-dependent manner with an IC50 of 27 μmol/L.

## In Vitro Effects on Action Potential Duration [Project J-PT-0002]

The effects of Project J on action potentials in isolated guinea-pig papillary muscles were studied at the concentrations of 3, 10, and 30 μmol/L.

No changes were noted when Project J was applied at the concentration of

3 μmol/L. At 10 μmol/L, only action potential duration between 90% and 30% repolarization (APD30-90) prolonged (5.5%). At 30 μmol/L, shortened action potential duration at 30% repolarization (APD30, −8.2%), and prolonged APD30-90 (13.7%) were noted, no effects were noted in action potential duration at 90% repolarization (APD90), resting membrane potential, action potential amplitude, or maximum rate of depolarization.

In conclusion, Project J does not affect the action potentials in isolated

guinea-pig papillary muscles at a concentration of 3 μmol/L, while it prolonged APD30-90 at 10 and 30 μmol/L, and prolonged APD30-90 at 30 μmol/L.

## In Vivo Effects on Cardiovascular and Respiratory Systems in Dogs [Project J-PT-0003]

Project J was orally administered to male beagle dogs at single doses of 1, 10, 100, and 1000 mg/kg.

Project J did not affect the general activity and behavior, body temperature, blood pressure, heart rate, electrocardiogram, respiration rate, blood gases, or blood electrolyte concentrations at doses of 1 and 10 mg/kg. At 100 mg/kg, vomiting, loose stool and decreased food consumption were noted. At 1000 mg/kg, vomiting and decreased food consumption were observed.

## In Vivo Effects on Central Nervous System in Rats [Project J-PT-0004]

Project J did not affect the general activity and behavior of male Crl:CD(SD) rats in the Irwin test following single doses of 10, 100, or 600 mg/kg.

## Pharmacodynamic Drug Interactions

No pharmacodynamics drug interaction studies were conducted.

## Other Pharmacology Studies

No other pharmacology studies were conducted.

# Toxicology

Toxicology studies were conducted using mice, rats, rabbits and dogs, or evaluated in vitro. All pivotal studies were performed in accordance with GLP.

## Single-Dose Toxicity

Single dose oral toxicity of Project J was evaluated in rats and dogs. A tabulated overview of single-dose toxicity studies are found in [End-of-Text Table 3.4].

## 4.3.1.1 Rat [Project J-TX-0005]

Crl:CD(SD) rats were given a single oral administration of Project J at 1000 and 2000 mg/kg for males and females.

There were no deaths in any group. No findings attributable to treatment were noted in general signs, body weights or gross pathological examination in any animals.

In conclusion, the approximate lethal dose level was considered to be greater than 2000 mg/kg in rats.

## 4.3.1.2 Dog [Project J-TX-0006]

Project J was administered once orally at dose levels of 1000 and 2000 mg/kg to 1 male and 1 female beagle dog per group.

No animal died or was euthanized due to moribundity, and no test article-related changes were noted in body weight, food consumption, hematology, gross pathology, or histopathology at either dose level.

At 1000 mg/kg, decrease in serum testosterone concentrations at 2 pm and 6 pm (sampling times for testosterone measurement; dosing times were: 9:29 am and 9:40 am) on Day 0 (dosing day), slight increases in blood urea nitrogen (BUN), inorganic phosphorus, and sodium in the male, and slight increases in creatinine, and total bilirubin in the female were noted on Day 1 (1 day after dosing). All changes had recovered by Day 7 (7 days after dosing).

At 2000 mg/kg, single incidences of vomiting were observed in both dogs. Slight increases in creatinine and slight decreases in total protein were noted in the male and female on Day 1, and all changes had recovered by Day 7.

In conclusion, the approximate lethal dose level was considered to be greater than 2000 mg/kg in dogs.

## Repeat-Dose Toxicity

Pivotal 4-week repeated dose oral toxicity studies of Project J were conducted in rats and dogs.

A tabulated overview of repeat-dose toxicity studies are found in [End-of-Text Table 3.5] (nonpivotal) and [End-of-Text Table 3.6] (pivotal).

## 4-Week Oral Toxicity in Rats [Project J-TX-0007]

Project J was orally administered once daily for 4 weeks at dose levels of 0 (control), 10, 100, 300, and 600 mg/kg to 10 male and 10 female Crl:CD(SD) rats per group in order to investigate its toxicity. Five males and five females were added to the control and highest dose groups to assess the reversibility of toxicity during a subsequent 8-week recovery period.

At 10 mg/kg, no test article-related changes were noted during or at the end of the dosing period.

At 100 mg/kg, a high reticulocyte ratio (only in females) was noted in hematology, and narrowing of the splenic marginal zone was observed microscopically.

At 300 mg/kg, suppression of body weight gain, low food consumption, and low body weight (only in males) were observed.

Body temperature was reduced (only in females) on Days 14 and 28 at approximately 2 hours after dosing.

Low hematocrit value, hemoglobin concentration and erythrocyte count (only in females); high reticulocyte ratio, platelet count, mean corpuscular volume (MCV, only in females) and mean corpuscular hemoglobin (MCH, only in females) were noted in hematology. Spleen weights were increased (only in females), and narrowing of the splenic marginal zone and dilatation of the splenic sinus with blood saturation were noted in histopathology. Low thymus weights (only in males) were noted without associated histopathological changes.

