## Safety Pharmacology

## In Vitro Assay on hERG Current

The effects on the hERG current were studied in hERG-transfected human embryonic kidney (HEK)293 cells using the whole-cell patch-clamp technique (Study Project D-PT-0001). The peak amplitude of tail currents was measured in 5 individual cells in each experimental group for 11 minutes after application of PROJECT D (0.073, 0.13 and 0.47 μmol/L), E-4031, an IKr inhibitor, as a positive control (0.1 μmol/L) or vehicle control (dimethylsulfoxide at 0.1% v/v). The background suppression rate in each cell was corrected for using the vehicle control group. Results of the positive control (87.6% suppression) confirmed the validity of this assay.

PROJECT D significantly suppressed hERG at 0.073, 0.13 and 0.47 μmol/L (46%, 64% and 90%, respectively). PROJECT D inhibited hERG current in vitro in a concentration-dependent manner, with an IC50 value of 49 ng/mL. This IC50 value was 15-fold higher than the non- protein bound Cmax at which prolonged QTc intervals were seen in an in vivo safety pharmacology study (Cmax 2150 ng/mL, Cmax,u 3.2 ng/mL, monkey fp 0.0015) (Study

Project D-PT-0002).

## Effects on CNS in Rats

Effects of PROJECT D on CNS functions after a single oral administration were evaluated in rats using modified Irwin’s observation method (Study Project D-PT-0003). In addition, toxicokinetics were analyzed from satellite groups.

PROJECT D was orally administered to 6 male and 6 female Sprague-Dawley (SD) rats each at doses of 0 (0.5% methylcellulose), 30 and 100 mg/kg (as free base equivalents). At the highest dose level of 300 mg/kg, 6 males and 3 females were allocated.

PROJECT D did not affect CNS functions at 30 mg/kg.

At 100 mg/kg, males showed decreased locomotor activities from 0.5 to 1 h after administration. Females showed decreased locomotor activities, prone position, eyelid closure and hypothermia at 0.5 to 4 h after administration.

At 300 mg/kg, 1 out of 6 males was found dead at 48 h postdose. All 3 females were sacrificed in a moribund state within 24 h after administration. In addition to the findings at 100 mg/kg, slight flaccidity for abdominal tone was recorded in both males and females, as well as decreased resistance for limb tone and low arousal in females.

Toxicokinetic analysis showed a dose-associated increase in exposure of PROJECT D [[Table 2](#_bookmark22)]. At 100 mg/kg (the highest non-lethal does), the plasma Cmax reached 2000 ng/mL.

PROJECT D had no effect on the CNS of rats ≤ 30 mg/kg where a plasma Cmax of 1120 ng/mL was achieved.

## Table 2 PROJECT D Toxicokinetic Parameters after Oral Administration to Rats

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Type of study** | **Dose (mg/kg)†** | **Mean Cmax (ng/mL)** | | **Mean AUC24 (ng·h/mL)** | | **tmax (h)** | |
| **Male** | **Female** | **Male** | **Female** | **Male** | **Female** |
| Effects on CNS in rats (Study Project D-PT-0003) | 30 | 639 | 1120 | 11200 | 15600 | 8.0 | 2.0 |
| 100 | 788 | 2000 | 16300 | 35200 | 24 | 2.0 |
| 300 | 2520 | 2920 | 39900 | 51300 | 24 | 8.0 |

Toxicokinetic data generated from 3 males and 3 females per dose group. CNS: central nervous system

†free base equivalents

## Effects on Cardiovascular and Respiratory Functions in Cynomolgus Monkeys

Cardiovascular and respiratory functions were assessed in 4 male cynomolgus monkeys after administration of PROJECT D at dose levels of 0 (vehicle), 1, 3, 10 and 30 mg/kg in a single ascending dose manner (Study Project D-PT-0002).

PROJECT D was suspended in a 0.5% methylcellulose solution. There was 7-day washout period between doses.

