### Safety Pharmacology

PROJECT 4 has been evaluated in 4 core safety pharmacology studies. A detailed description of these studies and their results can be found in End-of-Text Table 1.2. Key findings are discussed below.

### CNS Safety Pharmacology

**Rats**

The potential effect of PROJECT 4 hydrobromide on the central nervous system was evaluated in a GLP modified-Irwin test in rats (Company Report Project 4-PT-0001). Animals were administered vehicle (0.5% w/v MC suspension) or PROJECT 4 (10, 30 and 100 mg/kg) and were then monitored for general physical condition and behavior. PROJECT 4 concentrations in plasma were determined to detect systemic exposure.

PROJECT 4 at 10 mg/kg had no effect on general physical condition or behavior. However, at 30 mg/kg, an increase in locomotion and slight staggering gait were observed as early as 15 minutes after dosing. At 100 mg/kg, rats developed a prone position, an increase in locomotion with a slight or severe staggering gait, and loss of the righting reflex. These changes at 30 and 100 mg/kg disappeared by 2 and 6 hours after dosing, respectively. In the 100 mg/kg group, lacrimation was observed as early as 2 hours after dosing.

Toxicokinetics results for the 10, 30 and 100 mg/kg groups showed that Cmax and the area under the plasma PROJECT 4 concentration versus time curve from time 0 to 24 hours (AUC24) increased greater than dose proportionally. No dose-dependent difference was noted in the time to reach Cmax (tmax) of PROJECT 4.

It was thus concluded that under the conditions of this study, single oral dosing of PROJECT 4 hydrobromide at 30 mg/kg and greater to rats induced CNS effects.

**Cynomolgus Monkeys**

In a GLP safety pharmacology study conducted in cynomolgus monkeys, the effect of PROJECT 4 was evaluated in 4 unanesthetized males at doses of 0 (vehicle, 0.5% w/v MC suspension), 1, 10 and 30 mg/kg (Company Report Project 4-PT-0002). Dosing was performed in a crossover design on days 1, 8, 15 and 22 (first to fourth dosing). PROJECT 4 concentrations in plasma were measured to define systemic exposure.

There were no PROJECT 4-related changes to the intra-abdominal body temperature at 1 mg/kg, but at 10 and 30 mg/kg, intra-abdominal body temperature decreased between 1 and 8 hours after dosing. No behavioral abnormalities were observed in any animal at 1 mg/kg.

However, vomiting was observed between approximately 0.5 and 9 hours after dosing

10 mg/kg in 2 animals and between approximately 0.5 and 5 hours after dosing at 30 mg/kg in all 4 animals. In addition, between 1 and 4 hours after dosing, blunted responses to stimulation and slightly decreased spontaneous activity were observed in three animals and pale oral mucosa was observed in 1 animal.

Cmax and AUC24 increased less than dose proportionally. The tmax did not differ between dose levels.

It was concluded that, under the conditions of this study, PROJECT 4 at 1 mg/kg had no effect on intra-abdominal body temperature or clinical signs. At 10 and 30 mg/kg, decreased

intra-abdominal body temperature was observed. Vomiting was observed at PROJECT 4 doses

≥ 10 mg/kg and suppressed response to stimulation, slightly decreased spontaneous activity and pale oral mucosa were observed at 30 mg/kg.

### Cardiovascular Safety Pharmacology

**In Vitro Studies**

PROJECT 4 was evaluated in 2 in vitro GLP cardiovascular safety pharmacology studies: (1) IKr in hERG-transfected HEK293 cells using the whole-cell patch-clamp technique (Company Report Project 4-PT-0003) and (2) APD in isolated guinea-pig papillary muscles under a

surface-superfusing condition (Company Report Project 4-PT-0004).

In the IKr assay conducted in HEK293 cells, the peak amplitude of tail currents was measured in 5 separate cells in each experimental group and the suppression rate of the amplitude was calculated 10 minutes after application of PROJECT 4 (3×10−7, 3×10−6 and 3×10−5 M), positive- control substance (E-4031 at 1×10−7 M) and vehicle (dimethyl sulfoxide [DMSO] at 0.1% volume /volume [v/v]). The mean suppression rate of the vehicle-control group was used to compensate for variations in rundown in hERG current in determination of the suppression rate in each cell. The effects of PROJECT 4 and positive-control substance were evaluated using the compensated suppression rates.

The data indicate that in the vehicle-control group (DMSO; 0.1% v/v), the hERG current was reduced by 8.1% 10 minutes after application of PROJECT 4. The hERG-current compensated suppression rates of PROJECT 4 at concentrations of 3×10−7 and 3×10−6 M were 5.7% and 8.7%, respectively. No statistically significant difference was observed at these

2 concentrations when compared to the rate in the vehicle-control group. However, at the highest concentration tested in this study (3×10−5 M), the compensated suppression rate was 22.1%, which was statistically greater compared to the vehicle-control group. In the positive control group (E-4031), the compensated suppression rate was 86.3%, which was statistically greater compared to the vehicle-treated cells, confirming the validity of the test. These results indicate that PROJECT 4 does not affect the hERG current in hERG-transfected HEK293 cells up to a concentration of 3×10−6 M and that it suppressed the hERG current by approximately 22% (P<0.05) at the highest test concentration (3×10−5 M).