Elevations in total excretions and/or creatinine-corrected amounts of KIM-1, TIMP-1 (only in males), and cystatin C (only in females) were seen in urinalysis on Day 25; degenerative basophilic change in the renal proximal tubules was seen microscopically (only in a single male).

Whitening of the incisors at clinical observations and white discoloration of the upper incisors in gross pathology turned out to be single cell necrosis and/or degeneration of the papillary layer and/or ameloblasts at histopathology.

High inorganic phosphorus (only in males), low α1-globulin ratio (only in females) and high β-globulin ratio (only in females) were noted in blood chemistry. High liver and thyroid weights (both only in females) were noted without associated histopathological changes.

At 600 mg/kg, low body weight and food consumption, suppression of body weight gain, and body weight loss (only in females during Days 1 to 3) were noted. In clinical signs, salivation (only in males), decrease in stool volume (only in a single female), soiled (urine) perineal region (only in a single female), and trace of reddish rhinorrhea (only in a single female) were observed.

Body temperature was reduced in males on Day 28 and in females on Day 14, both at approximately 2 hours after dosing.

Low erythrocyte count, hematocrit value, and hemoglobin concentration; high reticulocyte ratio, platelet count, MCH concentration (only in males), MCV (only in females), MCH (only in females), leukocyte counts (only in females), lymphocyte counts (only in females) and large unstained cell count (only in females) were noted in hematology. Spleen weights were increased (only in females), and narrowing of the splenic marginal zone, dilatation of the splenic sinus with blood saturation, extramedullary hematopoiesis (only in females) were noted in histopathology. In immunohistochemistry examinations, narrowing of the CD45RA positive stain area in the splenic marginal zone was observed, which indicated decrease in

B-cells in this area. Low thymus weights (only in males) were noted without associated histopathological changes.

High urine volume, low specific gravity, elevations in total excretions and/or

creatinine-corrected amount of KIM-1, TIMP-1, lipocalin-2/NGAL, osteopontin (only in females), albumin (only in females) and cystatin C (only in females) were noted in urinalysis on Day 25. Degenerative basophilic change in the renal proximal tubules, dilatation of the renal tubules (only in females), and regeneration of the renal collecting duct (only in females) were observed in histopathology.

Low α1-globulin ratio, and globulin concentration (only in males); high inorganic phosphorus (only in males), albumin concentration and ratio (only in males), albumin/ globulin ratio (only in males) and β-globulin ratio (only in females) were noted in blood chemistry. High thyroid, liver and adrenal weights (only in females) weights were noted.

The above-mentioned findings noted at 600 mg/kg during or at the end of the dosing period completely recovered during the recovery period.

Whitening of the incisors in clinical observations, and white discoloration of the upper incisors in gross pathology were noted during or at the end of the dosing period; while whitening, defect, overgrowth of the incisor in clinical observations, white focus of the tip in the upper incisors (only in a single male), defect of the tip in the upper incisors (only in a single female) in the gross pathology were observed during or at the end of the recovery period. In the histopathological examinations, single cell necrosis and/or degeneration of the papillary layer and/or ameloblasts were observed in the incisors, which were not noted after the recovery period. Therefore, the changes in the incisors tended to recover.

Bilateral soft testes (only in a single male) was observed in gross pathology, tubular atrophy/ spermatid giant cell of the testis and ductal cell debris in the epididymis (both in 2 males) were noted in histopathology at the end of the treatment period. Bilateral soft and small testis at gross pathology, atrophy in the seminiferous tubules and hyperplasia of the Leydig cells in the testis, and ductal cell debris in the epididymis were noted in histopathology at the end of the recovery period in a single male. The reversibility of the changes in the male reproductive organs was unclear.

In conclusion, the NOAEL was 10 mg/kg for males and females because narrowing of the splenic marginal zone in both males and females and high reticulocyte ratio in females were noted at the lowest-observed-adverse-effect level (LOAEL) of 100 mg/kg and greater. All the changes noted during the dosing period recovered or tended to recover after 8-week drug withdrawal, but the reversibility of the histopathological changes in the testis was unclear.

## 4-Week Oral Toxicity in Dogs [Project J-TX-0008]

Project J was orally administered once daily for 4 weeks at dose levels of

0 (control), 1, 3, 30, and 300 mg/kg to 4 male and 4 female beagle dogs per group in order to investigate its toxicity. Three males and three females were added to the 300 mg/kg group to assess the reversibility of toxicity observed during the dosing period in a subsequent 4-week recovery period.

No toxicological changes were noted at 1 or 3 mg/kg. No test article-related changes were observed in body temperature or ophthalmology at any dose level.

At 30 mg/kg, vomiting (only in 1 male) and decreased body weight (only in 1 female) were observed. Decreased serum testosterone concentrations and atrophy of the prostate were noted.

