##### Safety Pharmacology

Safety pharmacology studies, conducted in accordance with Good Laboratory Practice (GLP) standards, assessed potential effects on the CNS, cardiovascular and respiratory systems. In all in vivo studies, Project L was dissolved in a 0.5 w/v% methylcellulose aqueous solution for administration. All dose levels and plasma concentrations are expressed as PROJECT L free base.

##### In Vitro Study: Effect on hERG Current

The effect of Project L on hERG current was studied in hERG-transfected human embryonic kidney (HEK)293 cells using the whole-cell patch-clamp technique

(Study Project L-PT-0002). Peak tail current amplitude for each experimental group was measured in 5 individual cells, and the change rates (suppression rates) of the amplitude were calculated 13 min after beginning the application.

At concentrations of 3, 10, and 30 μmol/L, PROJECT L exerted a significant suppressive effect on the hERG current (10.8%, 35.2%, and 63.8% [compensated suppression rate], respectively). The IC50 value of PROJECT L was estimated to be 18 μmol/L, indicating that PROJECT L suppressed hERG current in hERG-transfected HEK293 cells in a concentration- dependent manner (~10 μg/mL as PROJECT L).

##### In Vivo Studies

* + - * 1. **Effect on the Central Nervous System in Rats**

In order to investigate the effect of Project L on the CNS, general activity and behavior in male rats was assessed by the modified Irwin's method (Study Project L-PT-0001). A single oral dose of Project L was administered to groups of 6 male Crl:CD (SD) rats (6 weeks of age at administration) at 0 (vehicle control), 10, 30, 100, and 300 mg/kg under non-fasting conditions. The general activity and behavior of the animals were observed before and 1, 2, 4, 6, 8, 10, and 24 h after administration.

At doses of 10, 30, and 100 mg/kg, Project L did not affect the general activity or behavior of rats up to 24 h after administration. At a dose of 300 mg/kg Project L, a soiled perineal region was noted 24 h after administration in 2 animals. These animals had soft stool or diarrhea between 10 and 24 h after Project L administration.

Mean PROJECT L Cmax and AUC24 values increased nearly dose-proportionally up to 100 mg/kg. Mean time to reach Cmax (tmax) values ranged from 6 to 10 hours. Those

pharmacokinetic parameters tended to be saturated at a dose of 300 mg/kg. Cmax values at 10, 30, 100 and 300 mg/kg were 172.22, 918.98, 1995.85 and 2047.74 ng/mL, respectively.

AUC24 values at the corresponding doses were 1788.94, 8041.76, 24194.94, and 33909.61 ng·h/mL.

These data demonstrate that Project L had no effect on general activity or behavior after single doses of ≤ 100 mg/kg in this study, while doses of 300 mg/kg Project L resulted in a soiled perineal region, suggestive of soft stool or diarrhea.

##### Effects on Central Nervous, Cardiovascular and Respiratory Systems in Dogs

The purpose of this study was to investigate the effects of a single oral dose of Project L on the central nervous, cardiovascular, and respiratory systems (Study Project L-PT- 0003). Project L was orally administered to male, unanesthetized, beagle dogs (4/dose group) at doses of 0 (vehicle control), 3, 10, and 30 mg/kg Project L.

Animals were monitored via a telemetry system for effects on general activity and behavior, body temperature, blood pressure, heart rate, electrocardiogram, respiration rate, blood gases, and blood electrolyte concentrations.

At 3 mg/kg Project L, no treatment-related effects were noted. At 10 mg/kg Project L, the following findings were noted: vomiting in 2 animals, loose stool in 1 animal, an increase in the body temperature in 1 animal between 10 and 12 h after administration, and an increase in the heart rate in 1 animal between 10 and 16 h after

administration. At 30 mg/kg Project L, the following findings were noted: vomiting in all animals, syncope, salivation, incontinence of urine, mucous and bloody stool, loose stool, and anorexia in 1 animal, an increase in the body temperature in 1 animal between

6 and 48 h after administration, and an increase in the heart rate in 1 animal between 10 and 48 h after administration.

Mean PROJECT L Cmax and AUC values increased nearly dose-proportionally ≤ 10 mg/kg but less than dose-proportionally at a dose of 30 mg/kg. Mean PROJECT L Cmax values at dose of 3, 10, and 30 mg/kg were 72.73, 205.91, and 318.23 ng/mL, respectively. Mean AUC24 values at the corresponding doses were 945.35, 2722.63, and 4196.43 ng∙h/mL, respectively. Mean PROJECT L tmax values at the corresponding doses were 4.0, 4.0, and 3.5 h.

In this study, no treatment-related effects of Project L on the central nervous, cardiovascular, or respiratory systems were observed at 3 mg/kg; however, at 10 and 30 mg/kg Project L, various treatment-related effects were observed. No

arrhythmias or ECG abnormalities (PR, QT and QTc intervals, QRS duration) were noted at any dose level in dogs.