In the APD assay conducted in isolated guinea-pig papillary muscles, PROJECT 4 (3×10−7, 3×10−6 or 3×10−5 M), DMSO at 0.1% v/v (vehicle) and E-4031 at 1×10−7 M (positive control) were surface-superfused for 30 minutes over tissue preparations (5 muscles per experimental group). The resting membrane potential (RMP), action-potential amplitude (APA), rate of maximum upstroke velocity (dV/dtmax), and action potential duration at 30% and 90% repolarization (APD30 and APD90) were recorded before (baseline) and 30 minutes after treatment. The difference between APD30 and APD90 (APD30-90) and other changes from baseline in action potential parameters were then calculated.

The data indicate that there was no effect on any of the action-potential parameters in the vehicle control group and no significant effects were observed in the 3×10−7 M PROJECT 4 group. Neither PROJECT 4 nor E-4031 had any effect on RMP, APA or dV/dtmax at any of the concentrations tested. PROJECT 4 did not elicit prolongation of action potential duration, but rather, showed a concentration-dependent shortening of APD30, APD90, and APD30-90 at 3×10−6 M (−7.5%, −5.2% and −1.6%, respectively) and 3×10−5 M (−9.7%, −7.6% and −3.9%,

respectively). E-4031, the positive control, prolonged APD30, APD90 and APD30-90 by 19.7%, 33.5% and 58.1%, respectively, confirming the validity of the test. These results indicate that PROJECT 4 does not prolong action potential duration in guinea-pig papillary muscles.

**In Vivo Studies**

In a GLP safety pharmacology study conducted in cynomolgus monkeys, the effect of PROJECT 4 on cardiovascular parameters was evaluated in 4 unanesthetized males at doses of 0 (vehicle, 0.5% w/v MC suspension), 1, 10 and 30 mg/kg (Company Report Project 4-PT-0002). Dosing of PROJECT 4 was performed in a crossover design on days 1, 8, 15 and 22 (first to fourth dosing). Astemizole (10 mg/kg) was administered as a positive control for QTc prolongation subsequent to evaluation of the fourth dose of PROJECT 47 (on day 29).

PROJECT 4 concentrations in plasma were measured to determine systemic exposure.

The data indicated that a 1 mg/kg dose of PROJECT 4 did not affect any of the following: systolic, diastolic or mean blood pressure, heart rate or any electrocardiogram (ECG) parameters including PR interval, QRS duration, RR interval, QT interval and QTc corrected with Fridericia’s formula (QTcF). At the 10 and 30 mg/kg doses of PROJECT 4, heart rate was decreased in all 4 animals between 1 and 8 hours after dosing and was slightly reduced from the pre-dose rate in 3 of the animals. No clear changes in blood pressure (systolic, diastolic or mean) were observed at any either dose. QTc prolongation (10-12% compared to the pre- dose value) was observed 2 hours after dosing in 1 animal administered 10 mg/kg of PROJECT 4 and between 2 and 8 hours after dosing in 1 animal administered 30 mg/kg of PROJECT 4. In another animal, U waves were observed intermittently between 1 and 8 hours after administration of PROJECT 4 doses of 10 and 30 mg/kg, but they were not observed

2 hours after administration of the 30 mg/kg dose when tmax was observed, nor were any clear changes in QTc [observed (see Table 5](#_bookmark31)). There were no PROJECT 4-related changes in PR interval or QRS duration at 10 or 30 mg/kg. In contrast, astemizole prolonged the QT interval and QTcF; the maximum mean prolongation of QTcF from the pre-dosing value was 13.8%, which was observed at 1 hour after astemizole treatment.

### Table 5: Percentage Change in QTc

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Test Substance** | **Percentage Change in QTc (%)** | | | | | |
| **Pre** | **1 hour** | **2 hours** | **4 hours** | **8 hours** | **24 hours** |
| 0.5 % (w/v) Methylcellulose | 100.0 | 97.3 | 93.8 | 94.5 | 93.8 | 100.5 |
| PROJECT 4 hydrobromide 1 mg/kg† | 100.0 | 100.0 | 96.3 | 97.5 | 97.0 | 100.8 |
| PROJECT 4 hydrobromide 10 mg/kg† | 100.0 | 105.8 | 104.8 | 103.0 | 102.0 | 98.5 |
| PROJECT 4 hydrobromide 30 mg/kg† | 100.0 | 103.0 | 103.8 | 103.5 | 104.5 | 99.8 |
| Astemizole 10 mg/kg | 100.0 | 113.8 | 108.8 | 104.8 | 104.3 | 110.3 |

QTc: corrected QT interval; w/v: weight per volume.

† As PROJECT 4 freebase.

Source: Company Report Project 4-PT-0002.

At a dose of 1 mg/kg, there were no PROJECT 4-related changes in any electrolytes (ionized calcium, sodium, potassium and chloride). At PROJECT 4 doses of 10 and 30 mg/kg, plasma potassium decreased between 1 and 4 hours after dosing; however, there was no change in any other electrolyte.

It was concluded that PROJECT 4 at a dose of 1 mg/kg had no effect on the cardiovascular system or electrolytes. At doses of 10 and 30 mg/kg, PROJECT 4 decreased heart rate, prolonged QTcF and decreased plasma potassium levels.