Clinical signs, body temperature, blood pressure, heart rate, ECG, blood electrolytes (sodium, phosphate, chloride and ionized calcium), blood lactate levels, arterial blood pH and respiratory functions (respiratory rates, arterial pCO2, pO2 and hemoglobin O2 saturation) were monitored by telemetry in unanesthetized animals. In addition, toxicokinetics were analyzed.

There were no findings at 1 or 3 mg/kg. Doses of 10 and 30 mg/kg were associated with vomiting (5 to 21 times vomiting and 1 to 12 times retching per animal starting between

3 and 7 h after administration), prolonged group mean QT interval corrected by Fridericia’s formula (QTcF) at 10 h after administration, and decreased levels in blood sodium, chloride and ionized calcium.

No alterations in body temperature, blood pressure, heart rate, blood lactate levels, arterial blood pH or respiratory function parameters were noted at any dose level.

Toxicokinetic analysis showed a dose-associated increase in exposure of PROJECT D at doses

≤ 10 mg/kg. The plasma Cmax values after a dose of 10 and 30 mg/kg PROJECT D were comparable (approximately 2000 ng/mL) [[Table 3](#_bookmark24)].

In conclusion, PROJECT D did not affect cardiovascular or respiratory functions at doses

≤ 3 mg/kg where a plasma Cmax of 803 ng/mL was achieved. Vomiting, prolonged QTc intervals and blood electrolyte disturbances occurred at doses of 10 and 30 mg/kg.

## Table 3 PROJECT D Toxicokinetic Parameters after a Single Oral Administration to Cynomolgus Monkeys

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Type of study** | **Dose† (mg/kg)** | **Sex, No. of animals** | **Mean Cmax (ng/mL)** | **Mean AUC24**  **(ng·h/mL)** | **tmax (h)** |
| Effects on cardiovascular and respiratory functions in cynomolgus monkeys  (Study Project D-PT-0002) | 1 | M, 4 | 219 | 2900 | 7.0 |
| 3 | M, 4 | 803 | 10000 | 7.0 |
| 10 | M, 4 | 2150 | 27200 | 7.0 |
| 30 | M, 4 | 1960 | 25200 | 7.0 |

No.: number

†free base equivalents

## Pharmacodynamic Drug Interactions

No pharmacodynamic drug interaction studies of PROJECT D have been conducted to date.

## Other Pharmacology Studies

No other pharmacology studies of PROJECT D have been conducted to date.

# Toxicology

## Single-dose Toxicity

No single-dose toxicity studies of PROJECT D have been conducted to date.

## Repeated-dose Toxicity

## Preliminary 1-Week Study in Rats

In order to find an appropriate dose range for a 4-week study in rats, PROJECT D was suspended in 0.5% methylcellulose and orally administered to Sprague-Dawley rats

(5 rats/sex/dose) at dose levels of 0 (vehicle), 30, 100 and 300 mg/kg once daily for 1 week (Study Project D-TX-1001).

Clinical signs, body weight, food consumption, clinical pathology, necropsy, organ weights and histopathology were recorded.

At 30 mg/kg per day, suppressed body weight gain, increased blood lactate levels and microscopically cytoplasmic vacuolation in the macrophages in the lymphoid organs and lung (diagnosed as phospholipidosis by electron microscopy) were noted.

At 100 mg/kg per day, all females died or were sacrificed in a moribund state on day 6 or 7, while all males and females at 300 mg/kg per day died or were sacrificed in a moribund state between days 2 and 4. The rats showed decreased locomotor activity prior to moribundity or death.

In conclusion, a dose level of 30 mg/kg per day was considered to be the appropriate high dose level for a 4-week repeated dose toxicity study in rats.

## 4-Week Study with 4 Week Recovery Period in Rats

In order to evaluate the safety profile, PROJECT D was suspended in 0.5% methylcellulose and orally administered to Sprague-Dawley rats (10 rats/sex/dose) at dose levels of 0 (vehicle), 3, 10 and 30 mg/kg once daily for 4 weeks (Study Project D-TX-0001; [End-of-Text Table 3.2.1]). In order to assess the reversibility of any toxicity findings, an additional 5 rats/sex per dose were assigned to the 0, 10 and 30 mg/kg per day dose groups. Finally, satellite groups

(6 rats/sex/dose) were assigned to assess plasma PROJECT D concentrations on days 1, 14 and 28.