## Toxicology

All toxicity studies were conducted in accordance with GLP standards except preliminary Studies Project L-TX-0001, Project L-TX-0002, Project L-TX-0003 and Project L-TX-0004. PROJECT L

mesilate was used as a test article in all toxicology studies except the preliminary 1-week rat toxicity study (Study Project L-TX-0002) where PROJECT L free base was used. Pivotal toxicology studies were conducted with Project L prepared as a 0.5 w/v% methylcellulose (MC) solution for in vivo studies. The route of administration for all in vivo studies was oral. All dose levels and plasma drug concentrations are expressed as PROJECT L free base.

##### Single-dose Toxicity

##### Single Oral Dose Toxicity Study in Rats

A single oral dose of Project L was administered under fasted conditions at dose levels of 250, 500 and 1000 mg/kg to 5 male and 5 female Crl:CD(SD) rats per group (Study Project L-TX-0005). At 250 mg/kg Project L, no animals died and no toxic changes were observed on the dosing day (day 0). On the day following dosing (day 1), black stool (positive for occult blood), soft stool, and decreased stool volume were observed in both sexes, but these changes disappeared by day 4. Body weight loss or suppression of body weight gain was noted in both sexes on days 1 and 2, but body weight increased from

day 4 onwards. Unilateral swelling of the testes accompanied by histopathological dilatation of the seminiferous tubules was observed in 1 male.

At 500 mg/kg Project L, all rats died or were euthanized due to moribundity between days 0 and 4. At 1000 mg/kg Project L, 1 male died on day 0, and the remaining animals died or were euthanized due to moribundity on day 1. At 500 and

1000 mg/kg Project L, prior to mortality or moribundity, animals showed decreased spontaneous activity, prone position, gasping, hypothermia, pale skin, bradypnea, lacrimation, decreased stool volume, no stool, soft, black or mucus stool, soiled perineal region, and/or body weight loss. Gross pathology revealed retention of pale-yellow or pale-red content in the stomach, retention of dark-red or dark-brown content in the small and large intestines,

and discoloration of the heart in male rats. Histopathology revealed vacuolation of chief and parietal cells, focal necrosis of mucosa, focal hemorrhage and neutrophil infiltration in the lamina propria in the glandular stomach, vacuolation of the mucosal epithelium, accumulation of foam cells in the lamina propria or Peyer’s patches, atrophy of the villus or Peyer’s patches in the jejunum and ileum, foam cells in the lamina propria, vacuolation of the mucosal epithelium in the cecum; colon; and rectum, and focal necrosis of the villus tip in the duodenum.

Under the conditions of this study, the approximate lethal dose level of Project L was considered to be 500 mg/kg for male and female rats. Most acute changes observed in rats were classified as gastrointestinal disorders.

##### Single Oral Dose Toxicity Study in Dogs

A single oral dose of Project L was administered at dose levels of 30, 100, and

300 mg/kg to 1 male and 1 female beagle dog per group (preliminary Study Project L-TX-0001). No animals died or were euthanized due to moribundity during the observation period.

Vomiting was observed in both animals at ≥ 30 mg/kg Project L. Diarrhea was observed in the male dog at 2 and 8 h post dosing at ≥ 100 mg/kg Project L.

Aspartate aminotransferase (AST), blood urea nitrogen (BUN), and inorganic phosphorus increased in the male on day 1 postdose, but these changes recovered by day 7 postdose. No Project L-related changes in body weight, food consumption or hematology were noted at any dose level.

No clear increases in PROJECT L Cmax or AUC24 values were noted with dose increment. PROJECT L Cmax and AUC24 values at 300 mg/kg in the female were lower than in the male [End-Of-Text Table 3.4].

Vomiting was noted in males and females at all doses tested. Diarrhea and increased AST, BUN, and inorganic phosphorus were noted in males administered ≥ 100 mg/kg Project L.

##### Repeat-dose Toxicity

* + - 1. **2-Week Oral Dose Range-finding Toxicity Study in Rats**

ASProject L mesilate was orally administered to 5 male and 5 female Crl:CD (SD) rats per group at dose levels of 0 (vehicle control), 10, 30, and 100 mg/kg once daily for 2 weeks (preliminary Study Project L-TX-0003).

At 10 and 30 mg/kg per day Project L, no test article-related changes were observed. At 100 mg/kg per day Project L, 1 male was found dead on day 5,

2 females were found dead on days 10 and 12, and 2 males were prematurely sacrificed in a moribund condition on days 8 and 10. In males and females, including dead and prematurely sacrificed animals, soiled fur, incomplete eyelid opening and decreased body weight and food consumption were observed. In addition, decreased spontaneous motility, reddish substance around eyes, paleness, and watery feces were observed in males; and moist fur around the urethral orifice, soiled fur around eyes, and loss of hair were observed in females.

In the 10 and 30 mg/kg per day groups, the body weights of males and females increased similarly to those in the control group. The body weights of males and females in the 100 mg/kg per day group decreased. The food consumption of males and females in the

10 and 30 mg/kg per day groups was comparable to that in the control group. However, food consumption was decreased in rodents of both sexes in the 100 mg/kg per day group.