### Respiratory Safety Pharmacology

In a GLP safety pharmacology study conducted in cynomolgus monkeys, the effect of PROJECT 4 on respiratory safety parameters was evaluated in 4 unanesthetized males at doses of 0 (vehicle, 0.5% w/v MC suspension), 1, 10 and 30 mg/kg (Company

Report Project 4-PT-0002). Dosing was performed in a crossover design on days 1, 8, 15 and 22 (first to fourth dosing). Plasma concentrations of PROJECT 4 were measured to determine systemic exposure ([Table 6](#_bookmark33)).

### Table 6: Summary of Toxicokinetic Parameters after Single Oral Doses of PROJECT 4 in the Monkey Safety Pharmacology Study

|  |  |  |  |
| --- | --- | --- | --- |
| **Pharmacokinetics Parameters**† | **Dose of PROJECT 4**‡ | | |
| **1 mg/kg** | **10 mg/kg** | **30 mg/kg** |
| Cmax (ng/mL) | 32.005 | 177.160 | 363.170 |
| AUC24 (ng•h/mL) | 241.713 | 1,771.040 | 2,735.382 |
| tmax (h) | 1.3 | 1.5 | 1.5 |

AUC24: area under the concentration versus time curve from time zero to 24 hours after administration; Cmax: maximum concentration in plasma; tmax: time of Cmax.

† Calculation of toxicokinetic parameters was based on 3 animals/group/time point. Other pharmacokinetic parameters were calculated from the mean plasma PROJECT 4 freebase concentration versus time profiles.

‡ Dose levels are expressed as PROJECT 4 hydrobromide Source: Company Report Project 4-PT-0002.

There were no changes in respiratory rate or in any blood gas parameter (arterial blood pH, arterial oxygen and carbon dioxide tensions, hemoglobin and oxygen saturation) after administration of 1 mg/kg PROJECT 4. At doses of 10 and 30 mg/kg, PROJECT 4 slightly increased arterial carbon dioxide tension between 1 and 8 hours after dosing by about 2 to 7 mm Hg; however, there was no change in any other respiratory parameter.

It was concluded that PROJECT 4 at a dose of 1 mg/kg had no effect on the respiratory system. At doses of 10 and 30 mg/kg, PROJECT 4 increased arterial carbon dioxide tension.

## Toxicology

### Overview of the Toxicology Program

PROJECT 4 was evaluated in 5 exploratory and 9 pivotal toxicology studies. A tabulated overview of toxicology studies can be found in End-of-Text Table 3.1. A tabulated overview of toxicokinetic data can be found in End-of-Text Table 3.3.

The pivotal studies included single dose and 4-week repeated-dose studies both in rats and monkeys, 2 in vitro genotoxicity studies (bacterial reverse mutation and chromosomal aberration tests), and reproductive toxicity studies for embryo-fetal development in both rats and rabbits. All pivotal toxicology studies were conducted in accordance with the standards of Japanese Good Laboratory Practices (GLP) (Notifications and Ordinances, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare). These standards are also in accordance with Food and Drug Administration (FDA) GLP regulations.

### General Toxic Signs and Pathologic Effects

### Single Dose

Single-dose toxicity studies are summarized in End-of-Text Table 3.4. The acute toxicity of PROJECT 4 was evaluated in 2 GLP studies in rats (Company Report Project 4-TX-0001) and cynomolgus monkeys (Company Report Project 4-TX-0002). The animals were administered a single oral dose of PROJECT 4 dissolved in a vehicle of 0.5% w/v MC suspension. Key findings from these studies are discussed below.

### Rats

PROJECT 4 was administered once orally by gavage to Sprague-Dawley (SD) rats (5/sex/group) at 7 weeks of age, at dose levels of 0 (vehicle), 100, 300, 1000 and 2000 mg/kg

in males and 0, 30, 100, 300 and 600 mg/kg in females (Company Report Project 4-TX-0001). The following items were examined: mortality, clinical signs, body weight and gross pathology. Histopathologic analysis was reserved only for identified gross lesions.

One of 5 males in the 300 mg/kg group and all males in the 1000 and 2000 mg/kg groups (5 animals each) died on the day of dosing. Clinical signs observed in males on the day of dosing included the following: staggering gait, tremors and/or decrease in movement at all doses tested. Additionally, irregular respiration and/or prone position were noted in males administered PROJECT 4 doses of 300, 1000 and 2000 mg/kg. In addition, subnormal body surface temperature and scant feces were observed in the surviving males in the 300 mg/kg

group. Subnormal body surface temperature was also noted from 2 hours after dosing in 1 of the males in the 1000 mg/kg group prior to death. Gross pathology revealed a smudge around the nose in 2 dead males in each of the 1000 and 2000 mg/kg groups and a dark reddish change of the lungs in 1 dead male in the 1000 mg/kg group. Localized congestion was observed histopathologically in the lungs and is thought to account for the observed gross lesion.

Four of 5 females in the 300 mg/kg group died on the day of dosing. In the 600 mg/kg group, 1 of 5 females died on the day of dosing and the remaining 4 females died 1 day after dosing.