At 300 mg/kg, one male died in the morning on Day 24 of dosing, and 2 males and 1 female were sacrificed in moribund condition on Days 15, 17, and 24 of dosing. These animals showed decreased body weight or food consumption, with decreased spontaneous activity, abnormal position (prone position or lateral position), suppressed response to stimulation, emaciation, or no stool before death or sacrifice. Since notable body weight loss with reduction in spontaneous activity also occurred in further dogs at 300 mg/kg, dosing of this group was stopped prematurely at Day 25. The remaining 1 male and 3 females of the main group were sacrificed at Day 25, while the 3 males and 3 females allocated to the recovery group were necropsied following a recovery period of 32 days.

The following changes were additionally seen at 300 mg/kg, mainly, but not only in animals showing significant weight loss. Occasional vomiting and salivation were noted throughout the dosing period.

Decreased serum testosterone concentrations, low prostate weight, atrophy of the prostate, single cell necrosis of ductal epithelium of the epididymis, spermatid giant cell formation in the testis were noted.

Increased plasma BUN and creatinine were noted, and decreased plasma levels of calcium, sodium, potassium, and chloride were noted. Urinary excretion of sodium and chloride were reduced.

Increased aspartate transaminase, alanine transaminase, alkaline phosphatase, total bilirubin, total protein, and total cholesterol were noted in blood chemistry; high liver weight was noted; and atrophy, single cell and multifocal hepatocyte necrosis, or inflammatory cell infiltration were observed in the liver in histopathology.

Soft stool, mucous stool, abnormal stool color (reddish brown or dark red color and positive occult blood reactions in moribund animals only), diarrhea were noted in clinical observations. Microscopically, mucosal erosion, hemorrhage, and congestion and regeneration of glandular epithelium with inflammatory cell infiltration were observed in the duodenum, jejunum, cecum and rectum.

Decreased erythrocyte count, hemoglobin concentration, and hematocrit value, platelet count, leukocyte count, lymphocyte count, eosinophil count, basophil count, neutrophil count, monocyte count; increased neutrophil count (only in a single male) were noted in hematology. High spleen weight was noted, and hypocellularity in the sternal and femoral bone marrow, atrophy of the thymus and Peyer’s patch, and follicular atrophy of the spleen and mesenteric lymph node were observed in histopathology.

Inflammation and hemorrhage in the lungs, ductal dilatation of the sublingual gland, atrophy of muscle fiber in the femoral skeletal muscle, and increase in adrenal weight with decrease in lipid and hypertrophy of zona fasciculata were observed mainly in dead or moribund

animals; reduced heart rate with increase in QTc was noted only in 1 female on Day 22 of dosing and considered secondary to the poor condition of the dog.

All the findings observed at 300 mg/kg during the dosing period had disappeared at the end of the 4-week recovery period.

It was concluded that, the NOAEL was 3 mg/kg, the LOAEL was 30 mg/kg, and 300 mg/kg was considered to exceed the MTD. The changes observed during the dosing period recovered during the 4-week recovery period.

## Genotoxicity

The potential genotoxicity of Project J was evaluated by in vitro (reverse mutation test and chromosome aberration test) and in vivo (mouse micronucleus test and rat unscheduled DNA synthesis).

A tabulated overview of genotoxicity studies are found in [End-of-Text Table 3.7] (in vitro) and [End-of-Text Table 3.8] (in vivo).

## In Vitro Reverse Mutation Test [Project J-TX-0009]

The mutagenicity of Project J was examined in the presence and absence of S9 Mix using *Salmonella typhimurium* TA100, TA98, TA1535, and TA1537 and *Escherichia coli* WP2 uvrA.

The test article did not inhibit the growth of any strains. Precipitation of the test article on plates was observed in all strains at 625 μg/plate in the absence of S9 Mix and 1250 μg/plate in the presence of S9 Mix. The mean number of revertant colonies did not reach double that of the vehicle control group for any strain at any dose level, regardless of the presence or absence of metabolic activation.

These results indicate that Project J has no mutagenic potential.

## In Vitro Chromosome Aberration Test [Project J-TX-0010]

The potential for Project J to induce chromosomal aberrations was evaluated under three treatment conditions: 6-h treatment in the presence of S9 Mix and 6- and 24-h treatments in the absence of S9 Mix, using a Chinese hamster lung fibroblast (CHL) cell line. In the 6-h treatment groups in the presence of S9 Mix, chromosomal aberrations were analyzed over a concentration range of 60 to 100 μg/mL. The concentration ranges evaluated were 24 to 36 μg/mL and 7.5 to 12.5 μg/mL in the 6-h and 24-h treatment groups in the absence of S9 Mix, respectively.

Project J significantly increased the numbers of polyploid cells (numerical aberrations) and those of cells with structural aberration when compared to the vehicle control at concentrations of 100 μg/mL (with cytotoxicity of 42%) for the 6-h treatment group in the presence of S9 Mix.

These results indicate that Project J has the potential to induce structural and numerical chromosomal aberrations.

## Micronucleus Test in Mice [Project J-TX-0018]

Project J was orally administered to mice to examine its ability to induce micronuclei in bone marrow cells as an index for its potential to induce chromosome aberration in vivo. Project J was orally administered twice by gavage at a 24-hour interval to male Crlj:CD1(ICR) mice at 0 (control), 500, 1000 and 2000 mg/kg.