PROJECT L Cmax and AUC24 increased dose-dependently in males and females after a single dose (on day 1). In females, PROJECT L Cmax and AUC24 tended to decrease after repeated administration at doses ≥ 30 mg/kg per day as compared to single dose administration.

No study-drug related changes were noted in the 10 and 30 mg/kg per day dose groups in either males or females.

##### 4-Week Oral Dose Toxicity Study in Rats

Project L was orally administered once daily to 10 male and 10 female Crl:CD(SD) rats per group at dose levels of 0 (vehicle control), 3, 10, 30, and 60 mg/kg per day for

4 weeks (Study Project L-TX-0006). Six males and 6 females were added to the control, 30 and 60 mg/kg groups in order to assess the reversibility of toxicities observed during the dosing period in a subsequent 4-week recovery period.

No animal died at any dose level. At doses of ≥ 3 mg/kg per day Project L, low serum potassium was noted in both sexes. At doses of ≥ 10 mg/kg per day Project L, low serum albumin and albumin/globulin ratio were observed in males and high serum alanine aminotransferase (ALT) was observed in females. Vacuolation of mucosal epithelium in the cecum was observed in both sexes, atrophy of the mammary gland, vacuolar changes (high lymphocyte vacuolation ratio in peripheral blood and vacuolation of mucosal epithelium in the ileum) and atrophy of the splenic white pulp with low spleen weight were observed in males. Testicular toxicity was observed in males as follows: unilateral or bilateral small size, swelling, white focus, and/or softening of the testes, and unilateral or bilateral white nodule in the periepididymal tissue (head); low or high testes weight; degeneration/atrophy and dilatation of the seminiferous tubules, spermatic granuloma in the testes, spermatic granuloma in the efferent ductules of testes, intraluminal cell debris and decrease in sperm in the epididymis.

At doses of ≥ 30 mg/kg per day Project L, the following changes were observed in both sexes: low body weight, atrophy of the corneal epithelium, vacuolation (bile duct epithelium in the liver, glomeruli in the kidney, urothelium in the urinary bladder, and chief cells in the parathyroid) and foam cell accumulation (lung and lamina propria of the ileum), small thymus with low thymus weight, thickening of the epiphyseal cartilage in the femur, low urinary pH, low basophil count and ratio, and increased single cell necrosis of acinar cells in the pancreas.

At doses of ≥ 30 mg/kg per day Project L, the following changes were observed in males only: low food consumption, vacuolation (collecting duct epithelium in the kidney) and foam cell accumulation (mesenteric lymph nodes and lamina propria in the cecum), immune system-related changes (atrophy of the submandibular lymph nodes, mesenteric lymph nodes, and Peyer’s patches, hypocellularity in the femoral bone marrow, and low lymphocyte count and ratio in peripheral blood), urinary changes (high bilirubin, low total sodium excretion, increased incidence of no sperm in urinary sediment), low serum protein, and high serum inorganic phosphorus.

At doses of ≥ 30 mg/kg per day Project L, the following changes were observed in females only: vacuolation (mucosal epithelium in the duodenum, ileum, and rectum, and lymphocytes in the medulla of the submandibular lymph nodes), immune system-related changes (atrophy of the splenic white pulp with low spleen weight), liver-related changes (high serum AST and total bilirubin, low serum albumin concentration and ratio and albumin/globulin ratio, and multifocal necrosis of hepatocytes), urinary changes (positive occult blood and high ketone bodies), low hemoglobin concentration, low mean corpuscular volume (MCV), low mean corpuscular hemoglobin concentration (MCH), high neutrophil and monocyte counts and ratios, vacuolation of luteal cells in the ovary, ocular changes (unilateral corneal opacity, keratic precipitates at 30 and 60 mg/kg per day, unilateral enlargement of the eyeball and disappearance of light reflex, anterior and posterior synechiae at 30 mg/kg per day), and atrophy of the submandibular gland (with low submandibular gland weight) and Harderian gland.

At doses of 60 mg/kg per day Project L, the following changes were observed in both sexes: atrophy of the epidermis in the interscapular back of skin, vacuolation (proximal tubule epithelium in the kidney and mucosal epithelium in the jejunum) and foam cell accumulation (submandibular lymph nodes and lamina propria of the rectum), high lung weight, immune system-related changes (atrophy of the thymus and hypocellularity in the sternal bone marrow), liver-related changes (high serum ALP, periportal inflammatory cell infiltration and proliferation of the bile ducts), basophilic change of mucosal epithelium in the duodenum, urinary changes (low total potassium and chloride excretions), and soft stool (transient).

At doses of ≥ 60 mg/kg per day Project L, the following changes were observed in males only: vacuolation (mucosal epithelium in the duodenum and rectum, and lymphocytes in the medulla of submandibular lymph nodes), liver-related changes (high serum AST, ALT, and total bilirubin, and multifocal necrosis of hepatocytes), positive urinary occult blood, bone/cartilage-related changes (decrease in trabecular bones in the femur, and necrosis of the bone/cartilage and granuloma in the hip joint), mineralization in the muscular layers of the glandular stomach, high neutrophil and monocyte counts and ratios, high platelet count, atrophy of the submandibular gland (with low submandibular gland weight) and Harderian gland, and soiled perineal region and reddish urine (transient).