Clinical signs, body weight, food consumption, ophthalmology, urinalysis, hematology, blood chemistry, necropsy, organ weights and histopathology were recorded in this study. Blood lactate levels were monitored in a separate study (Study Project D-TX-0006).

At 3 mg/kg per day, no adverse findings were noted.

At 10 mg/kg per day, increased liver weight and cytoplasmic vacuolation in macrophages in the thymus, lymph nodes, Peyer’s patch, lung and uterus and in the epithelium of the bile duct were noted.

At 30 mg/kg per day, in addition to the findings observed at 10 mg/kg per day, salivation, suppressed body weight gain, decreased food consumption, low urinary pH, increased urine volume with low specific gravity, increase in urinary sodium and chloride excretion, decreases in erythrocyte counts, hemoglobin concentrations, and hematocrit values, increase in neutrophil and monocyte counts, higher ratio of lymphocytes with cytoplasmic vacuolation, increased levels of total cholesterol and inorganic phosphorus and decreased levels of blood chloride and globulin were noted. Spleen and lung weights were increased, while thymus weights were decreased. Vacuolation of macrophages in the bone marrow, spleen and testis as well as vacuolation of Kupffer cells were noted. Electron microscopy demonstrated that cytoplasmic vacuolation in the liver tissue was intracytoplasmic single membrane-bound lamellar bodies and this was diagnosed as phospholipidosis.

Following a 4-week recovery period in which no drug was administered, the observed toxicological findings described above were either not present or showed a tendency toward resolving demonstrating that the findings were reversible.

Systemic exposure of PROJECT D after repeated dosing, as measured by AUC24 and Cmax, increased dose-proportionally and was not remarkably different from single-dose exposure. PROJECT D exposure in females was 1.2- to 2.2-fold higher than in males (Cmax and AUC24, respectively).

In conclusion, the NOAEL of this study was 3 mg/kg per day.

## Table 4 PROJECT D Toxicokinetic Parameters after Oral Administration to Rats

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Daily Dose (mg/kg)†** | **3** | | **10** | | **30** | |
| **Male** | **Female** | **Male** | **Female** | **Male** | **Female** |
| **mean tmax (h): Day 1** | 2.0 | 2.0 | 4.0 | 4.0 | 4.0 | 8.0 |
| **Day 14** | 2.0 | 8.0 | 8.0 | 4.0 | 4.0 | 1.0 |
| **Day 28** | 4.0 | 4.0 | 4.0 | 4.0 | 2.0 | 4.0 |
| **mean Cmax (ng/mL): Day 1** | 97 | 122 | 205 | 303 | 623 | 729 |
| **Day 14** | 83 | 142 | 276 | 414 | 590 | 1180 |
| **Day 28** | 100 | 173 | 291 | 415 | 537 | 944 |
| **mean AUC24 (ng·h/mL): Day 1** | 1100 | 1600 | 2740 | 4680 | 9340 | 13000 |
| **Day 14** | 1150 | 2210 | 3930 | 6560 | 9060 | 19300 |
| **Day 28** | 1380 | 2460 | 4360 | 7060 | 7500 | 16300 |

Toxicokinetic data generated from 6 males and 6 females per dose group.

†free base equivalent

Source: Study Project D-TX-0001

## Preliminary 1-Week Study in Cynomolgus Monkeys

In order to find an appropriate dose range for a 4-week study in cynomolgus monkeys, PROJECT D was suspended in 0.5% methylcellulose and orally administered to cynomolgus monkeys (1 monkey/sex/dose) at dose levels of 0 (vehicle), 3, 30 and 100 mg/kg once daily for 1 week (Study Project D-TX-1002).

Clinical signs, body weight, food consumption, clinical pathology, ophthalmology, ECG, necropsy, organ weights and histopathology were recorded.

