### Safety Pharmacology

Safety pharmacology studies assessed the effect of PROJECT F on the hERG current in stably transfected HEK293 cells in vitro, the effects on the cardiovascular and respiratory systems in cynomolgus monkeys, and the effects on the central nervous system in mice. All studies were performed in accordance with Good Laboratory Practice (GLP).

### Safety Pharmacology Study of PROJECT F on the Central Nervous System in Mice (Project F-PT-0001)

In Project F-PT-0001 the effects of PROJECT F on the central nervous system were investigated in male Crl:CD1(ICR) mice following a single oral administration of PROJECT F at doses of 0, (methyl cellulose control), 30, 100, and 300 mg/kg.

PROJECT F had no observable adverse effects on general physical condition, behavior, and clinical signs at doses up to 300 mg/kg.

### Effects of PROJECT F on the hERG Current in HEK293 Cells (Project F-PT-0002)

In Project F-PT-0002 the effects of PROJECT F on the hERG current were assessed in hERG-transfected HEK293 cells by whole-cell patch-clamp.

PROJECT F inhibited the hERG current in a concentration dependent manner at concentrations of 3, 10, and 30 µmol/L. The IC50 value was 18.9 µmol/L (approximately 10.7 µg/mL).

### Safety Pharmacology Study of PROJECT F on Cardiovascular and Respiratory Systems in Cynomolgus Monkeys (Project F-PT-0003)

In Project F-PT-0003 in vivo assessments (conscious telemetered cynomolgus monkeys) showed that a single oral dose of PROJECT F (0, 10, 30, 100, or 300 mg/kg) had no effect on intra-abdominal body temperature, respiratory system, heart rate, PR interval, or QRS duration. At doses of 100 mg/kg or more, increased blood pressure, prolongation of QTc/QT interval and slightly decreased serum ionized calcium were observed. Vomiting was noted in the animals dosed at 300 mg/kg; thus exposure was limited in this group and there was little difference in exposure between the 100 mg/kg and 300 mg/kg groups. In toxicokinetics, the mean tmax was from 1.75 to 6.50 hours. The mean Cmax and AUC24 increased with increasing dose levels between 10 and 100 mg/kg, and were almost the same at 100 and 300 mg/kg.

## Toxicology

The current PROJECT F toxicology program consists of 1 toxicokinetic study, 3 preliminary 1-week repeated dose studies, 1 preliminary 2-week repeated dose study, 2 preliminary 13-week repeated dose studies, 2 pivotal 4-week repeated dose studies, 2 in vitro

genotoxicity studies, 1 in vivo genotoxicity study, 1 preliminary reproductive toxicity study (embryo-fetal development) and 1 in vitro phototoxicity study. All pivotal studies were performed in accordance with GLP. A tabulated overview of the nonclinical toxicology studies is provided in [End-of-Text Table 3.1]. All dose levels are described as the amount of the active ingredient of PROJECT F.

### Single Dose Toxicity

No single dose toxicity study was conducted, however, a single oral dose toxicokinetics study with PROJECT F was conducted in cynomolgus monkeys (1 male and 1 female per group) (Project F-TX-5004). Oral administration of PROJECT F at a dose of

1000 mg/kg resulted in one female monkey dying approximately 3 hours after dosing. The surviving male monkey at this dose showed salivation, vomiting, and decreased spontaneous motility. Vomiting was also noted in animals orally administered PROJECT F at

300 mg/kg.

### Repeated Dose Toxicity

The safety of PROJECT F had originally been evaluated in rats and cynomolgus monkeys, however, a pivotal 4-week repeated dose toxicity study was conducted in mice and cynomolgus monkeys, since mice were considered to be more appropriate species for rodent toxicity study based on the pharmacology studies [Section[s 4.1.1.1.5](#_bookmark12) and [4.1.1.1.6](#_bookmark14)].

### Preliminary 1-week Repeated Dose Toxicity Studies in Mice (Project F-TX-5005 and Project F-TX-5007, non-GLP)

In the initial non-GLP 1-week repeated dose mouse toxicity study (Project F-TX-5005), PROJECT F was administered once daily for 1 week at dose levels of 0, 30, 100, and 300 mg/kg per day orally to 10 male and 10 female Crl:CD1(ICR) mice per dose group.