Clinical signs observed in females included staggering gait at ≥ 30 mg/kg and tremors, decrease in movement, prone positioning and/or irregular respiration at ≥ 100 mg/kg on the day of dosing. In addition, the following findings were noted in females: reddish tears in the 100 mg/kg group 6 hours after dosing; subnormal body surface temperature, lateral positioning and/or lacrimation in the 300 and 600 mg/kg groups on the day of dosing; staggering gait and scant feces in the only surviving female in the 300 mg/kg group 1 day after dosing; cyanosis in 1 female in the 600 mg/kg group prior to death on the day of dosing; and no feces 1 day after dosing in females in the 600 mg/kg group, all of which subsequently died. Decreased body weight was noted in 1 female in the 300 mg/kg group 1 day after dosing. Gross pathology revealed dark reddish change of the lungs in 1 dead female in the 600 mg/kg group. Localized congestion was observed histopathologically in the lungs and is thought to account for the observed gross lesions.

As described above, treatment-related findings were noted at ≥ 100 mg/kg and deaths occurred at ≥ 300 mg/kg in both males and females. Therefore, the approximate lethal dose was estimated to be 300 mg/kg for both sexes.

### Cynomolgus Monkeys

PROJECT 4 at doses of 50 and 100 mg/kg was administered once orally by gavage to 1 male and 1 female cynomolgus monkey (Company Report Project 4-TX-0002). The following items were examined: mortality, clinical signs, body weight, food consumption, electrocardiography, hematology, clinical chemistry, gross pathology, organ weight and histopathology. Plasma PROJECT 4 concentrations were determined to detect systemic exposure (see [Table 6](#_bookmark33)).

At a dose of 50 mg/kg of PROJECT 4, staggering gait was observed in both the male and female monkey from 0.5 or 2 hours after dosing on the dosing day (day 0). Incomplete eyelid opening, decrease in spontaneous activity, abnormal positioning (lateral or sitting positioning) and blunted response to stimulation were also observed in the male; mydriasis and decreased spontaneous activity were observed in the female. These findings improved by 8 hours after dosing and had completely disappeared by the next morning. Food consumption decreased on day 0 in the male and from day 0 to day 2 in the female. Body weight was decreased slightly on day 1 in both animals, but recovered by day 4. No PROJECT 4-related changes were noted in other examinations.

At a dose of 100 mg/kg, staggering gait was observed in both the male and female monkey as early as 0.5 hour after dosing. Incomplete eyelid opening, mydriasis, vertical nystagmus, decreased spontaneous activity, abnormal positioning (lateral or supine positioning), blunted response to stimulation and unresponsiveness to touch, twitching, and clonic convulsions were also observed in the male; incomplete eyelid opening and decreased spontaneous activity were observed in the female. These findings improved by 8 hours after dosing and disappeared by the next morning with the exception of staggering gait observed in the male. Vomiting was observed in the female in the morning on day 1. No abnormalities were observed in either animal in the afternoon or thereafter. Food consumption decreased in the male and female on day 0 and body weight was decreased on day 1. Food consumption

recovered on day 1, body weight recovered by day 4 and no abnormalities were noted in food consumption or body weight thereafter. In blood chemistry, inorganic phosphorus, sodium and chloride increased in the male after administration of 100 mg/kg on day 1, but recovered by day 8. No PROJECT 4-related changes were noted in other examinations.

### Repeated Dose

Nonpivotal dose range finding repeated dose toxicity studies of PROJECT 4 conducted in rats and monkeys are summarized in End-of-Text Table 3.5. Tabulated results of PROJECT 4 pivotal 4-week oral repeated dose toxicity studies conducted in rats (Company

Report Project 4-TX-0003) and monkeys (Company Report Project 4-TX-0004) can be found in End- of-Text Tables 3.6.1 and 3.6.2, respectively. Key findings are presented below.

### Rats – 4 Week Study

PROJECT 4 was orally administered once daily (QD) for 4 weeks to male and female SD rats (10/sex/group) at 0 (vehicle, 0.5% w/v MC suspension), 10, 100 and 300 mg/kg for males and 0 (vehicle), 10, 30 and 100 mg/kg for females. Five animals of each sex were added to the control and highest dose groups (300 mg/kg for males and 100 mg/kg for females) to evaluate the reversibility of any toxicity observed during the dosing period in a subsequent

4-week recovery period. The following items were examined: mortality, clinical signs, body weight, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, gross pathology, organ weight and histopathology. Plasma PROJECT 4 concentrations were also measured on days 1, 14 and 28 to determine systemic exposure.

In the low dose group (10 mg/kg for males and females), no PROJECT 4-related toxic effects were evident in any animal.

In the middle dose group (100 mg/kg for males and 30 mg/kg for females), staggering gait and decreased body weight gain in males and females and salivation in males were noted. Staggering gait and decreased body weight gain were noted between days 1 and 4 of dosing only; salivation was noted on days 22 to 28 of dosing. Increased eosinophilic bodies in the epithelium of the proximal tubules in kidney were observed in males.

In the high dose group (300 mg/kg for males and 100 mg/kg for females), staggering gait, decreased movement, salivation, reddish tears and lacrimation were observed in almost all males and females either throughout the dosing period or during the first half of the dosing period. Transient irregular respiration was observed in one male. Decreased body weight gain was noted throughout the dosing period in males and on day 4 of dosing in females. Food consumption was decreased in males on day 4 of dosing, but was increased in females between days 11 and 28 of dosing. Decreased potassium excretion level/day in males was noted in the urinalysis. In addition, increased kidney weights in males and females, eosinophilic bodies in the epithelium of the proximal tubules in males and centrilobular hypertrophy of hepatocytes associated with increased liver weights in males and females were noted. Centrilobular hypertrophy of hepatocytes was found to be caused by a slight increase in smooth endoplasmic reticulum upon electron microscopic examination. The

findings during the dosing period were considered reversible since all of them had resolved during the recovery period.