At doses of ≥ 60 mg/kg per day Project L, the following changes were observed in females only: low food consumption, atrophy of the epidermis in the nipple in the abdominal skin and mammary gland, vacuolar changes (high lymphocyte vacuolation ratio in peripheral blood, and vacuolation of periportal hepatocytes, collecting duct epithelium in the kidney, splenic lymphocytes, and mucosal epithelium in the colon) and foam cell accumulation (mesenteric lymph nodes and lamina propria in the cecum), immune system-related changes (low lymphocyte count and ratio, small spleen, atrophy of the submandibular lymph nodes, mesenteric lymph nodes, and Peyer’s patches, and hypocellularity in the femoral bone marrow), liver-related changes (multiple dark red foci in the liver, high liver weight, focal necrosis of the bile ducts), high kidney weight, low urinary total sodium excretion, low hematocrit value and mean cell hemoglobin concentration (MCHC), high serum inorganic phosphorus, triglycerides, BUN, and globulin concentration (including high alpha2-globulin, beta-globulin, and gamma-globulin ratios), low serum total protein, reproductive organ changes (atrophy of the interstitial glands in the ovary, uterus, and mucosa in the vagina with small ovary and uterus, and neutrophil infiltration in the mucosa in the vagina), rough surface in the lung, atrophy of the sublingual gland, and decreased stool volume (transient).

During the recovery period at 30 and 60 mg/kg per day Project L, all changes observed during the dosing period recovered or tended to recover, except for testicular toxicity (such as small size, white focus, softening of the testes, degeneration/atrophy of the seminiferous tubules, spermatic granuloma in the testes, and spermatic granuloma in the efferent ductules), ocular findings (such as corneal opacity, keratic precipitates, enlargement of the eyeball, and anterior and posterior synechiae), and low lymphocytes and basophils in peripheral blood. The testicular toxicity and changes in peripheral blood did not appear to be aggravated from the end of the dosing period.

PROJECT L Cmax and AUC24 in both sexes increased more than dose-proportionally ≤ 60 mg/kg per day, except on days 14 and 28 in males, when both parameters were nearly dose proportional. PROJECT L Cmax and AUC24 at 30 and 60 mg/kg per day were slightly higher in females than in males, but there was no appreciable sex difference.

Under the conditions of this study, the NOAEL for males and females was < 3 mg/kg per day Project L due to low serum potassium level. The majority of changes observed during the dosing period showed full or partial recovery during the 4-week recovery period, with the exception of testicular toxicity, ocular findings, and low lymphocytes and basophils in peripheral blood.

##### 4-Week Oral Dose Toxicity Study in Dogs

Project L was orally administered once daily for 4 weeks at dose levels of 0 (vehicle control), 1.5, 5, and 15 mg/kg per day to 4 male and 4 female beagle dogs per group

(Study Project L-TX-0007). Three males and 3 females were added to the 5 and 15 mg/kg group to assess the reversibility of the observed toxicities; the recovery period was initially 4 weeks.

One male and 2 females from the 15 mg/kg per day group were sacrificed due to moribundity on days 16, 10 and 16 of dosing, respectively. Dosing at 15 mg/kg per day Project L was discontinued for males after day 20 and for females after day 17. Three males and 2 females were necropsied at the end of dosing, 3 males were necropsied after a recovery period of 36 days and 3 females were necropsied after a recovery period of 39 days.

At doses of ≥ 1.5 mg/kg per day Project L, decreased erythrocyte count, hematocrit value and hemoglobin concentration were observed in males and females, vomiting, increased ALP, prolonged APTT and decreased albumin were noted in males. At doses of

≥ 5 mg/kg per day Project L, decreased platelet count, decreased total protein, and erythrocytes in the sinus in the mesenteric lymph node were observed in males and females. Increased serum chloride, decreased albumin/globulin ratio, and thickening of the cartilage in the sternum in males, vomiting, increased ALP and prolonged APTT in females were noted.

At doses of 15 mg/kg per day Project L, similar changes were noted in male and female surviving and moribund animals. Decreased spontaneous activity, no stool, decreased body weight or food consumption was noted in surviving animals. In addition, prone position, tachypnea, panting, and suppression of touch response were noted in moribund animals. In males and females in surviving and moribund animals, soft stool, diarrhea, mucus stool or abnormal stool color with positive occult blood reactions was noted.