No statistically significant increases in micronucleated immature erythrocytes-incidences were detected in any group of the test article compared to the negative control group, showing a negative response in the micronucleus assay. The systemic exposures were appropriately confirmed at 2000 mg/kg.

Based on the results described above, Project J was judged negative in the bone marrow micronucleus test in mice under the conditions employed in this study, and was concluded to have no potential to induce chromosome aberration in vivo.

## Unscheduled DNA Synthesis in Rats [Project J-TX-0019]

In vivo unscheduled DNA synthesis (UDS) assay with rat hepatocytes using male Crl:CD(SD) rats was carried out to evaluate the DNA damaging effect.

There was no change in general condition nor dead animal in any dosing group. The results of the UDS test revealed that all mean numbers of net grains (NNG) in 500, 1000 and

2000 mg/kg PROJECT J dosed groups were less than two, while the mean NNG in the positive control group was more than two for both early and late sampling times. No significant dose dependent increase was observed in the mean NNG of Project J dosed groups for both early and late sampling times.

From these results, it was concluded that Project J does not have DNA damaging effect to the rat hepatocytes under these experimental conditions.

## Carcinogenicity

No carcinogenicity studies have been conducted with Project J.

## Reproductive and Developmental Toxicity

Pivotal studies for embryo-fetal developmental toxicity were conducted with Project J in rats and rabbits.

A tabulated overview of reproductive toxicity studies are found in [End-of-Text Table 3.10] (nonpivotal) and [End-of-Text Table 3.11] (pivotal).

## Effects on Embryo-Fetal Development in Rats [Project J-TX-0012]

Project J was administered orally to groups of pregnant Crl:CD(SD) rats at dose levels of 0 (control), 10, 100, and 600 mg/kg during the period from implantation until closure of the hard palate (Days 7 to 17 of gestation) to investigate its effect on dams and embryo-fetal development.

There were no changes in dams thought to be attributable to the test article in the 10 mg/kg group.

Maternal food consumption was significantly low from Days 7 to 12 of gestation at 100 mg/kg compared with the control group.

At 600 mg/kg, one dam which had shown decreased spontaneous motility, soiled fur around nose, and emaciation was found dead on Day 14 of gestation, and 2 dams exhibiting decreased spontaneous motility were sacrificed due to moribund condition on Day 16 of gestation. Marked decreased body weight and food consumption were observed in all 3 of these dams. In the gross pathological examination, 2 of these 3 dams showed small spleen and small thymus. Decreased spontaneous motility (2 dams, on Days 16 or 17 of gestation) and emaciation (1 dam: on Days 16 to 17 of gestation) were also observed to a lesser extent in the surviving dams. Statistical significance for either suppression of body weight gain on Days 7 to 18 of gestation or low values in body weight on Days 9 to 20 of gestation were noted. Food consumption was significantly low from Days 7 to 18 of gestation compared with the control group. In the gross pathological examination, one dam showed small spleen, obscure thymus, and enlarged adrenals.

Regarding the effects on fetuses, no changes attributable to the test article were observed in the 10 and 100 mg/kg groups.

In the 600 mg/kg group, low fetal body weight, skeletal abnormalities (bent radius and ulna) in 1 fetus, and increased incidence of skeletal variations (incomplete ossification of the cervical arch and wavy rib) were observed. These changes were considered to be closely related to the severe maternal toxicity, and accordingly Project J was considered to have no teratogenicity. No changes in postimplantation loss rate, number of live fetuses, sex ratio, placental weight or morphology, external abnormalities, visceral abnormalities or variations, or numbers of ossified sternebrae or sacro-caudal vertebrae were noted.

Based on these results, the NOAEL was judged to be 10 mg/kg for dams and 100 mg/kg for embryo-fetal development. No teratogenicity was identified.

## Effects on Embryo-Fetal Development in Rabbits [Project J-TX-0014]

Project J was administered orally to pregnant female Kbl:NZW rabbits/group at dose levels of 0 (control), 10, 100 and 300 mg/kg during the period from implantation until closure of the hard palate (Days 6 to 18 of gestation) to investigate its effect on dams and embryo-fetal development.

No death occurred in any dam. As the maternal toxicity, clinical changes, including decreased food consumption, scant/no feces or scant/no urine, were observed at 100 mg/kg and above. In the 300 mg/kg group, decreases in body weights and body weight gain were also noted. In addition, abortion due to malnutrition associated with decreased food consumption was noted in 3 and 11 dams in the 100 and 300 mg/kg groups, respectively. No changes suggestive of maternal toxicity were noted in the 10 mg/kg group.

No treatment-related effects were noted in any treated group on the post-implantation loss (%), the number, sex ratio, body weights, placental weights of live fetuses, the incidence of external, visceral or skeletal morphology, skeletal variation or ossification findings or the

number of ossified bones of fetuses. As described above, however, abortion occurred frequently in the 300 mg/kg group and the number of dams in this group decreased to 8. Therefore, this group could not be included for estimation of the NOAEL of PROJECT J for embryo-fetal development.

Based on the results above, the NOAELs were concluded to be 10 mg/kg for maternal toxicity and 100 mg/kg for embryo-fetal development. No teratogenicity was identified.