Plasma PROJECT 4 concentrations increased with dose. Females administered 100 mg/kg and males administered 300 mg/kg showed clear biphasic PROJECT 4 concentrations in plasma on dosing days 1, 14 and 28. The Cmax and AUC24 values in females administered 10 and

100 mg/kg were at least 2 fold higher than those in males. Cmax and AUC24 values on days 14 and 28 relative to day 1 were either comparable to or approximately 2-fold higher.

In this 4-week repeated oral dose toxicity study of PROJECT 4 in rats, the no observed adverse effect level (NOAEL) was 10 mg/kg/day. No PROJECT 4-related changes were noted after the recovery period.

### Cynomolgus Monkeys - 4 Week Study

PROJECT 4 was orally administered once daily for 4 weeks to cynomolgus monkeys (3/sex/group) at 0 (vehicle, 0.5% w/v MC suspension), 1, 3, 10 and 30 mg/kg. Three animals of each sex were added to the 30 mg/kg group to evaluate the reversibility of any toxicity observed during the dosing period in a subsequent 4-week recovery period. The following items were examined: mortality, clinical signs, body weight, food consumption, ophthalmologic parameters, electrocardiography, hematology, clinical chemistry, urinalysis, gross pathology, organ weight and histopathology. Plasma PROJECT 4 concentrations were also measured on days 1, 14 and 28 to determine systemic exposure.

No animals died in any group during the dosing or recovery period.

In the 1 and 3 mg /kg groups, no PROJECT 4-related changes were noted in any examination. At a dose of 10 mg/kg of PROJECT 4, symptoms were observed in all males and females.

Staggering gait was observed from day 1 to 2 in 2 males and 1 female, as was a decrease in spontaneous activity and/or incomplete eyelid opening. Staggering gait was also observed in 1 male and 2 females. These symptoms were observed from 1 hour after dosing and disappeared by 8 hours after dosing. Vomiting was observed in 2 females. Food consumption was decreased in all males and 1 female; these values were lower in males from day 1 to day 4. Body weight decreased transiently in 1 male.

At a dose of 30 mg/kg of PROJECT 4, symptoms were observed in all males and females. Staggering gait, decreased spontaneous activity and/or incomplete eyelid opening were observed from day 1 in all animals. Abnormal positioning (sitting, lateral or prone positioning), blunted response to stimulation with a loss of responsiveness to touch, somnolence, tremor, mydriasis, salivation and/or vertical nystagmus were also observed in 5 males and 2 females. In 1 female, staggering gait was the only sign observed for 11 days.

Most of these symptoms were observed by 1 hour after administration of PROJECT 4 and began to subside by 2.5 hours after treatment; however, staggering gait sometimes persisted for 8 hours. Vomiting was observed in 3 males and 4 females for 1 to 6 days and was most pronounced between 2.5 and 8 hours after dosing. Food consumption was decreased in 4 males and all females for 1 to 13 days during the first 2 weeks of the dosing period. A decrease was also noted in 2 males and 1 female on day 25. In 1 of these males, body weight decreased through the dosing period, but recovered by day 7 of the recovery period. There was a decreased or tendency toward decreased heart rate noted in males and females that occurred 2 hours after dosing on day 22 and individual heart rates were markedly decreased in 2 males and 1 female. Decreased erythrocyte count, hematocrit level and hemoglobin concentration were observed in 1 female on day 26. Decreased albumin and albumin/globulin ratio were observed in 1 female on day 26. No PROJECT 4-related changes were observed in ophthalmologic evaluations, urinalysis, gross pathology, organ weight or histopathology examinations.

There were no PROJECT 4-related changes observed in any of the animals in the recovery period.

Cmax and AUC24 increased with increasing dose in both sexes and showed no clear change after repeated dosing. There were no differences between doses or sexes for tmax.

In this 4-week repeated oral dose toxicity study of PROJECT 4 in cynomolgus monkeys, the NOAEL was 3 mg/kg/day.

### Genotoxicity

PROJECT 4 has been evaluated in 2 GLP in vitro genotoxicity studies: a bacterial reverse mutation assay (Company Report Project 4-TX-0005) and a chromosome aberration test (Company Report Project 4-TX-0006). Tabulated results for these studies can be found in End- of-Text Tables 3.7.1 and 3.7.2, respectively.

PROJECT 4 did not induce gene mutation when tested in 5 strains of bacteria (*Salmonella typhimurium* [TA98, TA100, TA1535 and TA1537] and *Escherichia coli* [WP2*uvrA*]). In addition, PROJECT 4 did not induce chromosomal aberrations in CHL/IU cells, regardless of treatment length or the presence or absence of metabolic activation. It was concluded that when tested under the conditions of these in vitro studies, PROJECT 4 does not exhibit genotoxic potential.

### Carcinogenicity

No carcinogenicity studies of PROJECT 4 have been conducted to date.

