### Safety Pharmacology

### Effects on Cardiovascular System

In 2 in vitro cardiovascular safety pharmacology studies, PROJECT 2 was shown to have no effect on the action potentials in isolated guinea-pig papillary muscles or on potassium current repolarization in hERG-transfected HEK293 cells up to the concentration of 1×10−6 mol/L (Studies Project 2-PT-0001 and Project 2-PT-0002).

In 2 in vivo cardiovascular safety pharmacology studies, there was no effect on the cardiovascular system of dogs following administration of oral PROJECT 2 at 300 mg/kg or intravenous PROJECT 2 at doses up to 0.4 mg/kg. As for the general activity and behavior, grayish stool (mixed with test substance) and loose stool were observed in dogs at 300 mg/kg following oral dosing. Loose stool and diarrhea was observed in the 0.12- and 0.4-mg/kg groups following intravenous dosing, respectively. However, it was judged to be incidental, since abnormal stool (loose stool or jelly like stool) was also observed in the control group (Studies Project 2-PT-0005 and Project 2-PT-0004).

### Effects on CNS

In vivo safety pharmacology studies were conducted in rats and dogs to evaluate the potential effects of PROJECT 2 on the CNS following oral or intravenous administration (Studies

Project 2-PT-0003, Project 2-PT-0004 and Project 2-PT-0005).

Following oral administration, PROJECT 2 at 300 mg/kg had no effect on the CNS of rats or dogs. As for the general activity and behavior, grayish stool and loose stool were observed in dogs (Study Project 2-PT-0005).

Following intravenous administration, PROJECT 2 at doses up to 0.4 mg/kg had no effect on the CNS of dogs. As for the general activity and behavior, vomiting was observed at

0.4 mg/kg at 6 hours post administration; however, blood concentration of PROJECT 2 was low at this time point. This effect was not considered attributable to PROJECT 2 (Studies

Project 2-PT-0004 and Project 2-PT-0005).

### 4.1.3.1.3 Effects on Respiratory System

In an in vivo respiratory safety pharmacology study in rats, PROJECT 2 at doses up to 300 mg/kg had no effect on respiratory rate, tidal volume or minute volume (Study Project 2-PT-0006).

### Pharmacodynamic Drug Interactions

No pharmacodynamic drug interaction studies have been conducted.

### Other Pharmacology Studies

No other pharmacology studies have been conducted.

## Toxicology

An overview of PROJECT 2 toxicology studies can be found in End-of-Text Tables 3.1 and 3.2. A summary of toxicokinetic data can be found in End-of-Text Table 3.3. PROJECT 2 was assessed in toxicology studies including single-dose toxicology, repeated-dose toxicology (up to 13 weeks in duration), genotoxicity (mutagenicity and clastogenicity) and developmental and reproductive toxicity (teratogenicity). All toxicology studies (with the exception of

dose-finding studies Project 2-TX-1001, Project 2-TX-1002 and Project 2-TX-1003) were performed in accordance with Japanese GLP standards (Notifications and Ordinances, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare); these standards are also in accordance with FDA GLP regulations.

### Single-dose Toxicity

A tabulated summary of the key findings from single-dose oral toxicity studies of PROJECT 2 in rats and dogs can be found in End-of-Text Table 3.4.

### Rat

In rats, a single oral dose of PROJECT 2 was administered at doses of 1000 or 2000 mg/kg. Following administration, clinical signs and mortality were observed, and body weight was measured. Gross pathology was conducted 14 days after dosing. There were no deaths in

any treatment group. No test material-related findings in clinical observation were noted in any of the groups. A slight decrease in body weight was observed in 3 females in the

2000-mg/kg group. Gross pathology showed that 2 males in the 1000-mg/kg group and 1 male in the 2000-mg/kg group had a large cecum. However, this finding was not

accompanied by abnormal histopathology [End-of-Text Table 3.4] (Study Project 2-TX-0004).

### Dog

In dogs, a single oral dose of PROJECT 2 was administered at doses of 500 or 2000 mg/kg. The plasma PROJECT 2 concentration was also measured to evaluate systemic exposure. During a 14-day period, general signs were observed; body weight and food consumption were measured; and hematology, clinical chemistry and toxicokinetic analysis were performed.

As the surviving animals were not euthanized, necropsy and histopathological examination were not performed [End-of-Text Table 3.4] (Study Project 2-TX-0003).

After a single oral administration of PROJECT 2 to dogs at doses of 500 and 2000 mg/kg, no deaths occurred in either group. Soft feces and increases in the total bilirubin level and GPT activity were observed. Additionally, vomiting and grayish white feces were noted in the 500- and 2000-mg/kg groups, respectively. All of the changes in blood chemistry resolved by day 14, with the exception of increased total bilirubin level in the males dosed with

500 mg/kg.

