### Safety Pharmacology

* + - 1. **In-vitro Effects on hERG Current (Project M-PT-0003)**

The effects of PROJECT M on the hERG current were studied in hERG transfected human embryonic kidney 293 cells (hERG-transfected HEK293 cells) by the whole-cell patch-clamp technique.

In the vehicle control group, the hERG current was reduced by 9.1% 11 min after start of the application. In the positive-control group (the rapid delayed rectifier current [IKr] inhibitor E-4031 at 0.1 μmol/L), the compensated suppression rate was 81.5%, and the rate was statistically significant when compared to the value in the control group. From these results, the validity of this study was confirmed.

The compensated suppressive rates of PROJECT M at the concentrations of 0.3, 3, and

30 μmol/L were 0.0%, 0.7%, and 13.8%, respectively; statistically significant suppression was noted at the concentration of 30 μmol/L when compared to the rate in the control group.

As described above, PROJECT M showed a suppressive effect on the hERG current at the highest test concentration of 30 μmol/L. Its compensated suppression rate was 13.8% at 30 μmol/L.

### In-vitro Effects on Action Potential Duration (Project M-PT-0004)

The effects of PROJECT M on action potentials in isolated guinea-pig papillary muscles were studied by the glass-electrode technique under a surface-superfusing condition. The effects of PROJECT M at concentrations of 0.3, 3, and 30 μmol/L on the resting membrane potential (RMP) and action potentials were tested. RMP, action-potential amplitude (APA), dV/dt max, and APD at 30% and 90% repolarization (APD30 and APD90) before and 35 min after beginning the application were recorded. The difference between APD30 and APD90 (APD30-90) and rates of change in the parameters including APD30-90 were calculated.

Dimethylsulfoxide at 0.1 vol% and E-4031, an IKr inhibitor, at 0.1 μmol/L were used as the vehicle control and positive-control, respectively.

The control substance did not affect the action-potential duration, RMP, or any other action-potential parameters in this study. In contrast, the positive-control substance statistically significantly prolonged APD30, APD90, and APD30-90 by 20.0%, 23.9%, and

31.7%, respectively. The IKr inhibitor had no effect on the RMP, APA, and dV/dt max. No effects were noted for the positive-control. From these results, the validity of this study was confirmed.

For PROJECT M, no effects on action-potential duration, RMP, APA, or dV/dt max were observed at any concentration.

These results show that PROJECT M does not affect the action potentials in isolated guinea-pig papillary muscles at concentrations up to 30 μmol/L.

### In-vivo Effects on Central Nervous System, Cardiovascular and Respiratory Systems in Dogs (Project M-PT-0002)

PROJECT M was orally administered to conscious male beagle dogs, and effects on the general activity and behavior, body temperature, blood pressure, heart rate, ECG, respiration rate, blood gases, and blood-electrolyte concentration were investigated. To evaluate the systemic exposure, plasma concentrations of PROJECT M were also assessed at dose levels of 10, 30, and 100 mg/kg.

PROJECT M showed no effects in any of the assessments at the dose of 10 mg/kg. Additionally, PROJECT M did not affect the general activity and behavior, body temperature, PR interval, QRS duration and QT interval in ECG, respiration rate, blood gases (pH, PaO2, PaCO2 and SaO2), or Na+, Cl-, and Ca2+ concentrations in blood at the dose up to 100 mg/kg. At

30 mg/kg, prolongation of QTc interval (Fridericia’s formula) and decrease in blood concentrations of K+ were noted. At 100 mg/kg, the following signs were noted: vomiting in 2 animals; decrease in blood pressure and an increase in heart rate were noted 4 h after administration in 1 animal; prolongation of QTc interval (Fridericia’s formula) was noted between 1 and 6 h after administration. Decrease in blood concentrations of K+ was noted.

No QTc prolongation was noted when corrected by Matsunaga’s formula. For the toxicokinetic parameters, tmax was 0.5 to 1.0 h at 10 and 30 mg/kg, and 0.5 to 4.0 h at

100 mg/kg. Cmax increased almost dose-proportionally between 10 and 30 mg/kg, and less than dose-proportionally between 30 and 100 mg/kg. AUC24 increased almost

dose-proportionally over the entire dose range of 10 to 100 mg/kg.

Sotalol hydrochloride at 10 mg/kg, which was used as the positive-control substance, prolonged QTc interval of the ECG from 0.5 to 8 h after administration.

These results indicate that PROJECT M has no effect on the CNS, cardiovascular, or respiratory systems at the dose of 10 mg/kg. At the dose of 30 mg/kg, prolongation of QTc interval (Fridericia’s formula) and a decrease in blood concentrations of K+ were noted. In addition, at the dose of 100 mg/kg vomiting, a decrease in blood pressure and an increase in heart rate were also noted.

### In-vivo Effects on Central Nervous System in Rats (Project M-PT-0001)

To investigate the effects of PROJECT M on the CNS, the general activity and behavior were evaluated by the modified Irwin's method. Six male rats were used in each testing group, and a single dose of PROJECT M was orally administered to the animals at doses of 30, 100, and 300 mg/kg. For the vehicle control, 0.5% (w/v) methylcellulose aqueous solution was administered. The general activity and behavior of the animals were observed before and after administration.

PROJECT M did not affect the general activity and behavior in any of the rats up to 24 h after administration at doses of 30, 100, or 300 mg/kg.

The results indicate that PROJECT M has no effect on the CNS up to 300 mg/kg.

### In-vivo Effects on Learning and Memory in Rats (Project M-PT-0005)

To assess the effects of PROJECT M on learning and memory in male rats using the Morris water maze test, PROJECT M at 0, 10, 30, 100, and 300 mg/kg per day was administered orally by gavage to rats for 5 days to examine its acute effect and for 28 days to examine its repeated dose effect. A vehicle control group was treated with 0.5 w/v% aqueous solution of methylcellulose. A positive control group was treated with scopolamine (0.5 mg/kg/dose).

Each test group consisted of 10 males.

In both the acute and repeated dose effect tests, there were no abnormal clinical signs, change in body weight, body weight gain, change in food consumption, or gross pathological findings in any group that received PROJECT M. No effects on learning or memory in the Morris water maze test were noted in any group that received PROJECT M. However, adverse effects on learning and memory in the Morris water maze test were noted in the acute effect test and learning and memory were impaired in the repeated dose effect test in the positive control group treated with scopolamine 0.5 mg/kg.

The results indicate that PROJECT M has no effects on learning and memory up to 300 mg/kg per day.

### Ex-vivo Effects on Choline Acetyltransferase Activities of 5-day or 28-day Treatment in the Rat Brain (Project M-PT-0006 and Project M-PT-0007)

The effects of PROJECT M on ChAT activities in the rat brain (hippocampus, basal nucleus of Meynert, and septal area) of male rats were measured. Five male rats were used in each test group and PROJECT M was orally administered at doses up to 300 mg/kg per day for 5 days to examine its acute effect and for 28 days to examine its repeated dose effect. For control, 0.5% (w/v) methylcellulose solution used as the vehicle was administered. No significant differences in ChAT activities were found between the control and PROJECT M test groups in any of the brain regions.

These results indicate that up to 28 days repeated doses of PROJECT M (up to 300 mg/kg per day) has no effect on ChAT activities in the rat brain.

### In-vivo Effects on Attention, Learning and Memory in Aged Rhesus Monkeys (Project M-PT-0009)

The purpose of this study was to evaluate the effects of PROJECT M on attention, learning and memory in 6 aged rhesus monkeys (19 to 27 years old) via a delayed match to sample (DMTS) task. PROJECT M was evaluated for acute (single dose) effects at 3 and 10 mg/kg and for subchronic (4-week repeated dose) effects at 10 mg/kg per day. In both cases, PROJECT M was mixed with chocolate as the vehicle (or with Prima Burger™ for only 1 animal in the

4-week repeated dose study) and then administered orally once daily to the test subjects. To confirm the validity of the behavioral test, the cholinergic antagonist scopolamine (a

well-documented memory impairing compound) was evaluated as a positive control for impairing performance of the DMTS task at 0.0025, 0.005 and 0.01 mg/kg administered intramuscularly.