Hemorrhage in the mucosa (lamina propria) or submucosa in the stomach, jejunum, ileum, cecum, colon, rectum and/or gallbladder, ulcer in the stomach, atrophy of the mucosa in the ileum, atrophy of lymphoid tissue of the cecum and rectum, cell debris in the crypts in the cecum, dilatation of the crypts in the colon and rectum were noted. A trace of reddish rhinorrhea, abnormal mucosa (abnormal color, maxillary oral mucosa or tongue), eye mucus, reddish conjunctiva, reddish oral mucosa, erosion, or interdigital inflammation, and histopathological lesions of mucosal or epithelial damages including atrophy, erosion, ulcer, or inflammatory cell infiltration in the tongue, esophagus, eyeball (conjunctiva/cornea/limbus), skin, eyelid, and oral mucosa were observed. Vacuolation or accumulation of foam cells in various organs and tissues (heart, tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, kidney, urinary bladder, prostate, uterus, eyeball (mucosa in the conjunctiva/cornea/limbus), skin, eyelid, mammary gland, or oral mucosa were observed. Increased lymphocyte vacuolation ratio in the peripheral blood was noted. Atrophy of the thymus, spleen, submandibular lymph node, mesenteric lymph node, Peyer's patch, or a decrease in zymogen granules in the acini in the pancreas was observed. Inflammatory cell infiltration, hemorrhage and edema in the alveoli, and thickening of the pleura in the lung were observed. Dilatation of the distal tubules, focal interstitial hemorrhage, and basophilic changes in the proximal tubules in the kidneys were observed. Decreases in the trabecular bone and thickening of the cartilage in the sternum, congestion in the spleen, erythrocytes in the sinus and histiocytosis in the sinus in the submandibular and mesenteric lymph nodes, hemorrhage in the myocardium and adrenals, and slight mineralization in the arterial wall in the heart (not in the coronary arteries) were observed. Additionally, high adrenal, spleen, lung, liver, or kidney weights, and gross pathological changes (swelling of interdigit in the hindlimb and forelimb, red discoloration in the conjunctiva, discoloration in the oral mucosa, red focus and discoloration in the tongue, enlargement in the spleen, dark red or red focus, or red, dark red, or black discoloration in the kidney, lung, stomach, jejunum, ileum, or gallbladder) were noted. Clinical pathology revealed decreased lymphocyte and eosinophil counts and decreased calcium and sodium levels. In addition, clinical pathology revealed increased monocyte and large unstained cell counts, AST and ALT levels, globulin, triglycerides, total cholesterol, BUN, creatinine and potassium levels. In moribund animals, increased glucose in urinalysis was noted in 1 female, and opacity in the cornea in the bilateral eyes was observed in 1 of the surviving male animals.

PROJECT L Cmax and AUC24 increased almost dose-proportionally ≤ 15 mg/kg per day in both sexes. Both parameters were slightly higher on day 14 (similar on day 28) as compared to day 1. There was no appreciable sex difference.

After a 4-week recovery period, inflammatory cell infiltration, edema and hemorrhage in the alveoli, or thickening of the pleura in the lung accompanying the gross lesion (dark red or red focus, or red or dark red discoloration in the lung) were still observed at doses of 5 mg/kg per day Project L; however, the severity and/or incidence had improved as compared to the end of the dosing period. At a dose of 15 mg/kg per day Project L, opacity in the cornea observed during the dosing period in 1 male did not worsen or reverse by the end of a 4-week recovery period. Other changes recovered or decreased in severity and/or incidence as compared to the interim mortality or the end of dosing period.

Under the conditions of this study, the NOAEL was < 1.5 mg/kg per day Project L for males and females. The changes observed during the dosing period recovered or tended to recover during the 4-week recovery period, except for opacity in the cornea in 1 male at 15 mg/kg per day.

##### Genotoxicity

##### In Vitro

* + - * 1. **Reverse Mutation Test**

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 a preincubation method with and without metabolic activation (Study Project L-TX-0008). Based on the results of the dose range-finding test at concentrations of 5 to 5000 μg/plate as PROJECT L with and without metabolic activation, the main test was performed at concentrations of 39.1 to 2500 μg/plate in TA100, and at concentrations of 9.77 to

625 μg/plate in TA1535, WP2*uvrA*, and TA98, and at concentrations of 2.44 to 156 μg/plate in TA1537 without metabolic activation, and at concentrations of 156 to 5000 μg/plate in TA100 and WP2*uvrA*, and at concentrations of 78.1 to 5000 μg/plate in TA1535, TA98, and TA1537 with metabolic activation.

There were no ≥ 2-fold increases in the number of revertant colonies observed in any test strain with or without metabolic activation as compared to negative control. No test article precipitation was observed on the plates with or without metabolic activation. Growth inhibition was observed at 78.1 μg/plate and greater in TA1537, and at 313 μg/plate and greater in TA1535, and at 625 μg/plate in WP2*uvrA* and TA98, and at 1250 μg/plate and greater in TA100 without metabolic activation, and at 2500 μg/plate and greater in TA98 and TA1537, and at 5000 μg/plate in TA1535 with metabolic activation.

Project L did not induce genetic mutations in bacteria under the conditions of this study.

##### Chromosomal Aberration Test

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 (Study Project L-TX-0009).

The dose levels for the chromosomal aberration test were based on the results of a dose-finding test for cell proliferation. Chromosomal aberrations were analyzed at the

following doses of PROJECT L: 0.25, 1, 1.5, and 3 μg/mL in short-term treatment without metabolic activation, 0.5, 3, 5.5, 6.5, and 8 μg/mL in short-term treatment with metabolic

activation, and 0.25, 0.5, 1, and 1.25 μg/mL in continuous treatment for 24 h. The number and incidence of cells with structural and numerical chromosomal aberrations were investigated.