In toxicokinetic analyses, Cmax for the male and female was 4.084 and 3.588 ng/mL, respectively, and AUC0-24 was 13.199 and 26.793 ng·h/mL, respectively, in the 500-mg/kg group. The tmax for the males and females were both 0.5 hour. In the 2000-mg/kg group, Cmax for the males and females was 5.835 and 9.958 ng/mL, respectively, and AUC0-24 was

73.429 and 65.299 ng·h/mL, respectively. tmax values for the males and females were 8 hours and 0.5 hour, respectively. Exposure was nonlinear with dose increases.

### 4.3.2 Repeated-dose Toxicity

An overview of repeated-dose toxicity studies can be found in End-of-Text Tables 3.1 and

3.2. A summary of repeated-dose toxicity findings can be found in End-of-Text Tables 3.6.1-3.6.6.

### 4.3.2.1 Rat

In a 2-week oral dose range-finding study in rats, PROJECT 2 was orally administered at doses of 0 (0.5% MC), 30, 100 and 300 mg/kg per day. Rats were monitored for mortality and clinical signs; food consumption and body and organ weights were measured; hematology and clinical chemistry assessments and gross pathological and histopathological examinations were performed. No PROJECT 2-related effects were noted for clinical signs, body weight, food consumption, hematology or clinical chemistry at PROJECT 2 doses of up to 300 mg/kg per day. At ≥ 30 mg/kg per day, large cecum and an increase in cecal weight (including the contents), which was considered to be due to the antibacterial effect of the PROJECT 2 (Study FDR090010), were observed with no histopathological changes in males and females. At 300 mg/kg per day, an increase in relative kidney weight with no association of histopathological abnormalities was observed in males, and a histopathological change of

mild basophilic tubules in the kidney was observed in 1 female. In toxicokinetic satellite groups that were included in this study, the plasma PROJECT 2 concentrations were measured following a single oral dose of 300 and 1000 mg/kg; toxicokinetic analyses showed no clear differences in the Cmax or AUC0-24 values between the 300- and 1000-mg/kg groups. Based on the results of this study, the highest dose was set at 300 mg/kg per day for the 4-week oral dose study in rats (Study Project 2-TX-1001).

In a 4-week oral dose study in rats, PROJECT 2 was administered at doses of 0 (0.5% MC), 30, 100 and 300 mg/kg per day for 4 weeks. Rats were monitored for general signs; body weight, organ weight and food consumption were measured; ophthalmology, hematology, clinical chemistry and urinalysis assessments were performed; and gross pathological and histopathological examinations were noted. No treatment-related effects were noted in clinical signs, body weight, food consumption, ophthalmology, hematology, clinical chemistry or urinalysis at any dose levels. Enlarged cecum and increased absolute and relative weights of the cecum (before and after removal of the contents) were noted for both males and females at doses of ≥ 30 mg/kg per day; however, no associated histopathological changes were observed. PROJECT 2 is reported to have a weak antimicrobial activity

(Study FDR090010). It is known that the compounds with an antibacterial effect (such as antibiotics) cause enlarged cecum in rodent, as a result of changes in cecal microflora [Greaves, 2007]. Thus, the findings in rats with PROJECT 2 were presumed to be caused by its antimicrobial activity and, therefore, not considered to have toxicological significance.

Minimal vacuolation in the squamous epithelium of the limiting ridge between the forestomach and the glandular stomach was observed in 2 females at 300 mg/kg per day. No PROJECT 2-related findings were observed in the kidney. Toxicokinetic satellite groups (PROJECT 2 and control groups) were included to determine the plasma PROJECT 2 concentrations. Toxicokinetic analyses were conducted on plasma samples collected on

day 1 and during week 4. On day 1, Cmax and AUC0-24 values could not be obtained, because a majority of the plasma PROJECT 2 concentrations were below or around the LLOQ for males and females at 30, 100 and 300 mg/kg per day, resulting in low Cmax and AUC0-24 values.

Although the Cmax and AUC0-24 values tended to increase during week 4 in a dose-dependent manner for the males and females, the plasma PROJECT 2 concentrations were only slightly above the LLOQ. No clear sex differences were observed in the Cmax and AUC0-24 values.

The NOAEL was judged to be 100 mg/kg per day [End-of-Text Table 3.6.1] (Study Project 2-TX-0006).