## Local Tolerance

No local tolerance studies have been conducted with Project J.

## Other Toxicity Studies

The potential phototoxicity of Project J was evaluated in vitro. The potential immunotoxicity of Project J was evaluated in rats with T-cell independent antibody response and T-cell dependent antibody response.

A tabulated overview of the phototoxicity study is found in [End-of-Text Table 3.14], and a tabulated overview of immunotoxicity studies are found in [End-of-Text Table 3.15].

## In Vitro 3T3 Phototoxicity [Project J-TX-0017]

An in vitro phototoxicity test of Project J was conducted using Balb/c 3T3 clone A31 cells derived from mouse embryo at 0.469, 0.938, 1.88, 3.75, 7.5, 15, 30, and

60 μg/mL.

As a result, the cell growth was not inhibited 50% or more at any concentrations under any treatment condition. The mean photo effect (MPE) was less than 0.1 (actual value: -0.057), and Project J was categorized into no phototoxic.

It was concluded that Project J did not induce phototoxic response to Balb/c 3T3 clone A31 cells under the condition of this study.

## Immunotoxicity: T-Cell Independent Antibody Response (TIAR) in Rats [Project J-TX-0015]

This study was conducted to assess effects of PROJECT J on the T-cell independent antibody response. Project J was orally administered to Crl:CD(SD) rats (10 rats of each sex per group) for 4 weeks at doses of 0 (control), 10, 100 and 300 mg/kg, and recovery group (recovery period of 4 weeks, at doses of 0 and 300 mg/kg) was set to evaluate the reversibility of any observed changes. The rats were immunized with

2,4,dinitrophenyl-aminoethyl carboxymethyl-ficoll (DNP)-ficoll (100 µg/animal) on Day 21 via the caudal vein, and anti-DNP IgM and IgG antibodies were measured on Day 29.

No significant differences were observed in the productions of anti-DNP IgM or IgG antibodies between test article and control groups at the end of the dosing period. Therefore, T-cell independent antibody response was not measured and evaluated in the recovery period.

During the dosing period, no treatment-related changes were noted in clinical signs, food consumption, organ weight or necropsy in any test article groups. The changes observed in

body weight gain were considered to be of little toxicological significance and not to affect the evaluation of the antibody production because the animals did not exhibit deterioration of the systemic condition or the changes were very slight. During the recovery period, overgrown teeth in the clinical sign and abnormal grown teeth in the necropsy were observed. However, it was considered to be of little toxicological significance to the antibody response because the animals did not exhibit deterioration of the systemic condition.

In conclusion, PROJECT J did not affect the T-cell independent antibody response up to 300 mg/kg under the conditions of this study.

## Immunotoxicity: T-Cell Dependent Antibody Response (TDAR) in Rats [Project J-TX-0016]

This study was conducted to investigate the immunotoxicity of Project J in rats. To examine the effect of Project J on the T-cell dependent antibody response, Crl:CD(SD) rats (10 animals/sex/group) were orally administered with Project J once daily to males and females over 4weeks at doses of 0 (control), 10, 100 and 300 mg/kg. To examine the reversibility of immunotoxicological changes, recovery groups (10 animals/sex/group) were added in the control or 300 mg/kg groups, and were subjected to a 4-week recovery period after 4-week administration. Keyhole limpet hemocyanin (KLH) was administered as antigen for immunization on Days 14 and 23 of dosing or Days 14 and 23 of recovery, and blood was collected on Days 20 and 29 of dosing or on Days 20 and 29 of recovery (6 days after immunization).

In the 10 mg/kg group, no abnormal changes thought to be attributable to the test article in any parameter were noted in males or females. In the 100 mg/kg group, decreased anti-KLH IgM antibody levels on Day 20 of dosing in males and decreased anti-KLH IgG antibody levels on Day 29 of dosing in females were noted. In the 300 mg/kg group, decreased

anti-KLH IgM antibody levels on Day 20 of dosing and anti-KLH IgG antibody on Day 29 of dosing were noted in males, and decreased anti-KLH IgG antibody levels on Day 29 of dosing were noted in females. Histopathological examination of the spleen revealed narrowing of marginal zone in the 100 mg/kg and 300 mg/kg groups at the end of dosing.

In the 300 mg/kg recovery group, no differences in levels of anti-KLH antibodies and histopathological changes were noted compared to those in the control recovery group in males and females. Abnormal incisor in both sexes, decreased absolute and relative thymus weight and increased relative adrenal gland weight were observed in males.

Based on the above results, the NOAEL of Project J for T-cell dependent antibody response under the conditions of the present study was concluded to be 10 mg/kg in males and females.

# Integrated Nonclinical Overview and Conclusion: Potential Clinical Relevance

## Pharmacological Properties

PROJECT J induces a significant analgesic effect in nonclinical models of OA pain: the inflammatory AIA model and non-inflammatory MIA model. The pharmacological active dose range is considered to be 0.63 (ED50) to 3 mg/kg in the AIA model. The analgesic effects of PROJECT J are not affected by confounding factors such as decreased locomotion. The exposures associated with 0.63 mg/kg are 427 ng/mL for Ceff and 8842 ng·h/mL for AUCinf.