No test article precipitation in the treatment medium was observed at the start or end of treatment. The cell proliferation ratio determined from population doubling showed

dose-dependent decreases under all treatment conditions. Significant increases in the number of cells with numerical chromosomal aberrations were noted when compared with the negative control group in all treatment conditions. The number of chromosomal aberrations also appeared to be dose-dependent. In conclusion, Project L has the potential to induce chromosomal aberrations in CHL/IU cells.

##### 4.3.3.2 In Vivo Micronucleus Test

Project L was orally administered once daily for 2 days at approximately 24-h intervals to groups of 5 male Crl:CD(SD) rats at dose levels of 0 (vehicle control), 125, 250, and 500 mg/kg per day Project L, to evaluate the potential to induce micronuclei in rat erythroblasts (Study Project L-TX-0010). Cyclophosphamide monohydrate was administered orally once at 20 mg/kg as a positive control. The number and incidence (%) of micronucleated immature erythrocytes (MNIE) per 2000 immature erythrocytes (IE) from each animal, and the ratio of IE per 500 total erythrocytes (IE + mature erythrocyte) from each animal were investigated in femoral bone marrow at approximately 24 h after the final administration.

No significant increase in the number of MNIE was noted in any test article group when compared with the negative control group. No significant decrease in IE% was noted in any test article group when compared with the negative control group. Mean MNIE% and IE% in both the negative and positive controls were within the range (mean ± 3SD) of the background data of the testing facility. Accordingly, it was judged that the present study met the acceptance criterion.

No animals died. Clinical evaluation revealed soft stool in 2 of 5 animals in both the 125 and 250 mg/kg per day groups on day 2. A decrease in stool volume was observed in 3 animals and all 5 animals in the 250 and 500 mg/kg per day groups, respectively, on day 3. A decrease in spontaneous activity was also observed in 1 animal in the 500 mg/kg per day group at 6 h after dosing on day 2. Decreases in body weight were noted in all test article groups. Project L did not induce micronuclei in rat erythroblasts.

##### Carcinogenicity

No carcinogenicity studies of Project L have been conducted to date.

##### Reproductive and Developmental Toxicity

No reproductive and developmental toxicity studies of Project L have been conducted to date.

##### Local Tolerance

No local tolerance studies of Project L have been conducted to date.

##### Other Toxicity Studies

*In Vitro: Phototoxicity*

The potential phototoxicity of Project L was investigated using cultured mammalian cells (Balb/c 3T3 cells) (Study Project L-TX-0011). A dose range-finding test was performed at PROJECT L concentrations of 0.781 to 100 μg/mL in the presence and absence of UV-A irradiation. The IC50 values for cell viability in the presence and absence of irradiation were determined to be 20.687 and 19.676 μg/mL PROJECT L, respectively. No test article precipitation in the treatment mixture was observed at the start or end of treatment at concentrations ≤ 100 μg/mL. Therefore, the main test was performed at concentrations of

1.86 to 50 μg/mL PROJECT L in the presence and absence of UV-A irradiation. A vehicle (water for injection)-treated and a chlorpromazine hydrochloride (CPZ)-treated group were used as negative and positive controls, respectively.

The IC50 value for cell viability was calculated in both the presence and absence of irradiation, and the photo-irritancy factor was < 2 (actual value: 1.257); therefore, PROJECT L was categorized as having no phototoxicity. No test article precipitation in the treatment mixture was observed at the start or end of treatment at ≤ 50 μg/mL PROJECT L. Project L showed no potential to induce phototoxicity to cultured mammalian cells.

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

Project L is a small molecule, irreversible EGFR TKI that inhibits EGFR containing the exon 19 deletion (del ex19) and the exon 21 (L858R) substitution mutation, as well as the T790M resistance mutation. In an in vitro kinase screen, Project L also inhibited several other kinases for which the clinical relevance is unknown. Project L inhibits EGFR phosphorylation in NCI-H1975 cells (NSCLC cell line expressing EGFR mutation T790M/L858R) and has shown efficacy (tumor regression) in an NCI-H1975 xenograft model. Results from the nonclinical studies described above support the investigation of Project L in patients with NSCLC whose tumors harbor EGFR mutations, including the T790M resistance mutation.

Project L showed more than 50% inhibition of each radioligand binding to the calcium channel (type L, dihydropyridine, rat), GABA A (BZ central, rat) receptor, histamine H3 (rat) receptor, sodium channel site 2 (rat) and sigma (non-selective, guinea pig) receptor at a 10 µmol/L concentration; the clinical relevance of these findings is unknown.