At 3 mg/kg per day, cytoplasmic vacuolation of macrophages in the bone marrow was noted. This finding was diagnosed as phospholipidosis by electron microscopy.

At 30 mg/kg per day, 1 male was sacrificed in a moribund state on day 7 after anorexia for

1. days. In the 30 and 100 mg/kg per day dose groups, vomiting, decreased food consumption, decreased heart rate and prolonged QTcB were noted. Phospholipidosis was observed in macrophages from various lymphoid organs.

In conclusion, 10 mg/kg per day was considered to be the appropriate high dose level for a 4-week repeated dose study in cynomolgus monkeys.

## 4-Week Study with 4 Week Recovery Period in Cynomolgus Monkeys

In order to evaluate the safety profile, PROJECT D was suspended in 0.5% methylcellulose and orally administered to cynomolgus monkeys (4 monkeys/sex/dose) at dose levels of 0 (vehicle), 1, 3 and 10 mg/kg once daily for 4 weeks. In order to assess the reversibility of any toxicity findings, another 3 monkeys/sex per dose were assigned to the 3 and 10 mg/kg per day dose groups (Study Project D-TX-0002; [End-of-Text Table 3.2.2]). Plasma PROJECT D concentrations were measured on days 1, 14 and 28.

Clinical signs, body weight, food consumption, ophthalmology, ECG, urinalysis, hematology, blood chemistry including blood lactate levels, necropsy, organ weights and histopathology were recorded.

At 1 mg/kg per day, no adverse findings were noted.

At 3 mg/kg per day, cytoplasmic vacuolation of macrophages in the bone marrow, thymus, spleen, Peyer’s patch and lung were noted. In addition, increased lymphocyte, large unstained cell, monocyte, eosinophil and basophil counts, elevated serum aspartate transaminase (AST), alanine transaminase (ALT) and triglyceride values, low blood sodium and chloride values and increased liver weight were noted at 3 mg/kg per day.

Administration of 10 mg/kg per day PROJECT D resulted in sacrifice due to moribundity in 1 male on day 24. Prior to moribundity, e.g., lateral position, hypothermia and a dull response to external stimulation, the male showed almost daily vomiting and a slight decrease in locomotor activities (continuously noted from day 16). In addition, a decrease in body weight (-0.65 kg [-10%]) during a 24-day period was noted. Slow heart rate, prolonged QTcB, increased neutrophil counts, increased lymphocyte counts with cytoplasmic vacuolation, increases in AST (209 IU/L versus a pretreatment value of 25 IU/L), ALT (144 IU/L versus a pretreatment value of 27 IU/L), glucose and urea nitrogen, and a decrease in chloride value (101 mEq/L versus a pretreatment value of 110 mEq/L) were recorded in the blood chemistry at the time of sacrifice. The blood lactate level (54.6 mg/dL) was within normal limits.

Histopathology for this sacrificed male showed cytoplasmic vacuolation of macrophages in the bone marrow, thymus, spleen, lymph nodes, Peyer’s patch and lung, and vacuolation of the myocardium and neurons. In addition, focal hepatocyte necrosis and hypertrophy of Kupffer cells and glomerular podocytes were noted.

At the 10 mg/kg per day dose level, the rest of the monkeys were sacrificed as scheduled after completion of a 4-week drug administration period. These animals showed slightly decreased locomotor activities, vomiting and salivation on limited occasions. Suppressed food consumption resulted in body weight loss. Heart rates were decreased and QTcB intervals at 6 h after administration were prolonged. Decreases in sodium and chloride excretion were noted in urinalysis. Hematology and blood chemistry showed increased fibrinogen levels, increased lymphocyte counts with cytoplasmic vacuolation, increased total bilirubin and triglyceride and decreased chloride levels. Blood lactate levels were unchanged on day 1 but were increased over baseline on days 14 (1.3-fold in females) and 28 (1.5- and 1.4-fold in females and males, respectively), 6 h post dose. There were no macroscopic findings. Increased organ weights were noted for the lung and adrenals.