No animal died or became moribund at any dose level and no test article-related toxic changes were noted in clinical signs, body weight, food consumption, ophthalmology, hematology, gross pathology, or organ weights. At the highest dose tested, 300 mg/kg per day, low triglycerides were noted in females and hypertrophy of the centrilobular hepatocyte in the liver was noted in both sexes.

Since the findings from the initial 1-week repeated dose toxicity study in mice were modest, a follow up non-GLP study was performed in which 5 male and 5 female Crl:CD1(ICR) mice (9 weeks old) per group were orally administered PROJECT F at a dose level of 1000 mg/kg per day once daily for 1 week (7 days) (Project F-TX-5007).

Mortality was observed in 2 males in the 1000 mg/kg per day group (1 on dosing day 4 and 1 on dosing day 6), and in 1 female (on dosing day 8). The deaths were preceded by decreased spontaneous motility (males and female) and tremor (males only). Postmortem, the males and females showed thymic changes (obscured or reduced size) and the female showed pale focus in the heart, dark red focus in the lungs, black spots in the glandular stomach, and pale color of the liver and kidneys.

In the surviving animals sacrificed at the study end, both males and females showed pale focus in the heart, small or obscured thymus, whitish crateriform raised lesions in the forestomach, and pale color of the liver and kidneys. Females also showed pale hearts and small spleens at terminal sacrifice.

Clinical observations included rough fur and decreased body weight as well as decreased food consumption (dosing day 4 and 7). The surviving females showed decreased spontaneous motility and tremor. The surviving females also showed increased body weight until day 4 followed by a decrease in body weight by day 7, reduced body weight gain, reduced food consumption on and after dosing day 4.

These studies showed that PROJECT F at a dose of 1000 mg/kg/day was lethal to mice. These studies also showed, based on the thymic and hepatocyte hypertrophy findings, that the no- observed-adverse-effect level (NOAEL) after the 1-week dosing of PROJECT F was 100 mg/kg/day for males and females in this study.

### Pivotal 4-week Repeated Toxicity Study in Mice with 4-week Recovery Period (Project F-TX-0001, GLP)

A tabulated summary of the results of the pivotal 4-week toxicity study in mice is presented in [End-of-Text Table 3.2.1]

Crl:CD1(ICR) mice were orally administered PROJECT F at a dose of 0, 30, 100, or 300 mg/kg per day with or without a 4-week recovery period. Systemic exposures to PROJECT F were determined on days 1, 14 and 28 using satellite groups of animals (3 animals/sex/group).

Slight decreases in red blood cells (RBC), hemoglobin (HGB) concentration and hematocrit (HCT) value were observed in males at doses of 100 or 300 mg/kg per day. In females similar findings were observed only at 300 mg/kg per day. In both sexes, the hematological findings were reversible by the end of the 4-week recovery period.

Based on the changes in the erythrocytic parameters, the NOAEL of PROJECT F was concluded to be 30 mg/kg per day for males and 100 mg/kg per day for females in this study.

### Preliminary 2-Week and Preliminary 13-Week Repeated Dose Toxicity Studies in Rats (Project F-TX-5001 and Project F-TX-5002, non-GLP)

Oral administration of PROJECT F to male Crl:CD(SD) rats at a daily dose up to 300 mg/kg per day for 2 weeks resulted in low serum albumin/globulin ratio, high serum

globulin concentration, high total cholesterol, and high liver weights (Project F-TX-5001). The increase in liver weight was associated with histopathological findings of granular degeneration and/or necrosis of the centrilobular hepatocytes and vacuolation of the periportal hepatocytes. The NOAEL was 100 mg/kg per day in this study.

In a preliminary 13-week study in Crl:CD(SD) rats were administered PROJECT F at doses of 0, 30, 100, 300 mg/kg per day (Project F-TX-5002). One male dosed at

300 mg/kg per day was found dead at approximately 3 hours after dosing on day 55. This animal showed decreased spontaneous activity before dosing as well as at 1 hour after dosing on the day of death. Gross pathologic examination revealed hemorrhagic fluid in the abdominal cavity, dilatation of the stomach and cecum and focal red discoloration at the cecal serosa. Histopathologic findings included hemorrhage in the cecum, centrilobular hepatocellular degeneration and/or necrosis and vacuolation of bile duct epithelium in the liver as well as hyaline droplet and degeneration and/or necrosis in proximal tubule in the kidney. There was no clear test article-related finding with relationship to the cause of death.