### Scopolamine Dose Effect Study

While the 0.0025 mg/kg dose of scopolamine did not affect DMTS task performance (performance accuracy: zero delay, short delay interval and long delay interval), the

0.005 and 0.01 mg/kg doses impaired performance at the short delays. No abnormal clinical signs, body weight changes, or effects on food consumption were noted with any of the doses of scopolamine. Therefore, the behavioral test using 6 aged rhesus monkeys was found to be valid for the purpose of assessing the cognitive effects of the test article, since scopolamine at

0.005 and 0.01 mg/kg impaired DMTS task performance.

### PROJECT M Single Dose Study

No effects on DMTS task performance were noted after the administration at 3 and 10 mg/kg PROJECT M. No abnormal clinical signs, body weight changes, or effects on food consumption were noted with either PROJECT M dose.

### PROJECT M 4-week Repeated Dose Study

No effects on performance of the standard DMTS task or the titrating DMTS task were noted after repeated administration of 10 mg/kg PROJECT M per day. No abnormal clinical signs, body weight changes, or effects on food consumption were noted with repeated PROJECT M administration at 10 mg/kg per day.

Toxicokinetic data showed that the mean plasma concentration at 2 h after administration increased dose-proportionally (4.1 times) for the 3 and 10 mg/kg dose levels.

These results indicate that PROJECT M has no effects on attention, learning and memory up to 10 mg/kg per day.

## Toxicology

The current toxicological data package for PROJECT M includes 2 single oral dose toxicity studies, one 4-week and three 13-week oral dose toxicity studies with recovery assessment, 2 additional histopathological studies, 3 genotoxicity studies, 2 studies of effects on fertility and early embryonic development to implantation, 2 studies of effects on embryo-fetal development, 1 study of effects on pre- and postnatal development, and 1 study of biomarker investigation for bile duct injury. All these studies were conducted under appropriate guidelines/guidances (ICH) and in accordance with Good Laboratory Practices (GLP) standards.

A tabulated overview of these studies may be found in [End-of-Text Table 3.1].

### Single-dose Toxicity 4.3.1.1 Rats (Project M-TX-0006)

PROJECT M was suspended in 0.5 w/v% methylcellulose solution and orally administered once while food was withheld to 5 male and 5 female Crl:CD(SD) rats per group at dose levels of 500 and 1000 mg/kg to investigate toxicity until 14 days after dosing.

One male and 4 females at 500 mg/kg, and 2 males and all females at 1000 mg/kg died or were sacrificed in moribund condition within 6 h after dosing. In these animals, a decrease in spontaneous activity, lateral position, ataxic gait and/or incomplete eyelid opening and bradypnea were observed from 2 h after dosing. In the surviving animals, decreased spontaneous activity and ataxic gait were observed after dosing. These symptoms disappeared by 2 days after dosing. No PROJECT M-related changes were observed in body weight or gross pathology.

The doses leading to mortality or morbidity of PROJECT M when administered once orally to rats was approximately 500 mg/kg in males and less than 500 mg/kg in females.

### 4.3.1.2 Dogs (Project M-TX-0007)

PROJECT M was suspended in 0.5 w/v% methylcellulose solution and administered orally once at 500 and 2000 mg/kg, to 1 male and 1 female beagle dog per dose group in order to investigate its toxicity until 14 days after dosing. Systemic exposure to PROJECT M was also assessed.

Vomiting was observed at both doses. In addition at 2000 mg/kg, eosinophil counts were decreased in the female, total bilirubin was increased in both the male and female, potassium was increased in the female, and chloride was decreased in the female on day 1, but these changes had recovered by day 7.

Toxicokinetic data showed that tmax values were 2.0 and 1.0 h for male and female dogs, respectively. Cmax values at both 500 and 2000 mg/kg were almost equal for males and females. AUC24 values at 2000 mg/kg were 1.4-times and 2.4-times higher than those at 500 mg/kg in the male and female, respectively.

The dose level leading to mortality or morbidity for male and female beagle dogs was greater than 2000 mg/kg.

### Repeat-dose Toxicity

Tabulated results of repeat-dose toxicity studies are presented in [End-of-Text Tables 3.5].

### 4.3.2.1 Repeat-dose Toxicity in Rats

**4.3.2.1.1 13-week Oral Toxicity with 4-week Recovery Period (Project M-TX-0008, Project M-TX-0023)**

PROJECT M was suspended in 0.5 w/v% methylcellulose solution and administered orally to

10 male and 10 female Crl:CD(SD) rats per group once daily for 13 weeks at 10, 30, 100, and 300 mg/kg per day. Five males and 5 females were added to the control and 300 mg/kg per day groups to assess the reversibility of any toxicity observed during the dosing period in a subsequent 4-week recovery period. Animals in the control group were administered

0.5 w/v% methylcellulose solution in the same manner as the PROJECT M groups. Microscopic evaluation of the dorsal root ganglion, superior cervical ganglion and specified brain regions were added to the standard histopathological evaluation. In addition, the modified Irwin observational battery was conducted towards the end of the treatment period. Satellite groups were added at each dose level to assess systemic exposure to PROJECT M.

No animal died in any group, and no PROJECT M-related changes were noted in the 10 or 30 mg/kg per day group.

At ≥ 100 mg/kg per day, liver weights were increased, and periductal crystal-like material and vacuolation of the bile duct cells were observed in the liver. Spleen weight was increased with extramedullary hematopoiesis. In addition, a high reticulocyte ratio, total bilirubin, and direct bilirubin were noted without an adverse effect on the total erythrocyte count.

At 300 mg/kg per day, the kidney weights were increased and contained hyaline droplets in the renal tubules, and tubular mineralization in the cortex. Testes weight was decreased with atrophy of the seminiferous tubules and epithelial vacuolation of the epididymis and cell debris in the lumen. Periductal mononuclear cell infiltration was seen in conjunction with the vacuoles in the bile duct cells. The vacuoles of the bile duct cells were negative for periodic acid–schiff stain and oil red O stain, and contained round droplets including laminated or amorphous structures on electron microscopic examination. These results suggested that material including phospholipids had accumulated in bile duct epithelial cells. There were no PROJECT M-related changes indicative of degeneration/necrosis or apoptosis in the superior cervical ganglion and dorsal root ganglion following staining with Fluoro-Jade B staining, immunohistochemical staining for cleaved caspase 3, TUNEL staining and

hematoxylin-eosin staining. It was concluded that PROJECT M did not cause degeneration/necrosis or apoptosis in these tissues up to the highest dose for 13 weeks.

Reversibility of liver, testes and epididymis toxicity was not observed; however, mononuclear cell infiltration in the liver, testes and liver weights showed a tendency toward　recovery following a 4-week recovery period. Other changes disappeared by the end of the recovery period.

Toxicokinetic data showed that Cmax of PROJECT M increased dose-proportionally from 10 to 30 mg/kg per day, but less than dose-proportional from 30 to 300 mg/kg per day. AUC24 of PROJECT M increased greater than dose-proportionally. Cmax and AUC24 in females were

1.42 to 2.91 and 1.24 to 3.08 times, respectively, higher than those in males. The tmax values for PROJECT M were 0.5 h at 10 and 30 mg/kg per day, and tended to increase to maximally 4 h at the higher doses.