In safety pharmacology studies, Project L had inhibitory effects on hERG current with an IC50 value of 18 μmol/L (~10 μg/mL as PROJECT L). Based on nonclinical efficacy studies of Project L, these plasma concentrations (18 µmol/L) are not anticipated to be achieved in cancer patients. In rats, Project L had no effect on the CNS at doses up to 300 mg/kg. Project L had no treatment-related effect at 3 mg/kg on the central nervous, cardiovascular or respiratory systems in dogs. No arrhythmias or ECG abnormalities (PR, QT and QTc intervals, QRS duration) were detected at any dose level tested (up to 30 mg/kg). At 10 mg/kg Project L, vomiting, loose stool, and increases in the body temperature and heart rate were noted. At 30 mg/kg Project L, syncope, salivation, urinary incontinence, mucous and bloody stool, and anorexia were noted in addition to the findings seen at 10 mg/kg.

In vitro studies suggest PROJECT L is a substrate for P-gp-mediated transport and is primarily metabolized by CYP3A4/5. Based on these results, the pharmacokinetics of PROJECT L may be affected by P-gp and/or CYP3A4 inhibitors. PROJECT L also showed an inhibitory effect on the P-gp-mediated transport of digoxin, and a direct and time-dependent inhibitory effect on CYP3A4. These results indicate that PROJECT L may affect the pharmacokinetics of P-gp and/or CYP3A4 substrates in humans. The direct inhibitory effects of PROJECT L on CYP1A2, 2C8, 2B6, 2C9, 2C19, and 2D6 were weak and thus, may not be clinically relevant.

In the single dose toxicity study in rats, the approximate lethal dose level was 500 mg/kg Project L for males and females. The major change noted in moribund animals was a gastrointestinal hemorrhagic disorder. In the preliminary single dose toxicity study in dogs, no animals showed mortality or moribundity at ≤ 300 mg/kg Project L.

Most of the toxicities observed with Project L in animals are similar to those seen with other EGFR antagonists. Major organ toxicities observed with Project L in the rat following repeated dosing were seen in the cornea, skin, lymphoid tissue, bone marrow, ovaries, testes, lung, liver and bone. In the dog, major organ toxicities included the gastrointestinal tract, cornea, skin, lymphoid tissue, lung, kidney and bone. Species differences with respect to the safety margin and associated plasma levels of PROJECT L are shown in [[Table 6](#_bookmark58)].

Gastrointestinal toxicity that included vomiting, hemorrhage (stomach, small intestine and large intestine) and ulcers (stomach) was observed in dogs only at the highest dose of

15 mg/kg per day. These findings were considered to be associated with moribundity and were reversible in surviving animals. Similar findings have been observed at high doses of EGFR antagonist, lapatinib (EMEA Assessment Report for TYVERB, Procedure No.

EMEA/H/C/795).

Reversible atrophy of the epithelium and mucosa of the cornea and skin was seen at doses

≥ 30 mg/kg per day in rats and at 15 mg/kg per day in dogs. Ophthalmological examination in rats and dogs indicated the presence of corneal opacities that were still present after a

4-week recovery period; however, no accompanying histopathology was identified. A similar lack of reversibility of corneal opacities in the dog has been reported with the EGFR antagonist gefitinib (EMEA Assessment Report for IRESSA, Procedure No.

EMEA/H/C/001016).

Reversible atrophy of lymphoid tissues was observed in male rats at doses ≥ 10 mg/kg per day, in female rats at doses ≥ 30 mg/kg per day and in male and female dogs at 15 mg/kg per day. Low lymphocyte counts in the peripheral blood and hypocellularity of the bone marrow were observed in male rats at doses ≥ 30 mg/kg per day and in females at 60 mg/kg per day. Lymphocyte counts remained low in rats after a 4-week recovery period; however, reversibility of this finding is anticipated because atrophy of the lymphoid organs and bone marrow hypocellularity was no longer observed after a 4-week recovery period. Lymphoid depletion in multiple tissues has also been reported for the EGFR antagonist, lapatinib (EMEA Assessment Report for TYVERB, Procedure No. EMEA/H/C/795). While there is a lack of EGFR expression on immune cells, immunomodulatory effects of EGFR inhibitors cannot be excluded entirely [Pollack, 2012].

Testicular changes, including degeneration/atrophy of the seminiferous tubules and spermatic granuloma, were observed in male rats at doses of ≥ 10 mg/kg per day. These findings did not reverse after a 4-week recovery period. While testicular toxicity has not been reported with other EGFR antagonists, epidermal growth factor receptors are expressed in the testes and have been reported to play a role in the regulation of mammalian spermatogenesis [Wunsch et al, 2004; Abé et al, 2008].

In female rats, reversible findings of atrophy of the ovary, uterus, and vagina were seen at 60 mg/kg per day, and vacuolation of the luteal cells was noted at ≥ 30 mg/kg per day.

Ovarian atrophy has also been reported in female rats with gefitinib and erlotinib (EMEA Assessment Report for IRESSA, Procedure No. EMEA/H/C/ 001016)[Tarceva® SPC, 2010].

Liver toxicity, consisting of multifocal necrosis of hepatocytes, periportal infiltration of inflammatory cells, and proliferation and necrosis of the bile ducts, was noted in rats at doses

≥ 30 mg/kg/day. Increased serum ALT and total bilirubin levels were also observed at

≥ 10 mg/kg and ≥ 30 mg/kg per day, respectively. In dogs, increased serum ALT was seen at the highest dose (15 mg/kg per day) but was not associated with histopathological changes.