### Reproductive and Developmental Toxicity

Nonpivotal dose range finding reproductive and developmental toxicity studies of PROJECT 4 conducted in rats and rabbits are summarized in End-of-Text Table 3.8. Tabulated results of pivotal reproductive and developmental toxicity studies conducted to evaluate the effects of PROJECT 4 on embryo fetal development conducted in rats (Company Report Project 4-TX-0007) and rabbits (Company Report Project 4-TX-0008) can be found in End-of-Text Tables 3.9.1 and 3.9.2, respectively. Key findings are presented below.

### Embryo Fetal Development in Rats

PROJECT 4 was administered orally to pregnant female SD rats (19-20/group) at daily doses of 0 (vehicle, 0.5% w/v MC suspension), 10, 30 and 100 mg/kg during the period from implantation until closure of the hard palate (gestational day [GD] 7-17). Animals underwent

Cesarean section on day 20 of gestation to evaluate the effects on dams and embryo-fetal development. A toxicokinetic satellite group consisting of 8 pregnant females (4 pregnant females in the control group) was set up for each dosage group to measure plasma PROJECT 4 concentration to evaluate systemic exposure.

General toxicological effects on dams were staggering gait at ≥ 30 mg/kg and increased irritability, reddish fur around the eyes and salivation in the 100 mg/kg group. In addition, suppressed body weight gain and decreased food consumption were observed at doses

≥ 10 mg/kg. There were no PROJECT 4-related effects observed in any group for necropsy findings, number of corpora lutea, number of implantations or implantation rate.

There were no PROJECT 4-related effects observed in the number of live fetuses, number of embryo-fetal deaths, post-implantation loss, fetal weights, placental weights, sex ratio or external/visceral/skeletal morphology in any group. These data indicate that PROJECT 4 showed no potential of fetal growth retardation, fetal lethality or teratogenicity.

Both Cmax and AUC24 increased with increasing dose levels on GD 7 and GD 17. Cmax and AUC24 were comparable on GD 7 and GD 17 in each treatment group, thus confirming systemic exposure of PROJECT 4 during the treatment period.

Based on these data, the NOAEL of PROJECT 4 was judged to be <10 mg/kg/day for dams and 100 mg/kg/day for embryo-fetal development.

### Embryo Fetal Development in Rabbits

PROJECT 4 was administered orally to pregnant female New Zealand White (NZW) rabbits (17-19/group) at doses of 0 (vehicle, 0.5% w/v MC suspension), 10, 30 and 100 mg/kg on GD 6 to GD 18, corresponding to the period from implantation to closure of the hard palate in rabbit fetuses, to evaluate the potential adverse effects on pregnant animals and embryo- fetal development (embryo-fetal death, growth retardation and external, visceral and skeletal malformations). Clinical observations and measurements of body weight and food consumption were conducted on the dams. On GD 29, all dams underwent Cesarean section and necropsy to determine the following parameters: numbers of corpora lutea, implantations, live and dead fetuses, body weight, placental weight, sex, and external, visceral and skeletal morphology of live fetuses. PROJECT 4 plasma concentrations were measured on the first day of dosing (GD 6) and on the last day of dosing (GD 18) to evaluate systemic exposure.

All dams survived the duration of the study. Decrease in movement, rapid respiration, prone positioning, lateral positioning, weakness and/or salivation were observed in the 100 mg/kg group after dosing. Two dams in the 30 mg/kg group aborted, but no abortion occurred in the 100 mg/kg group. In the 100 mg/kg group, although no treatment-related effects were evident in body weight or body weight gain, decreased food consumption was noted. No PROJECT 4-related effects were observed for the reproductive function of dams.

There were no PROJECT 4-related effects evident in the viability, growth or external/visceral/skeletal morphology of embryos or fetuses.

Plasma PROJECT 4 concentrations on GD 6 and GD 18 increased more than dose- proportionally. Cmax and AUC24 values on GD 18 were higher than those on GD 6 at each dose level.

Based on these data, the NOAEL of PROJECT 4 was considered to be 30 mg/kg/day for maternal general toxicity and 100 mg/kg/day for maternal reproductive function and embryo-fetal development.

### Local Tolerance

No local tolerance studies of PROJECT 4 have been conducted to date.

### Other Toxicity Studies

No other toxicity studies of PROJECT 4 have been conducted to date.

## Integrated Overview and Conclusions: Potential Clinical Relevance

The nonclinical toxicity profile of PROJECT 4 was evaluated for relevance to human risk and impact on clinical trial design. The minimum pharmacologically effective dose in humans is predicted to be approximately 0.3 to 10 mg/day based on pharmacological activity in various models of cognitive impairment in mice. Noteworthy findings of potential clinical interest observed in rat and monkey 4-week repeated-dose toxicity studies are presented in [Table 12](#_bookmark76). Potential PROJECT 4 target organ toxicity is discussed in Sections 4.5.1 through 4.5.5.