PROJECT J displayed selectivity to CB2 receptors compared for CB1 receptors, with respect to binding and functional agonistic activity. In addition, there was no evidence of a clinically relevant interaction of PROJECT J with 66 different kinds of receptors, ion channels, transporters and enzymes.

## The Rationale for Animal Selection for Toxicity Studies

Animal species used in the above-mentioned toxicity investigation (mouse, rat, rabbit and dog) were considered to be adequate to assess the safety of Project J in humans for the following reasons: no human specific metabolites were detected in the preliminary in vitro metabolic fingerprinting study using cryopreserved hepatocytes.

Pharmacological target of CB2 are expressed in rat, rabbit and dog. Crl:CD(SD) rat and beagle dog were used as a rodent and non-rodent species for repeated-dose toxicity studies, respectively. Kbl:NZW rabbit is a commonly used non-rodent species to assess toxicity of embryo-fetal development. Mice are commonly used for evaluation of genotoxic potential in vivo. In all four species, sufficient exposures were achieved compared to the anticipated clinically effective exposure.

## Target Organ Toxicity

The primary target organs identified in the safety pharmacology, single and repeated dose toxicity studies were the immune and male reproductive system, GI tract and erythropoietic system. Additional target organs/systems associated with higher doses were body temperature, kidney, liver and teeth. The toxicities in rats and dogs were generally reversible and adequate monitoring tools are available. In addition, adequate safety margin was obtained for toxicities associated with higher doses. A further discussion on clinical relevance of each target organ/system is given below. No teratogenicity was noted although maternal toxicity was observed. No in vivo genotoxicity and in vitro phototoxicity were identified.

Summary of target organ toxicity in repeat-dose toxicity studies is shown in [(Table 2](#_bookmark69)).

## Table 2 Summary of Target Organ Toxicity in Repeat-Dose Toxicity Studies

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Dose** | **Target organ** | **Findings** |
| Rat | ≥100 mg/kg [LOAEL] | immune system | narrowing of the splenic marginal zone |
| hematopoietic system | increase in reticulocyte |
| ≥300 mg/kg | general condition | low body weight, suppression of body weight gain, low food  consumption |
| CNS and clinical signs | decrease in body temperature |
| hematopoietic system | low hemoglobin, hematocrit, decrease in erythrocyte, high platelet, MCV, MCH, low thymus weight, high spleen weight,  blood saturation/ dilatation of splenic sinus |
| kidney | increase in urine KIM-1, TIMP-1, cystatin C, degenerative basophilic change of proximal tubule |
| liver and thyroid | decrease in serum α1-globulin, increase in serum β-globulin, high  liver and thyroid weight |
| incisor | whitening/ white discoloration of the incisor, single cell necrosis/  degeneration of papillary layer/ ameloblast |
| 600 mg/kg | general condition | decrease in stool volume, soiled perineal region, trace of reddish rhinorrhea |
| male reproductive organ | tubular atrophy/ spermatid giant cell of testis, ductal cell debris in  epididymis |
| CNS and clinical signs | salivation |
| hematopoietic system | increase in leukocyte and lymphocyte, extramedullary  hematopoiesis in spleen |
| kidney | increase in urine volume, low urine gravity, increase in urine  NGAL, osteopontin, albumin, dilatation of renal tubule, regeneration of collecting duct |
| Dog | ≥30 mg/kg [LOAEL] | general condition | decrease in body weight |
| male reproductive organ | low serum testosterone, atrophy of prostate |
| CNS and clinical signs | vomiting |
| 300 mg/kg [>MTD] | general condition | death, moribund, lateral/ prone position, suppressed response to  stimulation, emaciation, decrease in food consumption |
| male reproductive organ | low prostate weight, spermatid giant cell formation in testis,  single cell necrosis of ductal epithelium in epididymis |
| CNS and clinical signs | decrease in spontaneous activity, salivation |
| kidney | decrease in urine Na/ Cl, increase in serum BUN/ creatinine,  decrease in serum Ca/ Na/ K/ Cl |
| liver | increase in serum AST/ ALT/ ALP/ bilirubin/ total protein/ cholesterol, prolongation of PT/ APTT, high liver weight, multifocal/ single cell necrosis of hepatocyte, atrophy of  hepatocyte, inflammatory cell infiltration |
| gastrointestinal tract | soft/ mucous stool, reddish brown or dark red colored stool, diarrhea, mucosal erosion/ hemorrhage/ congestion/ mononuclear  cell/ neutrophil infiltration/ crypt abscess/ glandular epithelium regeneration of duodenum/ jejunum/ cecum/ rectum |
| cardiovascular system | decrease in heart rate, prolongation of QTc |
| other changes | decrease in erythrocyte, leukocyte, neutrophil, monocyte, eosinophil, basophil, low hemoglobin, hematocrit, platelet, high spleen weight, hypocellularity of sternal/ femoral bone marrow, atrophy of thymus/ Peyer’s patch, follicular atrophy of spleen/ mesenteric lymph node  increase in adrenal weight with decrease in lipid and hypertrophy of zona fasciculata, inflammation/ hemorrhage in lung, ductal dilatation of sublingual gland, atrophy of muscle fiber in skeletal  muscle |