Similar liver findings in the rat have been reported with gefitinib and erlotinib (EMEA Assessment Report for IRESSA, Procedure No. EMEA/H/C/ 001016)[ Tarceva SPC, 2010].

In kidneys, dilatation of the distal tubules, focal interstitial hemorrhage, and proximal tubules changes were observed in males and females in moribund and surviving dogs at a dose of

15 mg/kg per day. Similar findings have been observed in these species with gefitinib and erlotinib (EMEA Assessment Report for IRESSA, Procedure No. EMEA/H/C/ 001016) [Tarceva SPC, 2010].

Cartilage and bone changes (thickening of the epiphyseal cartilage and/or decreased trabecular bone of the femur) were observed in rats at ≥ 30 mg/kg per day. In dogs, thickening of the cartilage of the sternum and/or decreases in the trabecular bone were noted in males at 5 and 15 mg/kg per day and females at 15 mg/kg per day Project L.

Trabecular atrophy of the femur has been observed in rats with the EGFR antagonist lapatinib (EMEA Assessment Report for TYVERB, Procedure No. EMEA/H/C/795).

In the lung, inflammatory cell infiltration or hemorrhage in the alveoli was observed in dogs at 5 (1 female) and 15 mg/kg per day and was still present in the 5 mg/kg per day female after a 4-week recovery period. These lung effects may reflect exacerbation of spontaneous inflammation in the lung resulting in inflammatory lesions in the alveoli. EGFR antagonists have been reported to exacerbate acute lung injury or lung inflammation in mice [Harada et al, 2011; Inoue et al, 2008].

Project L did not induce genetic mutations in bacteria. Although Project L induced chromosomal aberrations in an in vitro test in mammalian cells, a micronucleus test in rats showed no potential to induce chromosomal aberrations in vivo. Project L showed no potential to induce phototoxicity in cultured mammalian cells (Balb/c 3T3 cells).

##### Potential Safety Concerns

Potential implications for human use associated with Project L based on the findings in the nonclinical studies are summarized in [Sectio[n 6.2](#_bookmark62), Guidance for the Investigator].

##### Safety Margins

The NOAEL was below the lowest dose tested in both rats and dogs (3 mg/kg and 1.5 mg/kg, respectively). The STD10 in rats was 60 mg/kg which equates to a human equivalent dose (HED) of 9.6 mg/kg. The HNSTD in dogs was 5 mg/kg which equates to an HED of

2.7 mg/kg [[Table 6](#_bookmark58)].

##### Table 6 Compilation of Doses and Systemic Exposure Data of PROJECT L at STD10/HNSTD/HED

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Species/ Study Duration** | **Dose** | **Sex (M/F)** | **HED†**  **(mg)** | **Total Plasma PROJECT L Concentration** | | | |
| **Cmax (ng/mL)** | | **AUC24 (ng･h/mL)** | |
| **First Dose** | **Last Dose** | **First Dose** | **Last Dose** |
| Rat/4-week | 60 mg/kg  (STD10) | M | 576 | 773.28 | 653.96 | 8841.30 | 15030.76 |
| F | 576 | 1089.27 | 791.90 | 10663.67 | 13693.09 |
| Dog/4-week | 5 mg/kg  (HNSTD) | M | 162 | 145.74 | 207.98 | 1615.77 | 2339.66 |
| F | 162 | 130.60 | 152.15 | 1295.66 | 1783.60 |

HED: human equivalent dose; HNSTD: highest non-severely toxic dose; STD10: severely toxic dose in 10% of animals

†The human equivalent dose levels were calculated by using the body surface area conversion factors (rat: 0.16 and dog: 0.54) and the human body weight of 60 kg

Source: Studies Project L-TX-0006 and Project L-TX-0007

*List of References*

Abé K, Eto K, Abé S. Epidermal growth factor mediates spermatogonial proliferation in newt testis.

Reprod Biol Endocrinol. 2008;6:7.

Harada C, Kawaguchi T, Ogata-Suetsugu S, Yamada M, Hamada N, Maeyama T, et al. EGFR tyrosine kinase inhibition worsens acute lung injury in mice with repairing airway epithelium. Am J Respir Crit Care Med. 2011;183:743-51.

Inoue A, Xin H, Suzuki T, Kanehira M, Kuroki Y, Fukuhara T, et al. Suppression of surfactant protein A by an epidermal growth factor receptor tyrosine kinase inhibitor exacerbates lung inflammation. Cancer Sci. 2008;99:1679-84.

Pollack, BP. EGFR inhibitors, MHC expression and immune responses: Can EGFR inhibitors be used as immune response modifiers? Oncoimmunology. 2012;1:71-4.

Tarceva (summary of product characteristics). Switzerland. F. Hoffmann-LaRoche Ltd, Basel, February 2010.

Wunsch A, Strothmann K, Simoni M, Gromoll J, Nieschlag E, Luetjens CM. Epidermal growth factor receptor pathway substrate 8 (Eps8) expression in maturing testis. Asian J Androl.

2004;6:195-203.