### Table 12: Noteworthy Findings of Potential Clinical Interest in Rat and Monkey 4-week Repeated-Dose Toxicity Studies

|  |  |  |
| --- | --- | --- |
| **System**  Noteworthy Findings | **Dose Level of Observed Response (mg/kg) *[HED]* (mg/kg)**† | |
| **Rat** | **Monkey (Cynomolgus)** |
| **4 Week** | **4 Week** |
| **Clinical Signs and General Condition** | | |
| Body Weight ↓ | -- | 30F *[9.6]*, 10 M *[5.4]* |
| Body Weight gain ↓ | 100 M *[16]*, 30 F *[4.8]* | -- |
| Decrease in movement | 100 F *[16]*, 300 M *[48]* | -- |
| Food Consumption ↓ | 300 M *[48]* | 10 M *[5.4]*, 10 F *[5.4]* |
| Food Consumption ↑ | 100 F *[16]*§ | -- |
| Decreased spontaneous activity | -- | 10 M *[5.4]*, 10 F *[5.4]* |
| Ataxic gait | -- | 10 M *[5.4]*, 10 F *[5.4]* |
| Staggering gait | 30 F *[4.8]*, 100 M *[16]* | -- |
| Incomplete eyelid opening | -- | 10 M *[5.4]*, 10 F *[5.4]* |
| Irregular respiration | 300 M *[48]* | -- |
| Lacrimation | 100 F *[16]*, 300 M *[48]* | -- |
| Vomiting | -- | 10 F *[5.4]*, 30 M *[9.6]* |
| Salivation | 100 M *[16]*, 100 F *[16]* | 30 F *[9.6]*, 30 M *[9.6]* |
| Tremor | -- | 30 F *[9.6]*, 30 M *[9.6]* |
| Reddish tears | 100 F *[16]*, 300 M *[48]* | -- |
| Response to stimulation ↓ | -- | 30 F *[9.6]*, 30 M *[9.6]* |
| Somnolence | -- | 30 F *[9.6]*, 30 M *[9.6]* |
| Vertical nystagmus | -- | 30 F *[9.6]*, 30 M *[9.6]* |
| Lack of response to touch | -- | 30 F *[9.6]*, 30 M *[9.6]* |
| Mydriasis | -- | 30 F *[9.6]*, 30 M *[9.6]* |
| *Table continued on next page* | | |

*Table 12 (continued)*

|  |  |  |
| --- | --- | --- |
| **System**  Noteworthy Findings | **Dose Level of Observed Response (mg/kg) *[HED]* (mg/kg)**† | |
| **Rat** | **Monkey (Cynomolgus)** |
| **4 Week** | **4 Week** |
| **Clinical Signs and General Condition** *(continued)* | | |
| Sitting position | -- | 30 F *[9.6]*, 30 M *[9.6]* |
| Prone position | -- | 30 F *[9.6]*, 30 M *[9.6]* |
| Lateral position | -- | 30 F *[9.6]*, 30 M *[9.6]* |
| **Electrocardiography** | | |
| Heart rate ↓ | -- | 30 F *[9.6]*, 30 M *[9.6]* |
| **Hematology** | | |
| Erythrocyte count ↓ | -- | 30 F *[9.6]* |
| Hemoglobin ↓ | -- | 30 F *[9.6]* |
| Hematocrit ↓ | -- | 30 F *[9.6]* |
| **Clinical Chemistry** | | |
| Glucose ↑ | 100 F *[16]* | -- |
| Creatinine ↓ | 30 F *[4.8]* | -- |
| Cholesterol ↑ | 300 M *[48]* | -- |
| Phospholipids ↑ | 100 M *[16]* | -- |
| Inorganic phosphorus ↑ | 300 M *[48]* | -- |
| Calcium ↑ | 100 M *[16]* | -- |
| Sodium ↓ | 300 M *[48]* | -- |
| Potassium ↑ | 300 M *[48]* | -- |
| Chloride ↑ | 100 F *[16]*, 100 M *[16]* | -- |
| Albumin | -- | 30 F *[9.6]* |
| Albumin/globulin ratio ↑ | 100 M *[16]* | 30 F *[9.6]* |
| **Urinalysis** | | |
| Potassium ↓ | 300 M *[48]* | -- |
| **Organ Weights-Relative** | | |
| Liver | 10 F *[1.6]*, 100 M *[16]* | -- |
| Kidney | 100 F *[16]*, 300 M *[48]* | -- |
| **Histopathology** | | |
| Liver (hypertrophy, hepatocyte, centrilobular) | 100 F *[16]*, 300 M *[48]* | -- |
| Kidney (increase, eosinophilic body,  epithelium, proximal tubule) | 100 M *[16]* | -- |

-- Adverse response not observed; F; female; HED: human equivalent dose; M: male.

† Lowest dose level at which a change in one or more of the listed parameters occurred as noted in report summary.

*HED* was based on human weight of 60 kg and scaling on the basis of mg/m2.

‡ Value taken from the recovery period only: 300 mg/kg M, *HED [48 mg/kg]*

§ Also observed during the recovery period: 100 mg/kg F, *HED [16 mg/kg]*

Source: Company Reports Project 4-TX-003 (4-week rat study) and Project 4-TX-004 (4-week monkey study).

### Effects on Liver

In the 4-week study in rats, liver weight was increased at PROJECT 4 doses ≥ 10 mg/kg in females and ≥ 100 mg/kg in males. In addition, centrilobular hypertrophy of hepatocytes was observed at the highest doses (300 mg/kg for male and 100 mg/kg for female). This histopathological change was considered to be due to metabolic enzyme induction because, upon electron microscopic examination, an increased number of hepatocytes with smooth endoplasmic reticulum were observed. These liver abnormalities were not accompanied by changes in hepatic enzymes or pathological lesions of other related organs and they were not observed after a 4-week recovery period. Furthermore, no such changes in the liver were

observed in the 4-week monkey study. Thus, the toxicological significance of this finding is considered to be minimal.