In conclusion, under the conditions of this study, the NOAEL of PROJECT M when administered orally once daily for 13 weeks to rats was 30 mg/kg per day, since periductal crystal-like material and vacuolation of the bile duct cells in the liver were observed in the 100 mg/kg per day group. The toxic changes observed during the dosing period were confirmed to be reversible, except for the changes in liver, testes, and epididymis.

### 4.3.2.1.2 13-week Oral Toxicity Followed by 13-week Reversibility in the Liver and Testes (Project M-TX-0018)

The reversibility of toxicity findings in the liver and testes when PROJECT M was repeatedly administered to Crl:CD(SD) rats for 13 weeks was investigated during a 13-week recovery period, since reversibility of liver and testes toxicity was not observed following a 4-week recovery period. PROJECT M was suspended in 0.5 w/v% methylcellulose solution and administered orally once daily to 20 males and 20 females at dose levels of 0, 100, and 300 mg/kg per day. Ten animals of each group were necropsied after the 13-week dosing period, and the other 10 animals following the 13-week recovery period. Animals in the control group were administered 0.5 w/v% methylcellulose solution in the same manner as the PROJECT M groups. Satellite groups were added at both dose levels to assess systemic exposure to PROJECT M.

At the end of treatment, the findings in the liver and testes following dosing were similar to those seen following the first 13-week study (Project M-TX-0008).

At ≥ 100 mg/kg per day, high liver weights were noted in females. Vacuolation of the bile duct cells and periductal crystal-like material in the liver were observed in both sexes after the treatment period, with periductal mononuclear cell infiltration at 300 mg/kg. In addition at 300 mg/kg, high total bilirubin in both sexes, and lactate dehydrogenase and sorbitol dehydrogenase activity in males were noted at the end of the dosing period.

At the end of the recovery period, high liver weight was noted in females at 300 mg/kg and vacuolation of the bile duct cells and periductal crystal-like material were still observed in both sexes, whereas the periductal mononuclear cell infiltration had disappeared. These hepatic changes, however, were considered to show a tendency toward recovery since their severity was lower than at the end of the dosing period. The changes in blood chemistry were no longer present by the end of the recovery period.

Atrophy of the seminiferous tubules in the testes was observed in males with an observation of small testes in 1 male. At the end of the recovery period, small testes and epididymis were　observed in 1 male with low testes weights. Atrophy of the seminiferous tubules in the testes was still observed; however, the severity and incidence were lower than at the end of the dosing period. Reversibility was shown for these lesions.

Systemic exposure (Cmax and AUC24) to PROJECT M was at the same level as in a previous 13-week repeated dose toxicity study (Project M-TX-0008).

### 4.3.2.2 Repeat-dose Toxicity in Dogs

**4.3.2.2.1 4-week Oral Toxicity (Project M-TX-0009)**

PROJECT M was suspended in 0.5 w/v% methylcellulose solution and administered orally once daily for 4 weeks at dose levels of 0 (vehicle), 3, 10, 30, and 100 mg/kg to 4 male and

4 female beagle dogs per group in order to investigate its toxicity. Microscopic evaluation of the dorsal root ganglion, anterior cervical ganglion and specified brain regions were added to the standard histopathological evaluation. Three males and 3 females were added to the

30 and 100 mg/kg per day groups in order to assess the reversibility of toxicity during a subsequent 4-week recovery period. Systemic exposure to PROJECT M was also assessed.

No treatment-related changes were noted in males or females in the 3 or 10 mg/kg per day group.

In 1 female at 30 mg/kg per day, PROJECT M-related changes were observed in the digestive tract and liver. Erosion, diffuse mucosal inflammation and regeneration of the epithelium were observed in the ileum. Liver findings consisted of degeneration and necrosis of centrilobular hepatocyte and centrilobular hemorrhage, hepatocyte hypertrophy and neutral fat containing cytoplasmic vacuoles. These changes were not observed in animals of the recovery group of 30 mg/kg per day.

At 100 mg/kg per day group, vomiting was observed during the dosing period. One female died on day 7 of dosing and 1 female was sacrificed in moribund condition on day 13 of dosing. Food consumption was notably decreased for 2 to 3 days before death/moribund sacrifice. Stool containing mucous or occult blood, lateral position, and/or hemorrhage in the lung and convulsions were observed prior to death. In addition, one male at 100 mg/kg per day died on day 6 of recovery. Reduced food intake and reduced spontaneous activity were observed from end of treatment until death. Lateral position, gasping, and vomiting were observed just before death. At necropsy, retention of white foamy fluid in trachea and bronchus was observed, and foreign body in the bronchus and bronchiole, alveolus with hemorrhage in the lung. The findings in the respiratory organs were considered to be caused by mis-swallowing due to vomiting in a lateral position.

The cause of moribundity/death in all 3 dogs was attributed to liver and gastrointestinal toxicity. The liver toxicity consisted of degeneration/necrosis, hypertrophy and/or cytoplasmic vacuoles containing neutral lipid in centrilobular and/or diffuse hepatocytes, and centrilobular hemorrhage. When available, high liver weights, and increases in hepatocellular/biliary parameters (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, direct bilirubin, total bile acid, and lactate dehydrogenase) were noted during treatment. In addition, triglycerides and total cholesterol　were increased, prothrombin time and activated partial thromboplastin time prolonged and albumin was reduced. Adverse changes throughout the digestive tract consisted of mucosal hemorrhage, inflammation and/or atrophy. Dilatation of the renal tubule and vacuoles in the tubular epithelium, which were positive for oil red O staining (neutral lipids), high kidney weights and increased blood urea nitrogen and creatinine were noted in 1 dog.

Few surviving animals of this dose group showed salivation, reduced body weight gain, reduced food consumption, and similar type of changes in liver, digestive tract and kidneys at the end of treatment.

Toxicokinetic data showed that the mean tmax values on each dosing day ranged from 0.5 to

1.7 h and tended to increase with increasing dose level. The mean Cmax values increased almost dose-proportionally between 3 and 30 mg/kg and less than dose-proportionally between 30 and 100 mg/kg per day in both sexes. In males, the mean AUC24 values increased more than dose-proportionally between 3 and 30 mg/kg and there was marked increase between 10 and 30 mg/kg per day, and less than dose-proportionally between 30 and 100 mg/kg per day. In females, the mean AUC24 values increased more than

dose-proportionally between 3 and 10 mg/kg per day, almost dose-proportionally between 10 and 30 mg/kg per day, and less than dose-proportionally between 30 and 100 mg/kg. For all dosing groups and dosing days, no sex difference was observed in the toxicokinetic parameters.

It was concluded that, under the conditions of this study, NOAEL was 30 mg/kg per day for males and 10 mg/kg per day for females. During the recovery period, general condition deteriorated in 1 male in the 100 mg/kg per day group. This male died, and reversibility in clinical examination could not be evaluated in this male. With the exception of the male that died during the recovery period, all changes noted during the dosing period in clinical sign and clinical examination recovered, and histopathological findings were not observed at the end of the recovery period.

### 4.3.2.2.2 13-week Oral Toxicity (Project M-TX-0019, Project M-TX-0024)

PROJECT M was suspended in 0.5 w/v% methylcellulose solution and administered orally once daily for 13 weeks at dose levels of 0 (vehicle), 3, 10, and 30 mg/kg to 4 male and 4 female beagle dogs per group in order to investigate its toxicity. Microscopic evaluation of the dorsal root ganglion, anterior cervical ganglion and specified brain regions were added to the standard histopathological evaluation. Three males and 3 females were added to the

30 mg/kg per day group to assess the reversibility of toxicity during a subsequent 4-week recovery period. Systemic exposure to PROJECT M was also assessed.

At 30 mg/kg per day, 1 male and 2 females were sacrificed moribund on day 35, 26, and 76 of dosing, respectively. These dogs showed aggravated general condition, evident by clinical signs such as a decrease in spontaneous activity, lateral position, hypothermia, dehydration signs, and crouching prior to sacrifice. Additionally, rhinorrhea, vomiting, and salivation　were observed in these dogs. Food consumption and glucose levels were decreased in all these animals, with body weight reduction in the male.