Histopathological examination showed cytoplasmic vacuolation of macrophages in the bone marrow, thymus, spleen, lymph nodes, Peyer’s patch, lung and vagina, and vacuolation of the myocardium and neurons in the brain. In addition, hypocellularity in the bone marrow, atrophy of the lymphoid follicles in the spleen, and hypertrophy of Kupffer cells and glomerular podocytes were noted. Electron microscopy in the liver and renal tissues demonstrated that the cytoplasmic vacuolation was intracytoplasmic single membrane-bound lamellar bodies which were diagnosed as phospholipidosis.

Following a 4-week recovery period in which no drug was administered, the observed toxicological findings described above were either not present or showed a tendency toward resolving demonstrating that the findings were reversible.

Systemic exposure of PROJECT D as measured by AUC24 and Cmax increased dose-proportionally.

There was no clear sex difference at 1 or 3 mg/kg per day. At 10 mg/kg per day, exposure in females was higher than in males. Systemic exposure on day 14 was higher than on day 1 but exposure on day 28 was comparable to day 14, suggesting that systemic exposure reached steady-state on day 14.

In conclusion, the NOAEL of this study was 1 mg/kg per day.

## Table 5 PROJECT D Toxicokinetic Parameters after Oral Administration to Monkeys

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Daily Dose (mg/kg)†** | **1** | | **3** | | **10** | |
| **Male n = 4** | **Female n = 4** | **Male n = 7** | **Female n = 7** | **Male n = 7‡** | **Female n = 7** |
| **mean tmax (h): Day 1** | 6.0 | 4.5 | 6.0 | 5.4 | 5.7 | 5.4 |
| **Day 14** | 5.5 | 6.0 | 6.0 | 5.7 | 5.1 | 5.4 |
| **Day 28** | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 5.7 |
| **mean Cmax (ng/mL): Day 1** | 345 | 352 | 1080 | 1550 | 3100 | 3670 |
| **Day 14** | 1330 | 784 | 3530 | 3670 | 8690 | 10200 |
| **Day 28** | 1310 | 924 | 2960 | 2780 | 7940 | 10500 |
| **mean AUC24 (ng·h/mL): Day 1** | 3020 | 3570 | 10000 | 13600 | 31000 | 40600 |
| **Day 14** | 14000 | 9140 | 34500 | 34500 | 99200 | 146000 |
| **Day 28** | 12900 | 9400 | 31800 | 29300 | 107000 | 132000 |

†free base equivalent

‡n = 6 on day 28

Source: Study Project D-TX-0002

## Genotoxicity

* + - 1. **In Vitro Reverse Mutation Assay**

The mutagenic potential of PROJECT D was assessed in a standard Ames assay using

5 different bacterial strains, *Salmonella typhimurium,* TA98, TA100, TA1535 and TA1537 and *Escherichia coli*, WP2*uvr*A (Study Project D-TX-0003). The assays were conducted with a preincubation method, with or without metabolic activation, at drug concentrations

≤ 5000 μg/plate. For metabolic activation, the plates were incubated with the S9 microsome fraction prepared from the livers of male Sprague-Dawley rats that were treated with phenobarbital and 5, 6-benozoflavone to induce metabolic enzyme activities. This assay was validated using positive controls.

No increases in the number of revertant colonies were noted at any drug concentration

≤ 5000 μg/plate, either with or without metabolic activation. These data indicate that there was no discernible mutagenic potential for PROJECT D.

## In Vitro Chromosome Aberration Assay

The clastogenic potential of PROJECT D was assessed in a standard mammalian cell in vitro assay using Chinese hamster lung cells (Study Project D-TX-0004). The results were obtained after 6- and 24-h incubations without metabolic activation (S9-) and after 6-h incubations with metabolic activation (S9+). The S9 liver microsomal fraction was prepared from rats that were treated with phenobarbital and 5, 6-benozoflavone to induce metabolic enzyme activities. The assay was validated using positive controls.

A statistically significant increase in the frequency of cells with numerical chromosome aberrations (4.0%) was noted at a drug concentration of 15 μg/mL after metabolic activation (incubation with S9+) where it showed relevant cytotoxicity (63.5% of vehicle control).