Surviving animals in the 300 mg/kg per day dose group showed low body weight, low hemoglobin concentration, low hematocrit values, high reticulocyte ratio and high platelet counts. Findings suggestive of liver injury included elevated liver enzymes (aspartate transaminase [AST] and alanine transaminase [ALT]), and elevated total cholesterol. These clinical chemistry findings were associated with histological findings of centrilobular hepatocellular degeneration and/or necrosis and vacuolation of bile duct epithelium. Gross liver weights were increased and thyroid follicular hypertrophy was also present. Renal findings included elevated urea nitrogen, kidney weights, hyaline droplet in proximal tubule, degeneration and/or necrosis in proximal tubule, and tubular basophilia in the kidney.

Liver and renal toxicity findings similar to those described above were also present in the 100 mg/kg per day dose group. In addition, erythrocytic parameter changes (low HGB concentration, HCT value, and high reticulocyte ratio) were noted.

Based on the 13-week repeated dose toxicity data, the NOAEL for PROJECT F was determined to be 30 mg/kg per day in this study.

### Preliminary 1-week Repeated Dose Toxicity Study in Cynomolgus Monkeys (Project F-TX-5006, non-GLP)

Cynomolgus monkeys (1 male and 1 female per dose group) were orally administered PROJECT F at a dose of 30, 100, or 300 mg/kg per day for 1 week.

In all drug treated groups, very slight or slight eosinophilic granules (termed hyaline droplets in the 4-week monkey study, below) were observed in the epithelia of the proximal tubules in the kidney. However, no other changes indicating renal dysfunction were noted at any dose level.

At 300 mg/kg per day, both monkeys (male and female) showed vomiting that occurred at approximately the tmax (2 or 4 hours). Vomiting was also noted in the female at the

100 mg/kg per day dose level.

Hematological findings of decreased erythrocyte count, HGB concentration, and HCT value were noted on day 8 in the female monkey at the 300 mg/kg per day dose level. Also noted in this animal was a high relative liver weight and an associated histopathological finding of slight diffuse vacuolation of the hepatocyte.

The NOAEL was determined to be 30 mg/kg per day, since vomiting was observed at doses of 100 mg/kg per day or greater in this study.

### 4-Week Repeated Dose Toxicity Study in Cynomolgus Monkeys with 4-Week Recovery Period (Project F-TX-0002, GLP)

A tabulated summary of the results of the pivotal 4-week toxicity study in cynomolgus monkeys is presented in [End-of-Text Table 3.2.2].

Cynomolgus monkeys (4 males and 4 females per dose group) were orally administered PROJECT F at a dose of 0, 3, 30, or 300 mg/kg per day for 4 weeks. In order to investigate the reversibility of any findings, 3 additional males and 3 additional females were included in the highest dose group and evaluated at the end of a 4-week recovery period.

Mortality or moribund animals were observed in females (1 sacrificed on day 13 and 2 on day 14 in a moribund state) administered 300 mg/kg per day, so the dose was reduced in the females to 100 mg/kg per day from day 15 through day 28. Further mortality and moribund sacrifice occurred in 2 males at the 300 mg/kg per day dose level (1 found dead on day 26 and 1 moribund sacrificed on day 28). The remaining animals in the high-dose treatment group (3 males and 2 females) and recovery group (2 males and 2 females) survived to scheduled termination.

In the animals that died or were moribund sacrificed, vomiting was observed from day 1 or day 8 of dosing and decreased food consumption was noted (females from week 1; males at week 4). Decreased body weight were noted in all moribund animals. Prior to death or moribund sacrifice, the animals showed slight or moderate decreased spontaneous activity, abnormal position (lateral position), hypothermia, suppressed response to touch, and/or suppressed response to stimulation. Hematological changes in these animals included increased leukocyte, neutrophil, and monocyte counts. Clinical chemistry observations from the moribund animals showed increased AST, increased ALT, increased total bilirubin, increased blood urea nitrogen (BUN), and creatinine. These clinical chemistry signs of liver and renal injury were accompanied by gross pathological findings of increased liver and kidney weights and histopathological findings of granular degeneration of hepatocytes and granular hypertrophy of Kupffer cells in the liver, and degeneration/necrosis and dilatation of the renal tubule and hyaline droplets in the proximal tubule in the kidneys. Urinalysis revealed increased protein and decreased chloride excretion. Finally, prolonged QT and/or QTc intervals were noted in these nonsurviving animals. It was considered that toxicity to the liver and/or kidney might have caused death or moribund condition observed in these　animals. Additional findings secondary to the moribund state are listed in [End-of-Text Table 3.2.2].