PROJECT M related changes were mainly observed in the liver and upper digestive tract. The liver toxicity was observed at 4 days prior to or at moribund sacrifice and consisted of changes in both hepatobiliary and hepatocellular parameters, similar to those seen in dogs showing liver toxicity in the 4-week study. In addition, an increase in biliary viscosity and mucus hypersecretion in gallbladder were observed.

Mucosal or abnormal stool color (reddish brown) was seen in 1 female. Mucosal atrophy in the tongue and esophagus were noted in all these animals, with ulcer in the oral mucosa or tongue in 2 dogs.

One surviving dog at 30 mg/kg per day showed abnormal mucosa (oral mucosa, pale yellow), decreased body weight, and food consumption towards the end of the treatment period.

Marked increases in plasma parameters for hepatocellular and hepatobiliary toxicity were noted on day 80 of dosing. These changes had recovered by the end of the dosing period (day 90). Additional changes in blood cell parameters, electrolyte levels, albumin and fibrinogen were considered secondary to the liver toxicity and general health condition. Treatment-related changes were not noted at 30 mg/kg per day during the 4-week recovery period.

There were no PROJECT M-related changes indicative of degeneration/necrosis or apoptosis in the anterior cervical ganglion and dorsal root ganglion following staining with Fluoro-Jade B staining, immunohistochemical staining for cleaved caspase 3, TUNEL staining and hematoxylin-eosin staining. It was concluded that PROJECT M did not cause degeneration/necrosis or apoptosis in these tissues up to the highest dose for 13 weeks.

Toxicokinetic data showed that mean tmax ranged from 0.5 to 1.3 h. Mean Cmax increased almost dose-proportionally on any dosing day in the dosing range of 3 to 10 mg/kg per day. In the dose range of 10 to 30 mg/kg per day, mean Cmax increased almost dose-proportionally on days 1 and 49 of dosing and slightly less than dose-proportionally on day 91 of dosing.

Mean AUC24 values increased more than dose-proportionally in the range of 3 to 10 mg/kg per day and almost dose-proportionally in the range of 10 to 30 mg/kg per day on any dosing day. Mean Cmax, AUC24, and tmax values were not influenced by repeated dosing in any dosing group. No sex differences were observed in any dosing group on any day.

It was concluded that, under the conditions of this study, the NOAEL of PROJECT M was 10 mg/kg per day for males and females, since effects on the liver and/or upper digestive tract were noted in 4 dogs at 30 mg/kg per day resulting in moribund sacrifice of 3 dogs. Treatment-related changes were not noted during the recovery period.

### Genotoxicity

The potential genotoxicity of PROJECT M was evaluated using in-vitro and in-vivo studies. Major findings from these studies where:

* + - * Study Project M-TX-0010 – PROJECT M does not induce gene mutation in bacteria [End-of-Text Table 3.6.1]
      * Study Project M-TX-0011 – PROJECT M does not induce chromosomal aberrations in Chinese hamster lung cells (CHL/IU) [End-of-Text Table 3.6.2].
      * Study Project M-TX-0020 – PROJECT M does not induce micronuclei in rats [End-of-Text Table 3.7]
      * It was concluded PROJECT M does not exhibit genotoxicity potential in in-vitro and in-vivo situations.

### In-vitro Reverse Mutation (Project M-TX-0010)

To assess the potential of PROJECT M to induce gene mutation, a bacterial reverse mutation test was performed with 5 test strains of bacteria [*Salmonella typhimurium* (TA100, TA1535, TA98, and TA1537) and *Escherichia coli* (WP2*uvrA*)], using the pre-incubation method with and without metabolic activation. The dose-finding test was performed at 15, 50, 150, 500, 1500, and 5000 μg/plate for all test strains with and without metabolic activation. Based on the results of the dose-finding test, the main test was performed at 156, 313, 625, 1250, 2500, and 5000 μg/plate for all test strains with and without metabolic activation.

PROJECT M precipitation was not observed at up to 5000 μg/plate upon addition of the PROJECT M formulation or on the plates after incubation for 48 h with or without metabolic activation.

Growth inhibition was not observed at up to 5000 μg/plate in any test strain with or without metabolic activation.

In comparison with the negative control, neither a 2-fold or greater number of revertant colonies per plate nor a dose-dependent increase in the number of revertant colonies was observed in any test strain in the dose-finding test or the main test, with or without metabolic activation.

It was concluded that PROJECT M does not induce gene mutation in bacteria.

### In-vitro Chromosome Aberration (Project M-TX-0011)

In order to assess the potential of PROJECT M to induce chromosomal aberrations, a chromosomal aberration test was performed with cultured mammalian CHL/IU cells in

short-term treatments for 6 h with and without metabolic activation, and continuous treatment for 24 h without metabolic activation.

The dose levels for the chromosomal aberration test were set based on the results of the

dose-finding test. The lowest dose that showed a cell proliferation ratio of less than 50% was set as the highest observational dose. When no dose showed a cell proliferation ratio of less than 50%, the highest dose was set as the highest observational dose. Chromosomal aberrations were analyzed at the following doses: 1250, 2500, and 5000 μg/mL in short-term treatments with and without metabolic activation, and 59.5, 107, 193, and 347 μg/mL in continuous treatment for 24 h. The number and incidence of cells with structural and numerical chromosomal aberrations were investigated.

No change in the color of the culture medium indicating a change in pH was observed when the PROJECT M formulation was added. PROJECT M precipitation in the treatment medium was observed at 2500 μg/mL and greater at the start and end of treatment.

The cell proliferation ratio determined from the number of viable cells and the relative mitotic index (for the continuous treatment for 24 h) showed dose-dependent decreases.

No significant increase in the number of cells with structural or numerical chromosomal aberrations was noted in any treatment group when compared with the negative control group.

It was concluded that PROJECT M does not induce chromosomal aberrations in CHL/IU cells, regardless of the presence or absence of metabolic activation, or treatment length.

### In-vivo Micronucleus Test in Rats (Project M-TX-0020)

To evaluate PROJECT M for its potential to induce micronuclei in rat erythroblasts in vivo, PROJECT M suspended in 0.5 w/v% methylcellulose solution (10 mL/kg) was orally administered once daily for 2 days to male Crl:CD(SD) rats (5 animals per group) at dose levels of 75, 150, and 300 mg/kg. As a negative control, the vehicle was administered in the same manner. As a positive control, cyclophosphamide monohydrate at a dose of 20 mg/kg was orally administered once. The animals were euthanized approximately 24 h after the final dosing, and femoral bone marrow specimens were prepared. The number of micronucleated immature erythrocytes per 2000 immature erythrocytes and the ratio of immature erythrocytes per 500 total erythrocytes were investigated. A satellite group was added to evaluate systemic exposure to PROJECT M.

No animal died in any group. No PROJECT M-related changes in clinical signs and body weight were noted in any group.

No statistically significant increase was noted in the number of micronucleated immature erythrocytes in any group when compared with the negative control group.

Significant decreases were noted in the ratio of immature erythrocytes in the 75, 150, and 300 mg/kg per day groups when compared with the negative control group. Therefore, PROJECT M was judged to inhibit bone marrow cell proliferation. The mean percentages of micronucleated immature erythrocytes and immature erythrocytes in the negative and positive control groups were within the range (mean ± 3 times standard deviation) of the control background data of the test facility. Accordingly, it was judged that the present study met the acceptance criterion.

Systemic exposure to PROJECT M was confirmed from toxicokinetic data after the final dosing. The Cmax, tmax, and AUC24 values were 24633.8 ng/mL, 1.0 h, and 303477.6 ng·h/mL, respectively, at 300 mg/kg per day.