In a 2-week intravenous study with a 2-week recovery period in rats, PROJECT 2 was administered at doses of 0 (vehicle: 5% ethanol in saline), 0.04, 0.12 and 0.4 mg/kg per day. To evaluate reversibility of any effects present following a 2-week nontreatment recovery period, additional rats were added to the vehicle and 0.4-mg/kg per day groups. The high dose level of 0.4 mg/kg per day was set for this study taking into account the solubility of PROJECT 2 in the vehicle as well as the effect of the vehicle on animals. Rats were monitored for clinical signs; food consumption and body and organ weights were measured; ophthalmology, hematology, clinical chemistry and urinalysis assessments were performed; and gross pathological and histopathological examinations were noted. Toxicokinetics were

also evaluated in toxicokinetic satellite groups. No PROJECT 2-related effects were noted in the main groups in clinical signs, body or organ weights, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, gross pathology or histopathology. No

PROJECT 2-related findings were observed in the kidney and the gastrointestinal (GI) tract in the main group and no findings were observed in the recovery group in any parameters evaluated at the end of the 2-week recovery period. In toxicokinetic satellite groups (PROJECT 2 and control) that were also included in this study, plasma PROJECT 2 concentrations were determined on day 1 and during week 2. Toxicokinetic analyses showed that the

C0 values were more than dose proportional on day 1 for both males and females, and less than dose proportional during week 2, except for the values for 0.12 mg/kg males. The AUC0-24 values were almost dose proportional on day 1 for both males and females, and less than dose proportional during week 2 for both males and females, except for the values for

0.12 mg/kg males. The NOAEL was judged to be 0.4 mg/kg per day, as 0.4 mg/0.5 mL/kg per day was the maximum concentration of soluble drug [End-of-Text Table 3.6.3]

(Study Project 2-TX-0008).

The epithelial vacuolation in the limiting ridge of the stomach in the 4-week oral dosing study was not observed in a 2-week intravenous study. These study results suggest that the cause of the vacuolation was not the result of the systemic exposure from intravenous dosing, but local irritation caused by oral dosing of the test article. Therefore, the change observed in the repeated oral dose study was suspected to have been caused by the irritability of the PROJECT 2.

In the 13-week oral dose study with a 4-week recovery period in rats, PROJECT 2 was administered at doses of 0 (0.5% MC), 30, 100 and 300 mg/kg per day. No treatment-related effects were noted in clinical signs, body weight, food consumption, ophthalmology, hematology, blood chemistry or urinalysis, gross pathology, or histopathology in any dose group. In all PROJECT 2 dose groups, increased absolute and relative weights of the cecum (before and after removal of the contents) were noted for both males and females, which was attributed to the known antibacterial activity effect of PROJECT 2 and considered to be toxicologically insignificant. No findings were observed in any parameters evaluated at the end of the recovery period. Toxicokinetics showed that, in general, mean Cmax and AUC0-24 increased in a less than dose proportional manner, and mean tmax ranged from 0.5 to 24 hours during the dosing period in all groups. Additionally, Cmax and AUC0-24 were independent of time elapsed during the dosing period on days 1, 49, and 91, and no clear sex differences were noted at any doses, except in the 30-mg/kg group in females on day 1 as a result of outliers observed in 2 females. The NOAEL was judged to be 300 mg/kg per day for both males and females [End-of-Text Table 3.6.5] (Study Project 2-TX-0011).

### 4.3.2.2 Dog

In a 7-day oral dose range-finding study in dogs, PROJECT 2 was administered at doses of

0 (0.5% MC), 30, 300 or 1000 mg/kg per day. Dogs were monitored for clinical signs; food consumption and body and organ weights were measured; hematology and clinical chemistry assessments and gross pathological and histopathological examinations were performed.

Toxicokinetics were determined on days 1 and 7. No PROJECT 2-related effects were observed except at 1000 mg/kg per day, at which the feces contained a white substance (considered to be unabsorbed PROJECT 2) was observed. There were no clear differences in the plasma Cmax and AUC0-24 values for PROJECT 2 among the dose levels. Based on the results of this study, the high dose for the 4-week oral repeated dose study in dogs was set at 300 mg/kg per day (Study Project 2-TX-1002).

In a 4-week oral repeated-dose study in dogs, PROJECT 2 was administered at doses of 0 (0.5% MC), 30, 100, and 300 mg/kg per day. Dogs were monitored for clinical signs; food consumption and body and organ weights were measured; ophthalmology, hematology, clinical chemistry and urinalysis assessments were performed; electrocardiography recordings were taken; and gross pathological and histopathological examinations were noted. Toxicokinetics were determined from the blood samples on day 1 and during week 4.