ALP: alkaline phosphatase; ALT: alanine transaminase; APTT: activated partial thromboplastin time; AST: aspartate transaminase; BUN: blood urea nitrogen; CNS: central nervous system; LOAEL: lowest-observed- adverse-effect level; MCH: mean corpuscular hemoglobin; MCV: mean corpuscular volume; MTD: maximum tolerated dose; NGAL: neutrophil gelatinase-associated lipocalin; PT: prothrombin time; QTc: corrected QT

## Effects on immune system

Narrowing of the marginal zone of the spleen (corresponding to reduction in CD45RA-positive B-cells) was observed in rat 4-week repeat-dose toxicity study, and

significant reduction in T-cell dependent antibody response was noted in rat immunotoxicity study. The reversibility of these findings was confirmed. CB2 receptor is reported to be particularly abundant in immune cells, namely B-cells > natural killer cells >> monocytes

> neutrophils > T-cells; and CB2 receptor is reported to mediate immunosuppressive or anti-inflammatory activity of endogenous cannabinoid such as anandamide [Cencioni et al, 2010; Galiègue et al, 1995; Xu et al, 2007]. Therefore, these findings may be related to the pharmacological action. The clinical risk for infection in human subjects is regarded to be low, based on the absence of changes in plasma differential leukocyte counts or globulin levels, no histopathological changes in the thymus, and no related findings in dogs.

## Effects on male reproductive organs

Reduced spermatogenesis was noted in the testis both in rats and dogs. This finding might be related to the pharmacological effect, because CB2 receptor is expressed in testis, and potentially involved in spermatogenesis [Maccarrone, 2008]. Moreover, cannabinoid

(∆9-tetrahydrocannabinol) is reported to induce male infertility, via lowering luteinizing hormone (LH) level in hypothalamus, and accordingly reducing testosterone production by Leydig cells [Fasano et al, 2009; Habayeb et al, 2002].

In dogs, decreased serum testosterone levels and atrophy of the prostate defined LOAEL (30 mg/kg). In addition, low prostate weight, single cell necrosis of ductal epithelium of the epididymis, spermatid giant cell formation in the testis were observed at the highest dose of 300 mg/kg. Spermatid giant cells are most often formed in conjunction with a slow

non-specific germ cell degenerative process. These findings were recovered after 4-week drug withdrawal.

In rats, tubular atrophy/spermatid giant cell of the testis and ductal cell debris in the epididymis were observed in the dosing period, and atrophy in the seminiferous tubules with hyperplasia of the Leydig cells in the testis and ductal cell debris in the epididymis were noted even after 8-week recovery period.

Leydig cell hyperplasia is considered to be related to increased LH signalling; the clinical relevance of rodent Leydig cell hyperplasia for human is considered low [Clegg ED et al, 1997]. Although in rats the relationship with testosterone reduction was less clear, as there was no effect on the prostate or seminal vesicle, the findings are suggestive of a

functional -hormonal - change in response to PROJECT J exposure. Such changes are typically reversible, but testicular recovery is known to take long (>8 weeks). Potential effects on spermatogenesis can be monitored by measuring serum testosterone, LH, follicle stimulating hormone (FSH) and inhibin B.

## Effects on CNS and clinical signs

Vomiting was noted to define LOAEL (30 mg/kg) in dogs, and suggested the potential for nausea in humans.

Transient and slight decrease in body temperature was observed at ≥ 300 mg/kg in rats. Hypothermia may reflect central CB1 receptor activation, although no further evidence of CB1 receptor-related clinical signs were observed.

## Effects on hematopoietic system

In rats, high reticulocyte ratio defined LOAEL (100 mg/kg), and hematological changes regarded as anemia were noted at ≥ 300 mg/kg. Although the direct effect on erythrocytes was suggested, the change was only slight, and anemia can be monitored by hematology, and reversibility was confirmed.

## Effects on kidney

In rats, degenerative/regenerative changes in renal tubules were identified. These changes might be related to the increases in urine nephrotoxicity parameters, and were reversible.

In dogs, no histopathological changes were identified, although slight and reversible increases in serum BUN and creatinine and changes in urine and serum electrolytes were noted.

## Effects on liver and thyroid

In dogs, hepatocellular necrosis was noted in the liver, accompanied by increases in AST, ALT, ALP and total bilirubin at 300 mg/kg. Changes were not noted after 4-week recovery period.

In rats, high liver and thyroid weights without histopathological findings were noted. The changes were reversible.

## Effects on gastrointestinal tract

In dogs, the effects on gastrointestinal tract were suggested by the changes in stool condition sporadically noted in clinical observations during dosing period at 300 mg/kg. However, histopathological findings such as mucosal erosion in lower digestive tract might be caused by aggravated general conditions, because the animals showed severe body weight decrease at the end of the dosing period. Both clinical and histopathological findings were reversible.

## Effects on incisors

In rats, necrosis of ameloblasts and degeneration of the papillary layer in the incisors were noted at ≥ 300 mg/kg. The changes were reversible after a 8-week recovery period and consistent with dental fluorosis [Lyaruu et al, 2008]. The relevance to human was considered to be low because the effects were observed only in rat incisors which grow continuously, whereas no findings were noted in the molars in rat repeat-dose study. Further, these findings would be monitorable by plasma fluoride. Plasma fluoride was considered to be derived from defluorination of PROJECT J.