In the surviving high-dose group (males, 300 mg/kg/day and females, 300 →100 mg/kg/day), vomiting was reported in males and females from day 1 and decreased food consumption (males from weeks 1; females from week 2). This was associated with decreased body weights in males and females from week 2 of dosing. One surviving female showed a slight decrease in spontaneous activity on day 14 and day 15 that recovered when the dose was reduced to 100 mg/kg per day. Hematological findings in the high dose surviving animals included decreased RBC, HGB, HCT and/or increased reticulocyte ratio, as well as increased leukocyte, neutrophil, and monocyte counts. Clinical chemistry changes were similar to that seen in the non-surviving animals, i.e., increased AST, ALT, and total bilirubin levels along with increased glucose. Urinalysis findings in the surviving high dose group were also similar to that of the nonsurviving animals. These indications of liver and kidney injury were accompanied by the same gross pathological and histopathological findings noted for the nonsurviving animals. In the liver of surviving animals, an increase of electron-dense structures with irregular size in hepatocytes and an increase of secondary lysosomes in the Kupffer cells were observed by electron microscopy in males and females.

Electrocardiographic changes of QT and QTc interval prolongation were also noted in the high-dose surviving animals. Finally, surviving animals in the high-dose group showed signs of poor physical condition similar to that seen in the nonsurviving animals [End-of-Text Table 3.2.2]. All of these findings were reversible by the end of the 4-week recovery period.

The presence of hyaline droplets in the proximal tubule was noted at 30 mg/kg per day, but no other changes indicating renal dysfunction were noted at this dose level.

It was concluded that the target organs of PROJECT F were the kidney and liver and the NOAEL was 30 mg/kg per day for male and female monkeys in this study.

### Preliminary 13-week Repeated Dose Toxicity Study in Cynomolgus Monkeys (Project F-TX-5003, non-GLP)

PROJECT F was administered to cynomolgus monkeys for 13 weeks at a dose of 3, or 10 mg/kg per day (n = 2 males and 2 females per group).

No animal died or was euthanized due to moribundity, and no test article-related changes were observed in clinical signs, body weight, food consumption, ophthalmology, electrocardiography, hematology, blood chemistry, gross pathology, or organ weights.

Histopathological findings were limited to very slight or slight narrowing of the marginal zone in the spleen observed at the 10 mg/kg per day dose level.

It was concluded that under the conditions of this study, the NOAEL was 3 mg/kg per day for males and females, since narrowing of the marginal zone in the spleen was observed in males and females at 10 mg/kg per day in this study.

### Genotoxicity

The genotoxicity potential was evaluated 2 in vitro studies and 1 in vivo study.

### Bacterial Reverse Mutation Test of PROJECT F (Project F-TX-0003, GLP)

In GLP Project F-TX-0003, bacterial reverse mutation test was performed on PROJECT F with 5 test strains of bacteria (*Salmonella typhimurium* [TA100, TA1535, TA98, and TA1537] and *Escherichia coli* [WP2uvrA]) using the preincubation method with and without metabolic activation (S9 mix).

The number of revertant colonies did not increase 2-fold or greater when compared with that in the negative control in any test strain with or without metabolic activation.

Therefore, it was concluded that PROJECT F had no discernible mutagenic potential.

### Chromosomal Aberration Test of PROJECT F in Cultured Mammalian Cells (Project F-TX-0004, GLP)

A chromosomal aberration test was performed with cultured mammalian (CHL/IU) cells in short-term treatment for 6 hours with and without metabolic activation (S9 mix) and a continuous treatment for 24 hours without S9 mix.

Based on the cell proliferation results, chromosomal aberrations were analyzed at PROJECT F concentrations of 100, 200, and 300 μg/mL in short-term treatment for 6 hours without S9 mix; at 300, 400, and 500 μg/mL in short-term treatment for 6 hours with S9 mix; and at 12.5, 25, and 50 μg/mL in continuous treatment for 24 hours without S9 mix.

The number of cells with structural chromosomal aberrations increased in a concentration- dependent manner reaching statistical significance at 500 μg/mL when compared with the corresponding negative control (6 hour incubation with S9 mix). No significant increase in the number of cells with numerical chromosomal aberrations was noted for any other treatment conditions.