It was concluded that PROJECT M has no potential to induce micronuclei in vivo.

### Carcinogenicity

Long-term carcinogenicity studies have not been conducted with PROJECT M.

### Reproductive and Developmental Toxicity

Studies for fertility and early embryonic developmental toxicity were conducted in male and female rats. Embryo-fetal developmental toxicity studies were conducted in rats and rabbits. A pre- and postnatal developmental toxicity study was conducted in rats.

### Fertility and Early Embryonic Development in Male Rats (Project M-TX-0021)

To assess the effects of PROJECT M on fertility of male and early embryonic development, PROJECT M suspended in 0.5% methylcellulose solution was administered orally once daily to Crl:CD(SD) male rats (20 males/group, 6 weeks old at initiation of administration) at dose levels of 30, 100 and 300 mg/kg for 9 weeks before the commencement of mating with nontreated female rats, throughout the mating period and up to the day before necropsy.

Control animals were given 0.5% methylcellulose solution. On day 13 of gestation, nontreated females were euthanized for Cesarean section and the effects on the fertility of males and early embryonic development were examined. Satellite groups (3 males in the control group and 9 males in each PROJECT M group) were also provided to evaluate systemic exposure to PROJECT M, and the plasma concentrations of PROJECT M were measured on days 1 and 63 of administration.

No PROJECT M-related effects were noted in the 30 or 100 mg/kg per day group. No PROJECT M- related effects on the body weight, food consumption or fertility were noted in any group.

In the 300 mg/kg per day group, salivation was observed in 11 males. Small testes and epididymis as macroscopic findings, and low testes weights were noted. In the histopathological examination, atrophy of the seminiferous tubule in the testes, cell debris in the duct of the epididymis, and decrease of the sperm in the epididymis were observed. In the sperm examination, low count of sperm, sperm motility and vitality of sperm, and high sperm form anomalies index (tailless) were noted.

No PROJECT M-related effects on early embryonic development (number of implantations, number of live embryos, pre-implantation loss index or postimplantation loss index) were noted in the 30 or 100 mg/kg per day group.

In the 300 mg/kg per day group, there were decreases in the number of implantations and live embryos and an increase in pre-implantation loss index.

Toxicokinetic data showed that tmax was 0.5 or 1.0 h at 30 and 100 mg/kg per day, and 4.0 h at 300 mg/kg per day on days 1 and 63 of administration. Cmax values on day 1 of administration increased slightly less than dose-proportionally between 30 and 300 mg/kg per day. On day 63 of administration, Cmax increased slightly less than dose-proportionally between 30 and 100 mg/kg per day and dose-proportionally between 100 and 300 mg/kg per day. AUC24 increased more than dose-proportionally between 30 and 300 mg/kg per day.

Cmax values on day 63 of administration were higher than those on day 1 of administration at 300 mg/kg per day and Cmax values were not influenced by repeated dosing at 30 and

100 mg/kg per day. AUC24 values on day 63 of administration were higher than those on day 1 of administration at 30 and 100 mg/kg/day and AUC24 values were not influenced by　repeated dosing at 300 mg/kg/day. The tmax values were not influenced by repeated dosing in any dosing group.

The NOAEL of PROJECT M was judged to be 100 mg/kg per day for general toxicity, reproductive ability in males and early embryonic development.

### Effects on Fertility and Early Embryonic Development in Female Rats (Project M-TX-0022)

To assess the effects of PROJECT M on female fertility and early embryonic development, PROJECT M suspended in 0.5% methylcellulose solution was administered orally once daily to Crl:CD(SD) female rats (20 females/group, 10 weeks old at initiation of administration) at dose levels of 30, 100 and 300 mg/kg for 2 weeks before the commencement of mating with nontreated male rats, throughout the mating period and up to day 7 of gestation. Control animals were given 0.5% methylcellulose solution. On day 13 of gestation, females were euthanized for Cesarean section, and the effects on the fertility of females and early embryonic development were examined. Satellite groups (3 females in the control group and 9 females in each PROJECT M group) were also used to evaluate systemic exposure to PROJECT M, and the plasma concentrations of PROJECT M were measured during the premating period on days 1 and 14 of administration.

No PROJECT M-related effects were noted in the 30 or 100 mg/kg per day group. No PROJECT M related effects on the estrous cycle, fertility or necropsy were noted in the 300 mg/kg group.

In the 300 mg/kg per day group, salivation was observed in 2 females during the gestation period. In addition, suppressed body weight gain and transient decreased food consumption during the gestation period were observed in same group.

No effects on early embryonic development (number of implantations, number of live embryos, pre-implantation loss index or postimplantation loss index) were observed in the 300 mg/kg group.

Toxicokinetic data showed that tmax values ranged from 0.5 to 2.0 h at all dose levels on days 1 and 14 of administration. Cmax values increased almost dose-proportionally between 30 and 100 mg/kg per day and slightly less than dose-proportionally between 100 and 300 mg/kg per. AUC24 values increased more than dose-proportionally between 30 and 300 mg/kg per day.

Cmax, AUC24 and tmax values were not influenced by repeated dosing in any group.

The NOAEL of PROJECT M was judged to be 100 mg/kg per day for general toxicity and 300 mg/kg per day for reproductive ability and early embryonic development in females.

### Effects on Embryo-fetal Development in Rats (Project M-TX-0013)

To assess the effects of PROJECT M on dams and embryo-fetal development, PROJECT M at 0, 30, 100, and 300 mg/kg per day was administered orally by gavage to female rats daily during the period of organogenesis (days 7 to 17 of gestation), and Cesarean section was performed on day 20 of gestation. A control group was treated with 0.5 w/v% aqueous solution of methylcellulose. Each test group consisted of 18 to 20 pregnant females. Systemic exposure was assessed by determination of the plasma concentration of PROJECT M on days 7 and 17 of

gestation. Satellite groups consisted of 4 pregnant females of the control group and 8 pregnant females each of the PROJECT M groups.

No dead dams, moribund dams, or dams that aborted or delivered prematurely were noted in any groups.

Suppressed body weight gain was noted in the dams of the 300 mg/kg per day group at the beginning of the dosing period. In this group, transiently-decreased food consumption was sporadically noted during the dosing period. No treatment-related changes were noted in the body weight, body weight gain, or food consumption in the 100 or 30 mg/kg per day group. No treatment-related changes were noted in the clinical signs, gross pathological findings, number of corpora lutea, or number of implantation sites in any PROJECT M group.

Decreased fetal body weights of both sexes, increased skeletal variations (short supernumerary rib and wavy rib), and delayed ossification (sternebrae) were noted in the fetuses at 300 mg/kg per day. No treatment-related changes were noted in fetal body weight of either sex, skeletal variations, or degree of ossified bones in the 100 or 30 mg/kg per day group.

No treatment-related changes were noted in the number of embryo-fetal deaths, index of embryo-fetal deaths, number of live fetuses, sex ratio, external abnormalities, visceral abnormalities, visceral variations, skeletal abnormalities, or placental morphology or weight in any PROJECT M group.

Toxicokinetic data showed that the mean tmax at 30 to 300 mg/kg per day was 0.5 to 1.0 h on day 7 of gestation and 0.5 to 4.0 h on day 17 of gestation. The mean Cmax increased slightly less than dose-proportionally on day 7 of gestation and increased almost dose-proportionally on day 17 of gestation. The mean AUC24 between the 30 and 100 mg/kg per day groups increased more than dose-proportionally on days 7 and 17 of gestation, and the mean AUC24 between the 100 and 300 mg/kg per day groups increased almost dose-proportionally on days 7 and 17 of gestation. The mean Cmax and AUC24 in each group on day 7 of gestation were comparable to the values on day 17 of gestation.