## Effects on embryo-fetal development

In the study of the effects on embryo-fetal development, no fetal abnormality was identified both in rats and rabbits at 100 mg/kg, and considered that PROJECT J has no teratogenicity. In rats, decreased fetal body weight, skeletal abnormality and increased skeletal variations were noted in fetus at 600 mg/kg, which were considered to be related to the severe maternal toxicity.

Evidence of maternal toxicity was noted, such as decrease in food consumption at

≥ 100 mg/kg both in rats and rabbits, death and moribundity at 600 mg/kg in rats, and abortion at ≥ 100 mg/kg in rabbits.

## Effects on cardiovascular system

In in vitro evaluations, hERG current inhibition was noted with an IC50 of 27 μmol/L (1467 times greater than the exposure at pharmacological effective dose), and APD prolongation was identified at 10 μmol/L (543 times greater than the exposure at

pharmacological effective dose) or more. However, these in vitro changes did not translate to in vivo changes in cardiovascular parameters following single dosing in dogs up to

1000 mg/kg (115 times greater Cmax than the value at pharmacological effective dose).

Significant reduction in heart rate with prolongation of QTc in a dog at 300 mg/kg was considered to be caused secondarily by aggravated general conditions, since changes were not seen in Week 1 when the dog was still in good condition. Standard cardiovascular monitoring is considered to be adequate.

## Exposure Assessment

Toxicokinetic parameters such as Cmax and AUC24 obtained by the toxicity studies in mice, rats, rabbits and dogs are presented in [End-of-Text Table 3.3]. Toxicokinetic parameters as well as exposure ratios to pharmacological effective dose in the definitive repeated dose toxicity studies in rats and dogs are presented in ([Table 3](#_bookmark71)).

In male mice, the systemic exposures were appropriately confirmed at 2000 mg/kg.

In rats, Cmax and AUC24 increased almost dose proportionally up to 600 mg/kg during the dosing period. Following repeated dosing, these parameters showed a tendency to remain almost constant. Exposures were slightly higher in females than in males.

In pregnant rabbits, Cmax and AUC24 increased less than dose proportionally up to 300 mg/kg both at first and last dosing. Following repeated dosing, these parameters increased approximately 2-fold at 10 mg/kg, almost constant at 100 mg/kg, and decreased at 300 mg/kg.

In dogs, Cmax and AUC24 increased almost dose proportionally up to 30 mg/kg at both first and last dosing, but increased less than dose-proportionally between 30 and 300 mg/kg.

Following repeated dosing, the parameters at Day 14 and 28 were slightly higher than those at Day 1. There was no apparent sex difference.

## Table 3 Exposure Ratios of Project J Based on Exposure at Pharmacological Effective Dose and Animal Cmax and AUC24 (at the Last Dose) in the Definitive Repeat-Dose Toxicity Studies in Rats and Dogs

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Species/ Study Duration** | **Dose** | **Sex (M/F)** | **Cmax** | | **AUC24** | |
| **Total concentrations (ng/mL)** | **Exposure ratios†** | **Total concentrations (ng·h/mL)** | **Exposure ratios†** |
| Rat/ 4 week po | 10 mg/kg  [NOAEL] | M | 785 | 13 | 2,540 | 3 |
| F | 2,067 | 35 | 16,942 | 18 |
| 100 mg/kg  [LOAEL] | M | 4,344 | 73 | 45,835 | 49 |
| F | 6,975 | 118 | 97,646 | 105 |
| 600 mg/kg | M | 17,065 | 289 | 187,085 | 201 |
| F | 17,097 | 289 | 213,845 | 230 |
| Dog/ 4 week po | 3 mg/kg  [NOAEL] | M | 1,983 | 29 | 27,077 | 25 |
| F | 2,466 | 36 | 38,316 | 35 |
| 30 mg/kg  [LOAEL] | M | 8,523 | 124 | 145,709 | 135 |
| F | 9,839 | 143 | 160,407 | 148 |
| 300 mg/kg  [>MTD] | M | 42,767 | 620 | 736,239 | 680 |
| F | 42,796 | 620 | 732,221 | 676 |
| Lewis AIA rat | 0.63 mg/kg  [ED50‡] | F | 563 | NA | 8,842 | NA |

LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level

† Based on the comparison using unbound concentration [unbound fraction in plasma: 0.10 for Lewis AIA rat,

0.14 for SD rat, 0.12 for dog], and species difference of 6.8 [rat CB2 ki: 36 nM / human CB2 ki: 5.3 nM]

‡ Pharmacologically effective dose (ED50) in Lewis AIA rat with Cmax value of 563 ng/mL and AUCinf value of 8,842 ng•h/mL [Project J-PH-0008].

## 4.4.5 Conclusion

PROJECT J showed robust signs of efficacy in models of both inflammatory (AIA) and

non-inflammatory (MIA) models of pain, indicating a potential use in the treatment of OA pain. There are no ADME-related or safety findings preventing the initiation of an PROJECT J single ascending dose (SAD) study.

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