It was concluded that PROJECT F has the potential to induce structural chromosomal aberrations in CHL/IU cells.

### Combination Study of Micronucleus Test and Comet Assay in Rats Treated with PROJECT F (Project F-TX-0005)

A combined micronucleus and comet study was performed with Crl:CD(SD) rats. In the

3 days preliminary test (1000 and 2000 mg/kg/day), a decrease in spontaneous activity, ataxic gait and prone position were observed at 2000 mg/kg per day. Soft stool and a soiled area around the anus were also observed. Based on these results, the highest dose for the main test was set at 1000 mg/kg per day. The main test was conducted by gavage at dose levels of 0, 250, 500, and 1000 mg/kg per day once daily for 3 consecutive days.

No abnormalities in clinical signs were observed in any test article group. A decrease in body weight gain was noted on day 2 and day 3 in the 1000 mg/kg per day group.

In the micronucleus test, no significant changes were noted in the number of micronucleated immature erythrocytes and immature erythrocytes % in any test article group.

In the comet assay, a statistically significant increase in the % tail intensity (0.31) was noted only in the 500 mg/kg per day group and the increase was dose-dependent. However, the　value was within the range of the negative control background data (mean ± 3SD: 0.00 to 0.79). A confirmatory assay was performed under the same conditions. The results showed a statistically significant increase in the % tail intensity only in the 1000 mg/kg per day group (0.41) and dose dependency. Although the slight increase in the % tail intensity was reproducible, the value was within the range of the negative control background data

(mean ± 3SD: 0.00 to 0.79). Therefore, it was concluded that these changes were not biologically meaningful, and the results of the comet assay were judged as negative.

It was concluded that PROJECT F had no potential to induce micronuclei and DNA damage in vivo.

### Carcinogenicity

No carcinogenicity studies have been conducted with PROJECT F as of the preparation of this Investigator’s Brochure.

### Reproductive and Developmental Toxicity

A preliminary study to evaluate the effects on embryo-fetal development has been conducted in mice.

### Dose-Range Finding Study for the Effects of PROJECT F on Embryo-fetal Development by Oral Administration in Mice (Project F-TX-5008)

PROJECT F was administered daily by oral gavage to pregnant mice (6 to 8 animals per dose level) at a doses of 0, 30, 100, or 300 mg/kg/day (as active ingredient) during the period of organogenesis (gestation day [GD] 6 to GD 15). Fetuses were evaluated after caesarean sections on GD 18.

No dams died, became moribund, aborted, or prematurely delivered their litters.

No treatment-related changes were noted in clinical signs, body weight, body weight gain, food consumption, gross pathological findings, the number of corpora lutea, or the number of implantation sites in any test article group.

No treatment-related changes were noted in the number of postimplantation losses, the postimplantation loss rate, the number of live fetuses, sex ratio of live fetuses, fetal body weight of either sex, or external abnormalities. Placental morphology and weight were not affected by test article administration. There were no visceral abnormalities or variations in the 300 mg/kg/day group. There were no treatment-related changes in skeletal abnormalities or variations or the degree of bone ossification in any test article group.

Based on these results, the NOAEL of PROJECT F in this study for general maternal toxicity and reproductive function was determined to be 300 mg/kg per day. The NOAEL for embryo-fetal development was also judged to be 300 mg/kg per day.

### Local Tolerance

No local tolerance studies have been conducted with PROJECT F as of the preparation of this Investigator’s Brochure.

### Other Toxicity Studies

**4.3.7.1 A Phototoxicity Study of PROJECT F Disuccinate in Balb/c 3T3 Cells (Project F-TX-0006)**

Balb/c 3T3 cells were treated with PROJECT F (9.49, 13.3, 18.6, 26.0, 36.4, 51.0, 71.4, and

100 μg/mL) or with chlorpromazine hydrochloride (0.391, 0.781, 1.56, 3.13, 6.25, 12.5, 25.0, and 50.0 μg/mL, positive control) or with dimethyl sulfoxide (negative control) for 1 hour followed by UV-A irradiation (5 J/cm2) or no irradiation while incubation continued. For the comparator, irradiation was not conducted. Cell viability was determined by Neutral Red extraction from cells (measurement of absorbance at 540 nm).

For the test article groups, the phototoxic potential was judged from only the mean photoeffect (MPE) because the IC50 for cell viability could not be determined in either the absence or presence of irradiation. The MPE was 0.036, and thus less than the phototoxic potential of 0.15. Therefore, PROJECT F was categorized as having no discernible phototoxic potential.