No effects on the frequency of cells with structural aberrations were noted at any dose levels.

## Carcinogenicity

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

## Reproductive and Developmental Toxicity

No reproductive or developmental toxicity studies of PROJECT D have been conducted to date.

## Local Tolerance

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

## Other Toxicity Studies

* + - 1. **In vitro Photosafety Test**

Phototoxic potential of PROJECT D was assessed by the relative reduction in viability of Balb/c 3T3 mouse fibroblasts exposed to the test article (≤ 30 μg/mL) and ultraviolet radiation (+UVR), as compared with the viability of fibroblasts exposed to the test article in the absence of ultraviolet radiation (-UVR) (Study Project D-TX-0005). Chlorpromazine was used as a positive control and confirmed the validity of this assay.

PROJECT D had a photoirritancy factor of 0.96 and mean photo effect of 0.00, thus it was judged to be non-phototoxic.

## Measurement of Blood Lactate Levels in Rats

In order to evaluate the effect of PROJECT D on blood lactate levels in rats, PROJECT D was suspended in 0.5% methylcellulose aqueous solution and orally administered once daily for 4 weeks to Sprague-Dawley rats (6 male rats/dose) at dose levels of 0 (vehicle), 3, 10 and

30 mg/kg (Study Project D-TX-0006); the same dose levels used in the 4-week repeated dose toxicity study (Study Project D-TX-0001). Time-course blood lactate levels were monitored on days 1 and 28. Clinical signs, body weight and food consumption were also recorded.

At 3 mg/kg per day, there were no significant changes in blood lactate levels.

At 10 and 30 mg/kg per day, a statistically significant elevation of blood lactate levels was noted on both days 1 and 28. The highest blood lactate value (19.3 mg/dL) was recorded at 4 h after dosing on day 1 in the 30 mg/kg per day dose group which corresponded with plasma tmax.

# 4.4 Integrated Nonclinical Overview and Conclusion: Potential Clinical Relevance

PROJECT D showed an inhibitory effect on human mitochondrial complex I activity in vitro. PROJECT D inhibited the growth of human breast cancer, NSCLC, CRC and DLBCL cells. In addition, PROJECT D demonstrated efficacy in nonclinical breast cancer and NSCLC models, with tumor regression and growth inhibition in xenograft mice. These results suggest the therapeutic potential of PROJECT D for the treatment of cancer.

A radioligand displacement study showed that PROJECT D had Ki values that were < 0.75

µmol/L for 7 targets (receptor/channel/transporter). These Ki values were within 100-fold of

the non-protein bound PROJECT D Cmax associated with the HNSTD observed in cynomolgus monkey toxicology studies (2780 to 2960 ng/mL). The clinical relevance of these findings is unknown.

Preliminary analysis of the pharmacokinetic profile of PROJECT D from the single dose

(1, 3, 10 or 30 mg/kg, po) safety pharmacology study in monkeys revealed that the terminal t1/2 ranged from 34.3 to 48.0h. Based on these nonclinical data, it is concluded that sequential blood sampling for the phase 1 clinical study needs to be conducted over a 72 h period in order to adequately characterize the human pharmacokinetics of PROJECT D.

Treatment-related noteworthy findings and safety margins are summarized in [[Table 6](#_bookmark59)] and [[Table 7](#_bookmark60)]. A series of nonclinical safety assessments of PROJECT D showed that noteworthy adverse findings were vomiting, QTc prolongation, decreased heart rate, blood electrolyte disturbance (i.e., low blood chloride levels), systemic cytoplasmic vacuolation (phospholipidosis) and elevation of blood lactate levels.

Potential clinical relevance is described in the [Sec[tion 6.2](#_bookmark65)] ‘Guidance for Investigator.’