Grayish white soft feces were observed at 100 mg/kg per day in 1 male for 1 day and grayish-white feces (including grayish-white watery feces in 1 female for 1 day) were noted in all animals at 300 mg/kg per day for 1 to 10 days. The grayish-white colored feces were considered to contain unabsorbed PROJECT 2. No PROJECT 2-related effects were noted in body weight, food consumption, hematology, electrocardiography, ophthalmology, clinical chemistry, urinalysis, gross pathology, organ weight or histopathology. Toxicokinetic analysis on day 1 and during week 4 showed no clear differences in the Cmax and AUC0-24 values for the doses in this study. Comparison of the Cmax and AUC0-24 values indicated no clear difference between values on day 1 and during week 4. The NOAEL was judged to be 300 mg/kg per day [End-of-Text Table 3.6.2] (Study Project 2-TX-0005).

In a 7-day intravenous dose range-finding study in dogs, PROJECT 2 was administered at doses of 0 (vehicle), 0.04, 0.12, or 0.4 mg/kg per day by bolus. Dogs were monitored for mortality and clinical signs; food consumption and body and organ weights were measured; hematology and clinical chemistry assessments and gross pathological and histopathological examinations were performed. Toxicokinetics were determined on days 1 and 7. A decrease in white blood cell counts was observed in the female at 0.12 mg/kg per day and in the male and female at 0.4 mg/kg per day. However, there were no associated changes in different white blood cell counts and histopathology in organs relevant to the hematopoietic system.

Histopathological examination revealed slight or moderate hypertrophy of straight proximal tubule in the males at 0.12 and 0.4 mg/kg per day and slight deposition of hyaline droplet of convoluted proximal tubule in the kidney in the female at 0.4 mg/kg per day. C0 and AUC0-24 values for PROJECT 2 increased in a nonlinear dose-dependent manner for the doses in this study and these values were similar on day 1 to day 7. Based on the results of this study, the solubility of PROJECT 2 in the vehicle (physiological saline containing 5% ethanol) as well as the effect of the vehicle on animals, the PROJECT 2 high dose level was set at 0.04 mg/kg per day for the 2-week intravenous study with a 2-week recovery period in dogs (Study

Project 2-TX-1003).

In a 2-week intravenous study with a 2-week recovery period in dogs, PROJECT 2 was administered at doses of 0 (vehicle), 0.04, 0.12 and 0.4 mg/kg per day. To evaluate the reversibility of any effects present following a 2-week untreated recovery period, additional dogs were used in the 0.4-mg/kg per day group. Dogs were monitored for clinical signs; food

consumption and body and organ weights were measured; ophthalmology, hematology, clinical chemistry and urinalysis assessments were performed; electrocardiography readings were taken and gross pathological and histopathological examinations were noted.

Toxicokinetics were determined from the blood samples collected on days 1 and 14. No PROJECT 2-related effects were noted in clinical signs, body weight, food consumption, electrocardiography, ophthalmology, hematology, clinical chemistry, urinalysis, gross pathology, organ weight or histopathology. No PROJECT 2-related findings were observed in the kidney and GI tract. No findings were observed in any parameters evaluated at the end of the recovery period.

Toxicokinetic analyses showed that C0 and AUC0-24 values tended to increase in a

dose-dependent manner in both males and females on days 1 and 14. There was no gender difference. The NOAEL was judged to be 0.4 mg/kg per day [End-of-Text Table 3.6.4] (Study Project 2-TX-0007).

In the 13-week oral dose study with a 4-week recovery period in dogs, PROJECT 2 was administered at doses of 0 (0.5% MC), 30, 100 and 300 mg/kg per day. No treatment-related effects were noted in clinical signs, body weight, food consumption, ophthalmology, electrocardiogram, hematology, blood chemistry or urinalysis, gross pathology, or histopathology in any dose group. No findings were observed in any parameters evaluated at the end of the recovery period. Toxicokinetics showed that mean Cmax and AUC0-24 increased in a less than dose proportional manner, and tmax was almost constant during the dosing period for each dose group. Additionally, there were no consistent gender differences and no obvious repeated dose effect. The NOAEL was judged to be 300 mg/kg per day for both males and females [End-of-Text Table 3.6.6] (Study Project 2-TX-0012).

### 4.3.3 Genotoxicity

The potential genotoxicity of PROJECT 2 was evaluated in vitro and in vivo.

### 4.3.3.1 In Vitro Reverse Mutation Test

A bacterial reverse mutation test was performed with 5 strains of bacteria (TA98, TA100, TA1535 and TA1537 [*Salmonella typhimurium*] and WP2*uvr*A [*Escherichia coli*]), using the pre-incubation method with and without metabolic activation in order to assess the potential of PROJECT 2 to induce gene mutation. The dose levels were set at 39.1, 78.1, 156, 313, 625, 1250 and 2500 mcg/plate in TA98 and WP2*uvr*A without metabolic activation, 19.5, 39.1,

78.1, 156, 313, 625 and 1250 mcg/plate in TA100, TA1535 and TA1537 without metabolic activation, 19.5, 39.1, 78.1, 156, 313, 625 and 1250 mcg/plate in TA98, TA100, TA1535 and TA1537 with metabolic activation, and 78.1, 156, 313, 625, 1250, 2500 and 5000 mcg/plate in WP2*uvr*A with metabolic activation. PROJECT 2 did not induce gene mutation in bacteria when tested under the conditions of the study [End-of-Text Table 3.7.1] (Study Project 2-TX-0001).