### Effects on Kidney

In the 4-week study in rats, eosinophilic bodies in the epithelium of the proximal tubules were observed in male rats at PROJECT 4 doses ≥ 100 mg/kg. This lesion was periodic acid- Schiff (PAS) negative and, therefore, considered to be related to alpha-2-microglobulin.

Accumulation of alpha-2-microglobulin in the kidneys of male rats is known to be a highly species- and sex-specific change [Uwagawa et al, 1999]. The toxicological significance of this renal change as well as the mechanism remain unclear; however, it is unlikely to be of relevance to humans.

### Effects on the Gastrointestinal Tract

In the 4-week study in monkeys, vomiting was observed at PROJECT 4 doses ≥ 10 mg/kg. Bouts of vomiting were transient and generally occurred during the first week of dosing. Vomiting was not associated with pathological lesion of the gastrointestinal tract. While vomiting was considered drug-related, some animals had no vomiting even at the highest doses of PROJECT 4. On the other hand, in the preliminary 1-week study, vomiting was observed at 3 mg/kg, which is the NOAEL in the 4-week study, suggesting that the sensitivity to vomiting may vary greatly among individual animals. The mechanism by which PROJECT 4 induces vomiting is unclear, but current data indicate that the exposure level at 3 mg/kg (approximately 12-15 times higher than the Cmax expected at the clinical dose) is the threshold at which vomiting may occur. In future clinical trials, symptoms related to nausea will be carefully assessed.

### Effects on the CNS

In the rat CNS study, neurological signs were observed at PROJECT 4 doses of 30 and 100 mg/kg and included staggering gait, increased locomotion, prone positioning and a

disappearance of the righting reflex. Similar findings were commonly observed in all species used in the toxicity studies and considered to be related to the pharmacological action of PROJECT 4. They were not accompanied by histopathological changes to the CNS and promptly disappeared during the recovery period. Generally, staggering gait was the most frequent sign observed at the lowest observed effect level (LOEL). The exposure level (Cmax) of LOEL is at least 36 times higher than that at the expected clinical dose. At higher exposure levels, decreased spontaneous activity or abnormal positioning (lateral or prone sitting) also were observed, and severe signs such as twitching and convulsions were induced at the highest exposure level, suggesting that the severity of neurological findings was dependent on exposure level. However, to ensure safety during clinical trials serious neurological adverse reactions could be avoided with careful monitoring for clinical signs.

### Effects on the Cardiovascular and Respiratory Systems

In the cynomolgus monkey telemetry study, prolongation of QTc was observed in 1 of 4 animals at PROJECT 4 doses ≥ 10 mg/kg, which corresponds to a Cmax approximately 25 times higher than that at the expected clinical dose. The QTc prolongation was slight (10-12%), but this exceeded the historical data range in the testing laboratory. While PROJECT 4 induced marginal inhibition in the hERG assay, it was at a concentration approximately 1,000 times higher than that at the expected clinical exposure level. In addition, the results of the 4-week and single dose toxicity studies in cynomolgus monkeys showed no evidence of QTc prolongation at exposure levels up to 600 times the expected clinical exposure level. Therefore, although the cause of QTc prolongation in 1 of 4 animals remains unclear, the safety concern for QT prolongation in humans is considered small.

Increased arterial carbon dioxide tension and decreased heart rate, body temperature and plasma potassium level were observed at 10 and 30 mg/kg PROJECT 4. The decrease in heart rate was also observed in the 4-week study and was likely related to a sedative effect of PROJECT 4, such as decreased spontaneous activity. The increase in arterial carbon dioxide tension and the decrease in body temperature may be related to such a sedative effect as well. The significance of the decrease in plasma potassium level, which was not detected in the 4- week monkey study, is unknown. Although these parameter changes may suggest some effects of PROJECT 4 on the cardiovascular and respiratory systems, the changes were all small and no serious effects on these organ systems were noted in the 4-week study even after repeated doses of PROJECT 4. Although the risk to humans is considered to be small, to avoid unexpected adverse reactions, ECG parameters as well as vital signs will be carefully monitored during future clinical trials.

### Conclusion

PROJECT 4 was evaluated in nonclinical safety pharmacology and toxicology studies at exposure levels similar to those expected for future clinical studies.

Findings of potential concern for clinical trials included neurological signs and vomiting. All toxicological findings were observed at doses that produced systemic exposures greater than the predicted human exposure at the estimated effective dose. None of the toxicological findings were accompanied with pathological changes and all were reversible. PROJECT 4 was not genotoxic or teratogenic in either in vitro or in vivo studies. PROJECT 4 weakly inhibited the hERG current but it shortened (not prolonged) the action potential duration at extremely high concentration (approximately 1,000 times higher than the expected clinical exposure level). However, PROJECT 4 induced some minor effects on the cardiovascular and respiratory systems in monkeys. Therefore, clinical signs, ECG parameters and vital signs will be carefully monitored during future clinical trials.

There were no serious toxicological findings that would prohibit starting phase 1 clinical trials of PROJECT 4.

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