## Table 6 Treatment-related Noteworthy Findings of Potential Clinical Interest in Safety Pharmacology and 4-Week Repeated Toxicity Studies of PROJECT D in Rats and Monkeys

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment-related Findings** | **Lowest Dose at Which Findings were Observed (mg/kg/day) [HED† (mg/man/day)]** | | | |
| **Rats** | | **Cynomolgus Monkeys** | |
| **Male** | **Female** | **Male** | **Female** |
| Vomiting | NA | NA | 10 [192] | 10 [192] |
| Prolonged QTc interval | NA | NA | 10 [192] | 10 [192] |
| Decreased heart rates | NA | NA | 10 [192] | 10 [192] |
| Low blood chloride levels | --- | 30 [288] | --- | 10 [192] |
| Low blood ionized  calcium levels‡ | --- | --- | 10 [192] | NT |
| Low blood sodium levels | --- | --- | 10 [192] | NT |
| High blood phosphate  levels | 30 [288] | --- | --- | --- |
| Cytoplasmic vacuolation (phospholipidosis) | 10 [96] | 10 [96] | 3 [58] | 3 [58] |
| Elevated blood lactate | 10 [96] | NT | 10 [192] | 10 [192] |

---: not observed; HED: human equivalent dose; NA: not applicable; NT: not tested

†Based on 60 kg/man and body surface area conversion factors (rats 0.16, monkeys 0.32).

‡ Observed only in the safety pharmacology study

Sources: Studies Project D-PT-0002, Project D-TX-0001, Project D-TX-0002 and Project D-TX-0006.

## Table 7 Safety Margins Based on Human AUC and Animal AUC of PROJECT D

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Species/Study Duration** | **Dose†** | **Sex (M/F)** | **AUC24**  **(ng·h/mL)** | **Safety Margin Based on 5 mg Human Dose** | **Safety Margin Based on 10 mg Human Dose** |
| Rat/4-week po | 3 mg/kg  (NOAEL) | M | 1380 | NA | NA |
| F | 2460 | NA | NA |
| 10 mg/kg (LOAEL) | M | 4360 | NA | NA |
| F | 7060 | NA | NA |
| Cynomolgus monkey/4-week po | 1 mg/kg (NOAEL) | M | 12900 | NA | NA |
| F | 9400 | NA | NA |
| 3 mg/kg (LOAEL) | M | 31800 | NA | NA |
| F | 29300 | NA | NA |
| Human | 5 mg | M | NA | NA | |
| F | NA |
| 10 mg | M | NA |
| F | NA |

LOAEL: lowest-observed-adverse-effect level; NA: not applicable; NOAEL: no-observed-adverse-effect level

†free base equivalent

Sources: Studies Project D-TX-0001 and Project D-TX-0002

*Dose determination for first in human clinical studies*

The starting dose and regimen for PROJECT D in Study Project D-CL-0001 is based upon data obtained from nonclinical studies. In addition, guidance from the FDA and EMA was also taken into consideration when determining the first dose.

Data from the 4-week repeated dose toxicity studies in rats and monkeys are shown in [[Table 8](#_bookmark61)]. Human equivalent doses based on body surface area conversion were calculated, and recommended safety factors (ICH S9 and FDA guidance) were applied.

## Table 8 Starting Dose in Humans Based on STD10 and HNSTD

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Toxicology finding** | **Species conversion**  **factor** | **Human equivalent dose** | **Safety factor** | **Starting dose†** |
| STD10 rats: > 30 mg/kg/day | × 0.16 | > 4.8 mg/kg/day | 1/10 | 30 mg |
| HNSTD monkeys: 3 mg/kg/day | × 0.32 | 0.96 mg/kg/day | 1/6 | 10 mg |

HNSTD: highest non-severely toxic dose; STD10: severely toxic dose in 10% of animals.

†assuming body weight of 60 kg

The starting dose for the first administration in humans is to be chosen from the most appropriate animal species; however, for PROJECT D, there is currently no information on which species is most relevant to humans. Therefore, monkey, as the more sensitive species tested, was used to establish the most conservative human starting dose of 10 mg/day for PROJECT D. In consideration of patient safety and the expected mechanism of action of PROJECT D, an additional reduction of the recommended human starting dose was made to

5 mg for this novel compound.