### In Vitro Chromosomal Aberration Test

A chromosomal aberration test was performed with cultured mammalian cells (CHL/IU cells), using short-term treatments for 6 hours with and without metabolic

activation and continuous treatment for 24 hours without metabolic activation in order to evaluate whether PROJECT 2 induces chromosomal aberrations. The dose levels were set at 300, 400, 500, 600, 700 and 800 mcg/mL in the short-term treatment with metabolic

activation, 100, 200, 300, 400, 500, and 600 mcg/mL in the short-term treatment without

metabolic activation, and 50, 100, 200, 300, 400 and 500 mcg/mL in the continuous treatment for 24 hours, and the incidence of cells having structural and numerical chromosomal aberrations was investigated. Vehicle (dimethyl sulfoxide) was tested as the negative control. Positive controls were a mitomycin C-treated group for treatment without metabolic activation and a benzo[a]pyrene-treated group for treatment with metabolic activation. PROJECT 2 did not induce chromosomal aberrations in CHI/IU cells, regardless of the presence or absence of metabolic activation, or treatment length under the conditions of this study [End-of-Text Table 3.7.2] (Study Project 2-TX-0002).

### Micronucleus Test in Rats

To examine clastogenic potency of PROJECT 2 based on an induction of micronuclei in rat erythroblasts, PROJECT 2 (4, 8 and 16 mg/kg) or vehicle (5% ethanol in saline) was intravenously administered to male and female rats once daily for 2 days. No significant increase in micronucleated immature erythrocytes was noted in any of the PROJECT 2 dose groups as compared to the control group. In both genders, a significant decrease in the ratio of immature erythrocytes was observed in the 16-mg/kg group when compared to the control group; therefore, PROJECT 2 was judged to have an inhibition effect on bone marrow cell proliferation. In the 16-mg/kg group, ataxic gait and decreased body weight were also observed in both sexes. In conclusion, under these conditions, PROJECT 2 did not induce micronuclei from chromosomal aberrations in male or female rats in vivo [End-of-Text Table 3.8.1] (Study Project 2-TX-0014).

### Carcinogenicity

No long-term carcinogenicity studies have been conducted with PROJECT 2.

### Reproductive and Developmental Toxicity

Studies to assess the teratogenic potential of PROJECT 2 were conducted in rats and rabbits.

### Fertility and early embryonic development to implantation in Rat

To examine effects of PROJECT 2 on the fertility of males and females and early embryonic development to implantation, PROJECT 2 (30, 100 and 300 mg/kg) or vehicle (0.5% MC) was administered to male and female rats once daily for 2 weeks before mating and throughout the mating period, and up to the day before necropsy for males and up to day 7 of gestation for females. No deaths or clinical abnormalities were observed in the control group or any dose group. Decreased food consumption was observed in all dose groups on day 4 and in the 300-mg/kg group on day 8. However, since these were transitory changes and no effect on body weight or general condition was observed in any dose group, this change was judged to be of little toxicological significance. Enlarged cecum was observed in the males of all dose groups, which was attributed to the known antibacterial activity of PROJECT 2. Enlarged cecum was not observed in any of the female dose groups, as necropsy was not performed

immediately after the end of PROJECT 2 administration. Due to the observed decreased food consumption, the NOAEL was estimated to be less than 30 mg/kg per day for general toxicity in males and females. Regarding fertility and early embryonic development in males and females, no PROJECT 2-related effects were noted in estrous cycles, days until copulation, copulation index, fertility index, number of corpora lutea, number of implantations, pre- and postimplantation loss or number of live embryos in any dosage group. Therefore, it was considered that the NOAEL for the fertility or early embryonic development for males and females was 300 mg/kg [End-of-Text Table 3.11.1] (Study Project 2-TX-0013).