The NOAEL of PROJECT M was considered to be 100 mg/kg per day for dams and embryo-fetal development.

### Effects on Embryo-fetal Development in Rabbits (Project M-TX-0015)

PROJECT M was administered orally by gavage to 17 or 19 pregnant Kbl:NZW rabbits per group at dose levels of 0 (0.5 w/v% methylcellulose solution), 100, 300, and 600 mg/kg per day during the period from implantation to closure of the hard palate (from day 6 to day 18 of gestation) to investigate effects of PROJECT M on pregnant females and embryo-fetal development. Systemic exposure to PROJECT M in dams was also assessed.

No dams died in any groups. Dose-dependent suppression of body weight gain and decrease in food consumption were noted during the dosing period in the 300 and 600 mg/kg per day groups. A decrease in stool volume in 3 dams and a decrease in body weight was also noted in the 600 mg/kg per day group. No PROJECT M-related changes were noted in clinical signs,

body weight, body weight gain, or food consumption in the 100 mg/kg per day group, or in the gross pathological findings, number of corpora lutea, or number of implantations in any PROJECT M group.

In the 600 mg/kg per day group, the number of embryo-fetal deaths and postimplantation loss rate were higher than the control group and the control background data. No PROJECT M- related changes were noted in the number of embryo-fetal deaths or postimplantation loss rate in the 300 or 100 mg/kg per day group. No PROJECT M-related changes were noted in the number of live fetuses, fetal body weight, placental weight, sex ratio, or external, placental, visceral, or skeletal findings in any PROJECT M group.

Toxicokinetic data showed that the tmax values at the dose of 100, 300, and 600 mg/kg per day ranged between 0.25 and 0.80 h. In 1 dam in the 300 mg/kg per day group, plasma concentrations of PROJECT M showed remarkably higher values than those of the other animals in the 300 and 600 mg/kg per day groups. The Cmax and AUC24 values showed no

dose-dependency over the entire dose range of 100 to 600 mg/kg per day on both days 6 and 18 of gestation. The Cmax and AUC24 values on day 18 of gestation were slightly higher than those on day 6 of gestation at 300 and 600 mg/kg per day. The reached exposures in terms of Cmax and AUC were very low compared to those reached in rats and dogs at similar doses and anticipated at the doses for clinical studies.

It was concluded that the NOAEL of PROJECT M was 100 mg/kg per day for dams and 300 mg/kg per day for embryo-fetal development.

### Effects on Pre- and Postnatal Development in Rats (Project M-TX-0012)

To assess the effects of PROJECT M on pre- and postnatal development and maternal functions, PROJECT M at 0, 30, 100, and 200 mg/kg per day was administered orally by gavage to female rats daily during the period from implantation to weaning (from day 7 of gestation to 20 days after delivery). A control group was treated with a 0.5 w/v% aqueous solution of methylcellulose. Each test group consisted of 18 or 19 pregnant females. Systemic exposure was assessed by determination of the plasma concentration of PROJECT M on day 7 of gestation and 20 days after delivery. Satellite groups consisted of 4 pregnant females of the control group and 8 pregnant females each of the PROJECT M groups.

There were no deaths or moribund dams; no dams aborted or delivered prematurely in any dose groups. In the 200 mg/kg per day dams, transiently-decreased food consumption was sporadically noted during the gestation period.

No treatment-related changes were noted in the clinical signs, body weight, gross pathological findings, gestation duration, number of implantation scars, gestation index, delivery conditions, or nursing conditions in any PROJECT M group.

In the 200 mg/kg per day group, pup viability index on day 4 after birth, weaning index and body weight at 21 days after birth were reduced. Development of some reflex functions was delayed (surface righting reflex, negative geotaxis, air righting reflex). Corneal and pinna reflexes were more severely affected and did not fully develop until weaning. Abnormal clinical signs included corneal opacity and drying, red eyeballs, decreased locomotor activity,

abdominal distension, hypothermia, moribundity, and death. No adverse effects of PROJECT M were noted in the number of live pups at birth, sex ratio, number of external abnormalities, incidence of pups with milk in the stomach up to day 4 of lactation, morphological differentiation, or pain response. All pups in the 200 mg/kg per day group were necropsied 21 days after birth, since it was considered impossible to evaluate postweaning functions due to the eye abnormalities.

No adverse effects of PROJECT M were noted in the number of live pups at birth, sex ratio, number of stillbirths, number of dead pups at birth, birth index, viability index, weaning index, number of external abnormalities, clinical signs, incidence of pups with milk in the stomach, body weight, food consumption, morphological differentiation, reflex functions, pain response, emotionality by the open-field test, motor coordination, learning ability by the water multiple T-maze test, or gross pathological findings in the 100 or 30 mg/kg per day group.

Regarding the reproductive functions of F1 pups, no adverse effects of PROJECT M were noted in the number of estrous cases during the premating period, copulation index, number of days until copulation after the start of pairing, fertility index, number of corpora lutea, number of implantation sites, implantation rate, number of pre-implantation losses, pre-implantation loss rate, number of embryonic deaths, postimplantation loss rate, or number of live embryos in the 100 or 30 mg/kg per day group.

In PROJECT M satellite groups, tmax values were 0.5 to 1.0 h both on day 7 of gestation and

20 days after delivery. Cmax values increased less than dose-proportionally, and AUC24 values increased almost dose-proportionally between 30 to 100 mg/kg per day, and less than

dose-proportionally between 100 to 200 mg/kg per day both on day 7 of gestation and

20 days after delivery. Cmax and AUC24 values on day 7 of gestation were higher than those on 20 days after delivery in each PROJECT M group.

The NOAEL of PROJECT M was judged to be 100 mg/kg per day in F0 dams for general toxicity, 200 mg/kg per day in F0 dams for maternal functions, and 100 mg/kg per day in F1 pups for development and reproductive functions.

### Local Tolerance

Local tolerance studies have not been conducted with PROJECT M.

### Other Toxicity Studies

A biomarkers investigation study for bile ductal injury in male rats was conducted.

### Biomarkers Investigation Study for Bile Ductal Injury in Male Rats (Project M-TX-0017)

PROJECT M was suspended in 0.5 w/v% methylcellulose solution and administered orally once daily for 4 weeks at dose levels of 30, 100, and 300 mg/kg in male Crl:CD(SD) rats. To explore biomarkers for bile duct injury, toxic changes of PROJECT M were evaluated in blood chemistry, organ weights and histopathology sequentially (at 1, 4, 8, and 24 h after dosing on day 1, at 4 and 24 h after dosing on days 7, 14, and 28) with 10 animals per examination

point. Animals in the control group were administered 0.5 w/v% methylcellulose solution in the same manner as PROJECT M groups.

No treatment-related death occurred in any group.

In the 30 mg/kg per day group, high kidney weight was noted at 24 h after dosing on day 28.

In the 100 mg/kg per day group, high direct bilirubin was noted at 1 hour after dosing on day 1. High liver weight was noted at 24 h after dosing on day 14. Vacuolation of the bile duct cells and periductular mononuclear cell infiltration in the liver were observed histopathologically at 4 and 24 h after dosing on day 28. Additionally, high kidney weight was noted at 24 h after dosing on day 28.

In the 300 mg/kg per day group, high total bilirubin and direct bilirubin were noted at 1, 4, and 8 h after dosing on day 1, and at 4 h after dosing on days 7, 14, and 28, but these changes recovered within 24 h after dosing. Low liver weight was noted at 4 and 8 h after dosing on day 1, but no histopathological lesions were observed. Also, high liver weight was noted at 24 h after dosing on days 7, 14, and 28. In histopathology, vacuolation of the bile duct cells was observed at 4 and 24 h after dosing on days 7, 14, and 28, periductular mononuclear cell infiltration and periductular crystal-like material in the liver were observed at 4 and 24 h after dosing on day 28, and hypertrophy of the hepatocytes was observed at 24 h after dosing on day 28. In the electron microscopic examination on the liver, round droplets including laminated or amorphous structures were observed in the bile duct cells at 24 h after dosing on days 14 and 28. Additionally, high kidney weight was noted at 4 h after dosing on days 7 and 14, and at 4 and 24 h after dosing on day 28.