## Integrated Nonclinical Overview and Conclusion: Potential Clinical Relevance

## Toxicology

Based on the toxicity findings noted in the pivotal nonclinical studies, the toxicological targets identified from the 4-week repeated oral dose toxicology studies in mice and cynomolgus monkeys were gastrointestinal tract, cardiac function, hematological findings, kidney and liver. These findings were reversible after a 4-week recovery period. In the preliminary 13-week cynomolgus monkey study, narrowing of the marginal zone in spleen was noted. Similar findings were not observed in the 4-week mouse or cynomolgus monkey repeated dose studies. Each of these findings are discussed in more detail below. The exposure ratios were calculated as unbound basis, using the effective unbound concentration (Ceff,u: 2.96 ng/mL) and effective unbound area under the plasma concentration-time curve (AUCeff,u: 35.5 ng·h/mL) at the effective dose (1 mg/kg twice daily) in the NZB/W F1 lupus prone mouse model.



### Aggravated General Conditions (death/moribundity)

In the 4-week cynomolgus monkey study, 3 females were moribund sacrificed on days 13 or 14 after treatment at 300 mg/kg per day. The dose for the remaining females in that dose group was subsequently reduced to 100 mg/kg per day (300→100 mg/kg per day dose level). One male died on day 26, and another male was moribund sacrificed on day 28 at 300 mg/kg per day. These animals showed vomiting and decreased food consumption on day 1 or day 2, and decreased body weight on day 7 in females or on day 14 in males. A decrease in spontaneous activity was observed up to 3 days before the moribundity or death. Lateral position, suppressed response to stimulation and hypothermia were observed before moribundity. No changes were noted in general condition during the 4-week recovery period in surviving animals.

These findings were observed at 300 mg/kg per day in male monkeys and at 300→100 mg/kg per day in females, and the exposure level at this dose is 1267-fold higher in males and

887-fold higher in females than that at the effective dose (1 mg/kg twice daily) in the NZB/W F1 lupus prone mouse model. The exposure ratio at 30 mg/kg per day is 179-fold in males and 128-fold in females as AUC.

The cause of mortality is unknown; however, contributing factors are likely liver or renal toxicity and an aggravated general condition due to overdose.

### Effect on the Gastrointestinal Tract

Vomiting was noted at 300 mg/kg per day (males) and 300→100 mg/kg per day (females) in the cynomolgus monkey 4-week repeated dose toxicity study (Project F-TX-0002) and at

300 mg/kg in the cynomolgus monkey telemetry study (Project F-PT-0003). There were no accompanied histopathological changes. Vomiting was not observed in the recovery period.

The exposure ratio at these doses was about 1000-fold higher than at the effective dose as Cmax base. The safety margin is 221-fold in the 4-week study and 484-fold in the telemetry study at 30 mg/kg per day, respectively.

The timing when vomiting was noted was approximately same as tmax (mainly 4 h after dosing), suggesting that the vomiting might be caused through the central nervous system.

### Effect on the Cardiovascular System

In an in vitro hERG current assay, inhibition of hERG channel conductance was observed in a concentration-dependent manner, with an IC50 of 18.9 µmol/L (10.7 µg/mL), and statistically significant differences were noted at 3 μmol/L (1.7 µg/mL) and higher. QT/QTc prolongations were observed in the cynomolgus monkey 4-week repeated dose toxicity study at 300 mg/kg per day (males) and 300→100 mg/kg per day (females), and at 100 and

300 mg/kg in the cynomolgus monkey telemetry study (Project F-PT-0003). This finding was not observed after recovery period, and was not accompanied with histopathological changes in cardiovascular system. No arrhythmia was noted at any dose.

The exposure ratios at 3 µmol/L and at IC50 were 575-fold and 3615-fold, respectively. QT/QTc prolongation was seen at about 1000-fold or higher exposure level as Cmax base in

the cynomolgus monkey study. The safety margins are 128-fold in the 4-week study, and 484-fold in the telemetry study at 30 mg/kg per day, respectively.

There is a wide safety margin, and cardiac function will be monitored in clinical studies.