### Embryo-Fetal Development in Rat

In the nonpivotal, 2-week, oral, repeated-dose toxicity study in rats, enlarged cecum and increase in the weight of the cecum, which was thought to be caused by antimicrobial activity of the test article (Study FDR090010), were observed for males and females at ≥ 30 mg/kg per day. In addition, oral absorption of the test article was low and there were no clear differences in PROJECT 2 Cmax or AUC0-24 between the 300-mg/kg per day and 1000-mg/kg per day groups after a single oral administration. Therefore, the doses in this definitive study were set at 0 (0.5% MC), 30, 100 and 300 mg/kg per day. PROJECT 2 was administered orally to pregnant rats on gestation day (GD) 7 to 17 to evaluate the effects on dams and embryo fetal development. Animals were sacrificed on GD 20 and necropsied. The following items were examined: the number of corpora lutea, number of implantations, index of embryo-fetal lethality, number of live fetuses, body weight, placenta weight or sex ratio of live fetuses, external appearance, skeletal and visceral observations of live fetuses. Toxicokinetics were determined from the blood samples collected on GD 7 and GD 17.

For dams, no deaths occurred in any group and there were no PROJECT 2-related changes or abnormalities in clinical signs or body weight. In all dose groups, food consumption was significantly lower than in the control group on GD 8. However, since it was a transitory change and no effect on the body weight was observed, this change was judged to be of no toxicological significance. In the 30-, 100- and 300-mg/kg per day groups, distention of cecum was observed in 1, 4 and 2 dams, respectively. The same change was also observed in the repeated toxicity study in rats, and it was thought to be caused by the antimicrobial activity of PROJECT 2 (Study FDR090010).

For embryo-fetal development, there were no PROJECT 2-related changes in the number of corpora lutea, number of implantations, index of embryo-fetal deaths, number of live fetuses, body weight of live fetuses or sex ratio of live fetuses. There were no abnormalities in the placenta or placenta weight in the dosage groups. In the external appearance, skeletal and visceral examinations of live fetuses, some abnormalities or variations were observed.

However, since all of them are observed spontaneously in rats and there were no PROJECT 2-related increase in the incidence of particular abnormalities or variation in the

external, visceral or skeletal examination in this study, it was judged that the test article did not show teratogenicity. No PROJECT 2-related effects were noted in the progress of ossification.

In toxicokinetic analyses, both Cmax and AUC0-24 increased with dose, but showed signs of leveling off at ≥ 100 mg/kg per day after the first administration. After the final administration, both Cmax and AUC0-24 were higher than those after the first administration. Cmax and AUC0-24 were comparable between dose levels at 100 and 300 mg/kg per day. The NOAEL of PROJECT 2 was estimated to be 300 mg/kg per day for general toxicity in dams and embryo-fetal development [End-of-Text Table 3.11.2] (Study Project 2-TX-0010).

### Embryo-Fetal Development in Rabbit

In a nonpivotal 5-day repeated oral dose study in nonpregnant rabbits, PROJECT 2 was administered at dose levels of 10, 30, 100 and 300 mg/kg per day. Since toxic effects of the test article were not clear for rabbits, the highest dose level was set at 300 mg/kg per day, which was the same as the highest dose in an exploratory study using rats. Since rabbits are generally known to be more sensitive to antibacterial agents than rats the lowest dose of

10 mg/kg per day was added. The following items were examined: clinical signs, body weight, food consumption and necropsy.

Decreased amount of feces lasting 2 or more successive days was observed in all animals at dose ≥ 30 mg/kg per day. All animals in the 100- and 300-mg/kg per day groups had brownish urine on the day of necropsy. The decrease in the group mean body weight was statistically significant throughout the administration period including on day 6 (necropsy) at 100 and 300 mg/kg per day and on days 5 and 6 of administration at 30 mg/kg per day. The decrease in the group mean food consumption was statistically significant throughout the administration period including on day 6 at 100 and 300 mg/kg per day and from days 4 to 6 of administration at 30 mg/kg. The food consumption on day 2 in the 100- and 300-mg/kg groups was very low (< 10 g/day; normal mean consumption is 100 to 200 g/day). However, there were no deaths in any group. Based on findings such as significantly lower food consumption in the 100- and 300-mg/kg per day groups, 30 mg/kg per day, which is expected to produce slight toxic effects in dams, was judged to be suitable for the highest dose in the definitive study [End-of-Text Table 3.11.3] (Study Project 2-TX-0009).

The dose levels in the pivotal study were set at 0 (0.5% MC), 3, 10 and 30 mg/kg per day in which PROJECT 2 was administered orally to pregnant rabbits on GD 6 to 18 to evaluate potential effects on dams and embryo fetal development. Animals were sacrificed on GD 28, and then necropsied. The following items were examined: the number of corpora lutea, number of implantations, index of embryo-fetal lethality, number of live fetuses, body weight, placenta weight or sex ratio of live fetuses, external appearance, skeletal and visceral observations of live fetuses. Toxicokinetics were determined from the blood samples collected on GD 6 and GD 18.