No PROJECT M-related changes were noted in clinical signs, body weight, food consumption, urinalysis, hematology, or gross pathology. No changes were seen in the following liver parameters: aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyltransferase (GGT), glutamate dehydrogenase (GLDH) or total bile acids.

Toxicokinetic data showed that C4 (plasma concentration of PROJECT M at 4 h after dosing) increased more than dose-proportionally (7.7 to 15.2 times) between 30 and 100 mg/kg per day, and almost dose-proportionally (3.1 to 4.7 times) between 100 and 300 mg/kg per day on each dosing day. C4 at 30 mg/kg per day tended to increase by repeat dosing, while those at 100 and 300 mg/kg per day showed no effect of repeat dosing.

In conclusion, increased total and direct bilirubin were noted from the first dosing at 300 mg/kg per day with or without vacuolation of the bile duct cells.

## 4.4 Integrated Nonclinical Overview and Conclusion: Potential Clinical Relevance

PROJECT M is an orally available small molecule TrkA inhibitor. PROJECT M inhibited kinase activities (substrate phosphorylation) of the kinase domains of human TrkA, B and C with IC50 values of 0.155, 1.41 and 1.09 μmol/L, respectively. Of the other 64 kinases examined, more than 50% inhibition by PROJECT M (10 μmol/L) was detected in activity of FLT3 (78.6%)

and AXL (53.1%). The IC50 values were 2.73 and 8.00 μmol/L for FLT3 and AXL, respectively. In addition, PROJECT M demonstrated no appreciable interaction with 57 different kinds of receptors, ion channels, transporters, and enzymes.

PROJECT M showed inhibitory effects on NGF-induced plasma extravasation at ≥ 3 mg/kg orally in rats, indicating that PROJECT M inhibits the biological action of NGF. Furthermore, 7-day repeated administration of PROJECT M at 3 mg/kg orally was effective in a rat model of OA pain (MIA model). In the MIA model Cmax and AUC24 on day 7 of 3 mg/kg PROJECT M

were 242.24 ng/mL and 331.62 ng·h/mL, respectively. When taking into account that in rats 55% of PROJECT M is protein-bound (the mean value at 0.05 to 50μg/mL), the free effective concentration (Ceff,u) was 109 ng/mL (approximately 0.2 μmol/L). The results indicate that the effective plasma concentration in this model was higher than the in vitro human TrkA inhibitory concentration, but not the human TrkB and TrkC inhibitory concentrations.

Moreover, the weak or absence of effect on off-target activities in vitro and lack of obvious effect on the central and peripheral nervous system in toxicology studies up to high doses, suggests that potential off-target activity on the other receptors would be minimal at predicted efficacious exposures.

The current pharmacology package provides proof of mechanism (inhibition of TrkA) for the pharmacological activity of PROJECT M and also provides nonclinical proof of concept by demonstrating analgesic activity in a nonclinical pain model.

Pharmacokinetics after single intravenous and oral administration of PROJECT M were investigated in rats and dogs. After a single oral administration of PROJECT M to rats (1, 3 and 10 mg/kg) and dogs (0.3, 1 and 3 mg/kg), Cmax and AUCinf increased more than

dose-proportionally. The elimination t1/2, after intravenous administration (1 mg/kg), was relatively short, being 1.27 h in rats and 1.44 h in dogs. Absolute F was 29.3% (1 mg/kg) in rats and 44.9% in dogs (0.3 mg/kg). PROJECT M showed about 10% penetration in the brain of rats and rhesus monkeys. PROJECT M is likely to be a substrate of P-gp.

The in-vitro protein binding ratios in plasma spiked with [14C]PROJECT M at final concentrations of PROJECT M between 0.05 and 50 μg/mL were 79.1% to 71.1% in mouse plasma, 56.7% to 52.8% in rat plasma, 57.5% to 39.2% for rabbit plasma, 56.7% to 40.1% for dog plasma, 95.8 to 38.6% in monkey plasma and 96.8% to 42.0% in human plasma. The in-vitro protein binding ratio remained nearly identical across the tested concentration range in mouse and rat plasma. In rabbit, dog, monkey and human plasma, the plasma protein binding was inversely related to PROJECT M concentration, demonstrating a

concentration-dependent protein binding profile. The protein binding ratio in monkey plasma was comparable with that in human plasma. α1-AGP primarily contributes to the protein binding of PROJECT M in human plasma, and to a lesser extent human serum albumin, γ-globulin, LDL and HDL.

PROJECT M showed weak direct and/or time-dependent inhibitory potency in vitro for CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A. The IC50 values of

PROJECT M ranged from 107 to > 300 μmol/L. PROJECT M inhibited P-gp-mediated transport of

digoxin with an IC50 of 34.4 μmol/L. Based on these data, the risk of a DDI via CYP and/or P-gp inhibition by PROJECT M are considered low due to the relatively high IC50 values.

In-vitro metabolite fingerprinting did not reveal any human-specific PROJECT M metabolites. The most prominent metabolite in human hepatocytes was a hydrolyzed form, which was detected in very small quantities in human liver microsomes. These results suggest that non- CYP enzymes primarily contribute to hydrolysis of PROJECT M in human hepatocytes. In addition to hydrolysis, based on recombinant human CYP data, CYP3A4 and CYP3A5 also appear to contribute to the metabolism of PROJECT M. However, as PROJECT M metabolism is thought to be primarily by hydrolysis mediated by non CYP enzyme(s), the risk of a DDI related to inhibition or induction of CPY3A is considered low.

Retention of radioactivity was noted in rat thoracic aorta after a single oral dose of 3 mg/kg. No adverse changes were noted at histopathological evaluation of the aorta up to high doses (300 mg/kg per day for 13 weeks in rats, 100 mg/kg per day for 4 weeks and 30 mg/kg per day for 13 weeks in dogs) in repeated dose toxicity studies. Therefore the retention of PROJECT M-related material in the aorta after a single dose was considered of low toxicological relevance. After a single oral dose of [14C]-PROJECT M to rats, radioactivity was excreted mainly in the feces via bile.

Although PROJECT M has limited potential to penetrate the brain (PROJECT M is a P-gp substrate and showed approximately 10% penetration in rats and rhesus monkeys), Blocking NGF signaling may theoretically be associated with adverse effects on learning and memory by affecting the maintenance of cholinergic neurons. For that reason, several additional studies were conducted in rats at high multiple doses and in aged rhesus monkeys. In these studies, PROJECT M did not induce functional changes in memory or learning, as assessed by the Morris water maze in rats and DMTS testing in aged rhesus monkeys. Further, PROJECT M did not produce changes in ChAT levels in specified rat brain areas related to cognition. Moreover, PROJECT M did not induce histopathological changes in cholinergic regions in rat or dog brain in the toxicity studies at high doses.

Anti-NGF monoclonal antibody compounds in development for the treatment of various pain syndromes had been on partial clinical hold in the US based on peripheral nervous system effects observed in animal studies conducted with anti NGF monoclonal antibodies [Tiseo et al, 2014]. This clinical hold was lifted in early 2015 [Pfizer, 2015]. No microscopic changes (no degeneration/necrosis or apoptosis) were observed in the superior (anterior) cervical ganglion and dorsal root ganglion of rats and dogs in repeated-dose toxicity studies of PROJECT M up to 13 weeks using additional staining (Fluoro-Jade B staining, immunohistochemical staining for cleaved caspase 3, TUNEL staining and hematoxylin- eosin staining).