### Effect on Erythrocytes

Decreases in the RBC, HGB and HCT were observed at 100 mg/kg per day in male mice and at 300 mg/kg per day in both sexes in the mouse 4-week study, and at 300 mg/kg per day (males) and 300→100 mg/kg per day (females) in the cynomolgus monkey 4-week study. In the cynomolgus monkey 4-week study, an increase in reticulocyte ratio was also observed. No findings suggesting hemolysis were observed and no histopathological changes were noted in the hematopoietic organs/tissues. The findings were not observed in the recovery period.

The exposure ratio at 100 mg/kg per day was 177-fold in the mouse study, and at 300 mg/kg per day (males) and 300→100 mg/kg per day (females) it was 887-fold in the cynomolgus monkey study. The safety margins at 30 mg/kg per day were 40-fold in the mouse study as AUC. Erythrocyte parameters are monitorable in clinical studies.

### Effect on the Kidney

At doses of 30 mg/kg or more, hyaline droplets in the proximal tubule were observed in the 4-week cynomolgus monkey study. At doses of 300 mg/kg per day (males) and

300→100 mg/kg per day (females), increases in BUN, creatinine, urine protein and glucose, a decrease in urine chloride excretion, degeneration or necrosis in the renal tubules, dilation of the renal tubules, and cytoplasmic vacuolation in the proximal tubular epithelium were observed. No renal changes were observed after the 4-week recovery period.

At 300 mg/kg per day (males) and 300→100 mg/kg per day (females), the exposure ratio is 887-fold. The safety margin is 128-fold as the AUC base in the 4-week cynomolgus monkey study at 30 mg/kg per day.

Hyaline droplets in the renal proximal tubules were observed microscopically, and electron microscopic examination confirmed an increase in secondary lysosomes in the renal proximal tubular epithelium. In the preliminary 13-week cynomolgus monkey study, no renal changes were noted at up to 10 mg/kg per day, the highest dose tested.

The findings were reversible; however, renal function parameters will be monitored in clinical studies.

### Effect on the Liver

Granular degeneration of the hepatocytes, granular hypertrophy of the Kupffer cells, and vacuolation of the hepatocytes were observed in the 4-week cynomolgus monkey study. Serum AST, ALT and total bilirubin were increased. These changes showed reversibility in the 4-week recovery period.

These findings were observed at 300 mg/kg per day (males) and 300→100 mg/kg per day (females) and exposure ratio is 887-fold. The safety margin is 128-fold as AUC in the

4-week cynomolgus monkey study at 30 mg/kg per day.

Electron microscopic examination confirmed an increase in electron-dense structures with irregular size in the hepatocytes, an increase in secondary lysosomes in the Kupffer cells, and cytoplasmic vacuolation in the hepatocytes.

The findings were reversible; however, liver function will be monitored in clinical studies.

### Effect on the Spleen

Narrowing of the marginal zone was observed at 10 mg/kg per day in the preliminary

13-week cynomolgus monkey study, although it was not observed at up to the highest dose tested, 300 mg/kg per day (males) and 300→100 mg/kg per day (females) in the 4-week cynomolgus monkey study.

The exposure ratio at 10 mg/kg per day was 20-fold in the 13-week cynomolgus monkey study. The safety margins were 4-fold in 13-week study, and 887-fold in 4-week study at 300 mg/kg per day (males) and 300→100 mg/kg per day (females) as AUC base.

This finding was considered related to the pharmacological effect. The splenic change was not observed in cynomolgus monkeys administered with PROJECT F for 4 weeks, which is longer than planned dosing period in the phase 1 clinical trial (2 weeks). This observation will need to be further assessed in the longer toxicology study for the phase 2 clinical trials.

Toxicokinetics data in the 4-week toxicity studies in mice and cynomolgus monkeys (Project F-TX-0001 and Project F-TX-0002), and comparison with the exposure at the effective dose (1 mg/kg twice daily) in NZB/W F1 lupus prone mouse model are summarized in [[Table 2](#_bookmark70)].

A summary of target organ toxicities is listed in [[Table 3](#_bookmark71)].