For dams, no deaths were observed in any treatment group including the control, with the exception of 1 death at 30 mg/kg per day on GD 17. Three dams at 10 mg/kg per day aborted. General toxicological effects included suppression of body weight gain, decrease in food consumption, decreased fecal amount and brownish urine discharge at 10 and 30 mg/kg per day. No PROJECT 2-related findings were observed in general signs, measurement of body

weight, food consumption or gross pathological examination in any dam treated at 3 mg/kg per day.

As for the effects on embryo-fetal development, 30 mg/kg per day produced apparent evidence for embryo-fetal lethality (high values for index of embryo-fetal death and low values for number of live fetuses). No treatment-related effects on external abnormalities, visceral abnormalities (except at 30 mg) or variations or skeletal abnormalities or variations as well as placental abnormalities were noted in any treated group.

Embryo-fetal lethality was considered to be induced by PROJECT 2 as a result of suppression of body weight gain and degeneration of general condition. PROJECT 2 is thought to have antibacterial activities that induced changes in rabbit gut microflora (Study FDR090010). It is thought that these changes were manifested as poor appetite and that the consequent suppression of body weight gain and degeneration of general condition results in embryo- fetal lethality. However, this may be species-specific effect, as rabbits are known to be sensitive to intestinal effects from antibacterial agents [Clark et al, 1986].

In toxicokinetic analyses, no definitive description could be made because the data had large inter-individual differences. However, plasma PROJECT 2 concentration appeared to be

dose-dependent. The NOAEL of PROJECT 2 was judged to be 3 mg/kg per day for general toxicological effect in dams and 10 mg/kg per day for embryo-fetal development

[End-of-Text Table 3.11.3] (Study Project 2-TX-0009).

### Local Tolerance

No local tolerance studies of PROJECT 2 have been conducted to date.

### Other Toxicity Studies

No other toxicity studies of PROJECT 2 have been conducted to date.

## Integrated Nonclinical Overview and Conclusions: Potential Clinical Relevance

PROJECT 2 is a new molecular entity discovered by Astellas Pharma Inc. that has an agonistic effect on the melanocortin receptor and shows suppression of inflammatory cytokine production. PROJECT 2 is considered to act directly on inflammatory lesions in the colon, thereby possibly avoiding systemic adverse events commonly associated with other UC treatments.

Data from nonclinical toxicokinetic and metabolic studies conducted with PROJECT 2 indicate that adequate doses were tested in appropriate in vitro and in vivo models to sufficiently evaluate the potential toxicity profile of PROJECT 2 in human studies. All findings were evaluated for relevance to human risk assessment and impact on clinical trial design.

PROJECT 2 was poorly absorbed when administered orally to rats and dogs with normal colons. The absolute bioavailability in rats and dogs was < 0.02% and < 0.2%, respectively. The Cmax and AUC0-24 were low in the oral dose studies. However, PROJECT 2 is believed to act directly on the mucosa in the colon. Therefore, exposure in plasma is not indicative of

efficacy, and results from a nonclinical adsorption, distribution, metabolism and elimination study suggest that PROJECT 2 is available in the gastrointestinal tract based on the recovery of intact drug from feces.

It is not known whether PROJECT 2 can be absorbed systemically in patients with UC lesions or whether the permeability of the colonic mucosa is higher at lesion sites. Therefore, intravenous repeated-dose studies were conducted to further investigate the potential systemic toxicity of PROJECT 2. No toxic changes were observed in any of the treatment groups in the intravenous repeated-dose studies. As a result, the potential toxicity of PROJECT 2 in clinical trials is considered to be low. There were no potential toxic effects of PROJECT 2 on the CNS, cardiovascular, respiratory or immune systems nor was there evidence of potential liver toxicity, clastogenicity or teratogenicity.

Potential adverse effects to the kidney were observed in repeated-dose studies in rats and dogs. In a nonpivotal 2-week oral repeated-dose study in rats, the finding of basophilic tubules was noted at 300 mg/kg per day. In the subsequent pivotal 4- and 13-week oral dose studies, this finding was not reproduced. This result suggests that the renal change suggestive of repair from cell damage occurred at an early stage of test article treatment and it recovered within the 4-week repeated dose period. Therefore, this change was considered to be of marginal toxicological significance.

In a nonpivotal 1-week intravenous repeated-dose study in dogs, hypertrophy of proximal straight renal tubules was seen at 0.12 and 0.4 mg/kg per day and hyaline droplet in the proximal convoluted renal tubules was observed at 0.4 mg/kg per day. However, these findings were not reproduced in the subsequent pivotal 2-week intravenous study. In general, these types of findings were considered to persist so far as drug treatment is continued.

Since, the changes observed in 1-week study were not seen in a longer 2-week study, they are considered to have little toxicological significance.