The primary target organs identified in the safety pharmacology, single and repeated dose toxicity studies were the cardiovascular system, liver, and the GI tract. Additional target organs associated with higher doses were kidneys and testes.

Reduced blood pressure, increased heart rate, reduced potassium and/or QTc prolongation were noted at ≥ 30 mg/kg in the dog telemetry study. Suppression rate on the hERG current was 13.8% at 30 μmol/L. Clinical data from the SAD study with single doses up to 240 mg showed essentially no change from baseline in QTcF values or heart rate with increasing PROJECT M plasma concentrations. Close monitoring for these toxicities is considered adequate to minimize risk in clinical studies.

In the 4- and 13-week repeated dose toxicity studies in dogs, mortality or moribund sacrifice occurred at 100 mg/kg per day and 30 mg/kg per day, respectively. These dogs showed clear evidence of liver and GI tract toxicity. In addition, increase of biliary viscosity and mucus hypersecretion in gallbladder were observed in the 13-week study. These hepatocellular and hepatobiliary changes were associated with increases in plasma hepatocellular and hepatobiliary markers. In addition, 1 dog in each study showed episodes of clear increases in liver function markers, which fully recovered before terminal sacrifice. The findings of the GI tract comprised mucosal hemorrhage, inflammation, atrophy, or erosion throughout the digestive tract. Mucous stool and stool containing blood were considered to be associated with these GI tract findings. These GI tract changes were not noted during the 4-week recovery period.

Administration of ≥ 100 mg/kg per day PROJECT M to rats for up to 13 weeks was associated with dose-dependent vacuolation of bile duct epithelial cells containing amorphous and phospholipid structure. In addition, periductal crystal-like material and an inflammatory response were seen. A tendency towards recovery was noted during the 13-week recovery period. Increases in bilirubin during the treatment period were noted, but in the absence of further changes in hepatobiliary markers, the association of this safety biomarker with the histopathological bile epithelial findings is not clear. In addition to monitoring for liver function biomarkers, an exposure limit on the mean clinical exposure will remain below the unbound exposure at the NOAEL in the rat.

At higher doses/exposures in the rat (13 weeks at ≥ 100 mg/kg per day) and dog (4 weeks at 100 mg/kg per day) repeated dose toxicity studies, adverse effects on kidneys, erythropoiesis and testes were observed. These comprised increased kidney weight and tubular changes at 300 mg/kg per day in rats and moribund dogs, increased reticulocyte count without affecting red blood cell count at ≥ 100 mg/kg in rats, and seminiferous tubular atrophy at 300 mg/kg per day in rats. Risk mitigation actions for these findings are included in the clinical studies.

Exposure margins obtained during the repeated dose toxicity studies in rats and dogs are provided in [[Table 3](#_bookmark68)].

The testes findings in the 13-week repeated dose toxicity study in rats were consistent with the changes in the male rat study for the effects on fertility and early embryonic development to implantation. Atrophy of the seminiferous tubule in the testes, with low sperm count, motility and vitality, and high sperm form anomalies index (tailless) were observed at

300 mg/kg per day. Consequently, a decreased number of implantations and live embryos, and an increased pre-implantation loss index were observed. Risk mitigation actions for these findings will be included in clinical studies.

In pregnant rats PROJECT M administration was associated with fetal growth retardation at 300 mg/kg per day, and clear postnatal developmental toxicity at 200 mg/kg per day,

consisting of pup mortality and decreased body weight gain. In addition, opacity or drying of the cornea was observed after eye opening. Development of reflex functions was retarded.

Comparable postnatal findings are seen in TrkA receptor knockout mice [Smeyne et al, 1994]. Women of childbearing potential may be included in the clinical trials conducted with PROJECT M provided that the women are informed about this potential risk, are confirmed to be not pregnant prior to treatment and use 2 forms of highly effective methods for birth control.

### Table 3 Exposures in Animal Studies and Comparison with Estimated Highest Clinical Exposure in Phase 2a Study Project M-CL-0022 Based on Unbound Cmax and AUC

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study No.** | **Species/ Study Duration** | **Dose (mg/kg/day)** | **Sex** | **Cmax,u † (ng/mL)** | **AUC24,u †**  **(ng·h/mL)** | **Remarks** | **Exposure Margin†** | |
| **Cmax,u** | **AUC24,u** |
| Project M- TX-0008 | Rat/ 13 weeks | 30 (NOAEL) | M | 2550 | 7249 | LOAEL based on changes in erythropoiesis  and liver toxicity. | 7.6 | 2.5 |
| F | 3865 | 15124 | 12 | 5.1 |
| 100  (LOAEL) | M | 4096 | 24452 | 12 | 8.3 |
| F | 11493 | 75239 | 34 | 26 |
| Project M- TX-0019 | Dog/ 13 weeks | 10 (NOAEL) | M | 2082 | 7501 | LOAEL based on liver toxicity | 6.2 | 2.5 |
| F | 2608 | 7783 | 7.8 | 2.6 |
| 30 (LOAEL) | M | 3792 | 22210 | 11 | 7.5 |
| F | 4907 | 21646 | 15 | 7.3 |
| Project M- TX-0012 | Rat/ pre-and postnatal development | 100 (NOAEL) | F | 6735 | 47316 | NOAEL based on pup mortality  & developmental changes | 20 | 16 |
| Project M-CL-  0022 | Human/  4 weeks | 100 mg bid/  subject ‡ | M/F | 335 | 2946 | NA | NA | NA |

F: female; LOAEL: lowest-observed-adverse-effect level; M: male; NA: not applicable; NOAEL: no-observed- adverse-effect level; u: unbound.

† Due to differences in protein binding among species, unbound PROJECT M values were used to calculate exposure margins. Protein binding for rat = 55% and dog = 51.3%, concentration-dependent for human (97% at an PROJECT M concentration of 50 ng/mL to 74% at 5000 ng/mL).

‡ The predicted median steady state unbound exposure for the highest planned (May 2015) dose of 200 mg bid in the multiple ascending dose study (Project M-CL-0002) is 5454 ng•h/mL; this is 1.3-fold below the exposure limit (mean unbound exposures of the NOAEL) in the 13-week rat toxicology study (AUC24,u 7250 ng•h/mL).

The dataset available justifies conducting clinical studies of up to 13 weeks of duration in men and women provided that highly effective methods for birth control are used according to ICH-M3 guideline.

A summary of potential safety concerns, as well as recommended risk minimization actions is provided in [[Table 9](#_bookmark101)] in [Section [6.3](#_bookmark96)].

#### List of References

Bove SE, Calcaterra SL, Brooker RM, Huber CM, Guzman RE, Juneau PL, et al. Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis. Osteoarthritis Cartilage. 2003;11:821-30.

Fernihough J, Gentry C, Malcangio M, Fox A, Rediske J, Pellas T, et al. Pain related behaviour in

two models of osteoarthritis in the rat knee. Pain. 2004;112:83-93.

Matson DJ, Broom DC, Carson SR, Baldassari J, Kehne J, Cortright DN. Inflammation-induced reduction of spontaneous activity by adjuvant: a novel model to study the effect of analgesics in rats. J Pharmacol Exp Ther. 2007;320:194-201.

Pfizer, Inc. Pfizer And Lilly Preparing To Resume Phase 3 Chronic Pain Program For Tanezumab.

Press release, March 23, 2015.

Smeyne RJ, Klein R, Schnapp A, Long LK, Bryant S, Lewin A, et al. Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene. Nature. 1994;368:246-9.

Tiseo PJ, Kivitz AJ, Ervin JE, Ren H, Mellis SJ. Fasinumab (REGN475), an antibody against nerve growth factor for the treatment of pain: Results from a double-blind, placebo-controlled exploratory study in osteoarthritis of the knee. Pain. 2014;155:1245-52.