### Table 2 Mean Plasma Exposure Levels of Safety Studies

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study No.** | **Species/ Study**  **Duration/ Route** | **Dose (mg/kg)** | **Sex** | **Sampling point** | **Cmax (ng/mL)** | **AUC24**  **(ng·h/mL)** | **Exposure Ratio†** | |
| **Cmax,u based** | **AUC24,u**  **based** |
| Project F- TX- 0001 | Mice/ 4 weeks/  po | 30  NOAEL for M | M | Day 28 | 3440 | 8230 | 202 | 40 |
| F | 3540 | 8190 | 208 | 40 |
| 100  NOAEL for F | M | Day 28 | 7670 | 36100 | 451 | 177 |
| F | 8460 | 35200 | 497 | 173 |
| 300 | M | Day 28 | 11200 | 135000 | 658 | 662 |
| F | 16500 | 146000 | 970 | 716 |
| Project F- TX- 0002 | Cynomolgus monkey/  4 weeks/ po | 3 | M | Day 28 | 410 | 1150 | 66 | 15 |
| F | 115 | 394 | 18 | 5 |
| 30  NOAEL | M | Day 28 | 1870 | 13400 | 299 | 179 |
| F | 1380 | 9600 | 221 | 128 |
| 300 | M | Day 28 | 6950 | 95100 | 1111 | 1267 |
| 300‡ | F | Day 14 | 5990 | 79500 | 957 | 1059 |
| 100‡ | F | Day 28 | 6140 | 66600 | 981 | 887 |

AUCeff,u: effective area under the plasma concentration-time curve for unbound drug; AUC24,u: area under the plasma concentration-time curve from time 0 to 24 hours for unbound drug; Ceff,u: effective observed plasma concentration for unbound drug; Cmax,u: maximum observed plasma concentration for unbound drug; NOAEL: no observed adverse effect level; fp: plasma free fraction.

† The exposure ratios were calculated as unbound basis, using Ceff,u: 2.96 ng/mL and AUCeff,u: 35.5 ng·h/mL at the effective dose of 1 mg/kg in the NZB/W F1 lupus prone mouse model. fp = 0.174 (mouse) and 0.464 (monkey).

‡ Mortality or moribund animals were observed in females on day 13 or 14 administered 300 mg/kg per day, so the dose was reduced in the females to 100 mg/kg per day from day 15 through day 28.

### Table 3 Summary of Nonclinical Safety Observations and Potential Human Relevance

|  |  |  |
| --- | --- | --- |
| **Key Safety Targets** | **Key Observations** | **Potential Relevance to Human Usage** |
| **General Condition** | Cynomolgus monkey: vomiting, decreased spontaneous activity, lateral position, suppressed response to stimulation, hypothermia, decreased body weight and food consumption, and death or  moribund | Possibly relevant Careful monitoring of clinical signs |
| **Liver** | Cynomolgus monkey: Granular degeneration of the hepatocytes, granular hypertrophy of the Kupffer cells, and vacuolation of the hepatocytes. Increased AST, ALT and total  bilirubin. | Possibly relevant Standard monitoring of liver function parameter |
| **Kidney** | Cynomolgus monkey: Degeneration/necrosis in the renal tubules, cytoplasmic vacuolation in the proximal tubular epithelium and dilation of the renal tubules and hyaline droplets in the proximal tubule. Increased BUN and creatinine. Increased urine protein, glucose and decreased  chloride excretion. | Possibly relevant Standard monitoring of renal parameter |
| **Heart / CV (incl QT)** | In vitro: hERG inhibition.  Cynomolgus monkey: Prolongation of QTc | Possibly relevant Standard monitoring of electrocardiogram  parameters |
| **Blood** | Mouse and cynomolgus monkey: decrease in RBC, HGB and HCT  Cynomolgus monkey: increase in reticulocytes | Possibly relevant Standard monitoring of hematological  parameters |
| **Spleen** | Cynomolgus monkey: narrowing of marginal  zone (preliminary: only in 13-week study) | Not yet determined |
| **Gastrointestinal tract** | Cynomolgus monkey: vomiting | Possibly relevant  Careful monitoring of clinical signs |
| **Genotoxicity** | PROJECT F has the potential to induce structural chromosomal aberrations in mammalian CHL/IU cells with metabolic activation (S9 mix) | Not relevant, because the reverse mutation, in vivo micronucleus and comet tests were  negative |
| **Reproductive toxicity** | No effect on embryo-fetal development in  preliminary mice study | Not yet determined |
| **Carcinogenicity** | Not yet determined | Not yet determined |

ALT: alanine transaminase; AST: aspartate transaminase; BUN: blood urea nitrogen; CV: cardiovascular; hERG: human ether-a-go-go-related gene; HCT: hematocrit; HGB: hemoglobin; RBC: red blood cells.

### List of References

Weening JJ, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, Balow JE, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. J Am Soc Nephrol. 2004;15(2):241-50.