Potential adverse effects to GI tract were observed in repeated dose studies in rats. In the pivotal 4- and 13-week oral repeated dose studies in rats, enlarged cecum and increased weight of the cecum were observed for males and females (at ≥ 30 mg/kg/day). However, these changes were not associated with related histopathological lesions. PROJECT 2 is reported to have a weak antimicrobial activity (Study FDR090010). It is known that the compounds with an antibacterial effect (such as antibiotics) cause enlarged cecum in rodent, as a result of changes in cecal microflora [Greaves, 2007]. Thus, the findings in rats with PROJECT 2 were presumed to be caused by its antimicrobial activity. Therefore, these findings were not considered to be of toxicological significance. No diarrhea or other toxicological finding associated with antimicrobial activity was observed in the repeated toxicity studies in rats and dogs. In this pivotal 4-week study, minimal vacuolation in the squamous epithelium of the limiting ridge between the forestomach and the glandular stomach was observed at 300 mg/kg per day. However, this finding was not reproduced in the subsequent pivotal 13-week study. As this change was not observed in the pivotal 2-week intravenous study in rats, the change was attributed to irritability associated with oral administration of PROJECT 2. Therefore, findings of potential concern for clinical trials may include GI adverse effects.

A summary of the findings of potential clinical interest observed in rat and dog repeated-dose toxicity studies and a human repeated-dose phase 1 study is presented in [Table 8.](#_bookmark68) None of the findings in nonclinical safety pharmacology and toxicology studies or the single- and multiple-dose phase 1 studies described within this Investigator’s Brochure would preclude further development of PROJECT 2. These data suggest that careful monitoring of GI symptoms should be performed during clinical trials of PROJECT 2.

### Table 8: Margin Compared to Systemic Exposure between Animals and Human

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Study**  Treatment-Related Change | | **Nonclinical Toxicokinetic data†** | | | | **Margin compared to 1000 mg (bid) in human‡** | |
| **Cmax or C0 (iv)**  **(ng/mL)** | | **AUC0-24**  **(ng·h/mL)** | | **Cmax** | **AUC** |
| **Male** | **Female** | **Male** | **Female** |
| **Rat 4-wk oral toxicity study** | **mg/kg** |  | | | | | |
| Enlarged Cecum§ | ≥30 | 0.798 | 0.503 | 4.645 | 1.252 | 0.22 | 0.17 |
| NOAEL | 100 | 2.052 | 1.105 | 7.734 | 4.258 | 0.49 | 0.57 |
| Vacuolation in squamous epithelium of the limiting  ridge of stomach | 300 | -- | 1.965 | -- | 16.101 | 0.87 | 2.16 |
| **Rat 13-wk oral toxicity study** |  |  | | | | | |
| Enlarged Cecum§ | ≥30 | 0.738 | 0.786 | 2.596 | 6.017 | 0.33 | 0.35 |
| NOAEL | 300 | 1.957 | 1.880 | 30.460 | 21.744 | 0.84 | 2.92 |
| **Rat 2-wk (iv) toxicity study** |  |  | | | | | |
| NOAEL | 0.4 | 77.044 | 108.958 | 64.749 | 68.221 | 34.3 | 8.7 |
| **Dog 4-wk oral toxicity study** |  |  | | | | | |
| Grayish white feces§, †† | ≥100 | 3.451 | 8.406 | 24.043 | 35.602 | 1.5 | 3.2 |
| NOAEL | 300 | 3.526 | 8.342 | 27.219 | 49.570 | 1.6 | 3.7 |
| **Dog 13-wk oral toxicity study** |  |  | | | | | |
| Grayish white feces§, †† | >100 | 2.754 | 2.553 | 13.873 | 30.664 | 1.2 | 1.9 |
| NOAEL | 300 | 3.243 | 3.156 | 17.200 | 17.130 | 1.4 | 2.3 |
| **Dog 2-wk (iv) toxicity study** |  |  | | | | | |
| NOAEL | 0.4 | 261.191 | 306.396 | 151.647 | 169.310 | 116.1 | 20.4 |

--: not observed; NOAEL: no observed adverse effect level.

† Toxicokinetic data obtained at the last dose was used in each study.

‡ Cmax: 2.249 ng/mL, AUC: 7.448 ng·h/mL (Study Project 2-CL-0002, n = 9, day 14, AUC0-12h x2). Margins were calculated using lower values of male or female animals in each study (underlines: values used).

§ These findings were evaluated to be secondary changes caused by the antibacterial effect of PROJECT 2.

†† Feces containing test article-like material.

Source: Studies Project 2-TX-0005 (4-week oral dog); Project 2-TX-0006 (4-week oral rat); Project 2-TX-0011 (13-week oral rat); Project 2-TX-0012 (13-week oral dog); Project 2-TX-0007 (2-week iv dog); and Project 2-TX-0008 (2-week iv rat)