## Safety Pharmacology

Safety pharmacology studies, conducted in accordance with Good Laboratory Practices (GLP) standards, consisted of a hERG current assay, APD assay, and studies on the CNS in rats and the CNS, cardiovascular and respiratory systems in dogs. Overall, these studies indicated that PROJECT U had no inhibitory effects on hERG current or APD in vitro and no effects on CNS, cardiovascular and respiratory systems in vivo [see End-of-Text Table 1.2].

## Effects of PROJECT U on hERG Current in hERG-transfected HEK293 Cells

PROJECT U monohydrate at concentrations up to 10 mcmol/L had no effect on the hERG current in hERG-transfected HEK293 cells using the whole cell clamp method [Project U-PT-0002]. The compensated % inhibition after application of PROJECT U at 0.1, 1, and 10 mcmol/L were

0.7 ± 1.6 %, 1.9 ± 1.3 %, and 1.6 ± 3.6 %, respectively.

## Effects of PROJECT U on APD in Isolated Guinea Pig Papillary Muscles

PROJECT U monohydrate at concentrations of 0.1, 1, and 10 mcmol/L did not affect resting membrane potential (RMP), action potential amplitude (APA), maximal upstroke velocity (Vmax), action potential durations at 30% repolarization (APD30), 90% repolarization (APD90), or the difference between APD30 and APD90 in guinea pig papillary muscles in a study using the glass microelectrode technique [Project U-PT-0001].

## Effects of PROJECT U on CNS in Rats

The effects of a single oral dose of PROJECT U on the general activity and behavior in rats were determined using the modified Irwin’s method [Project U-PT-0004]. PROJECT U 10, 100, and

1000 mg/kg did not affect the general activity or behavior in any of the rats up to 24 hours after administration.

## Effects on CNS, Cardiovascular and Respiratory Systems in Dogs

A single oral dose of PROJECT U (10, 100, or 1000 mg/kg po) did not affect the general activity and behavior, body temperature, blood pressure, heart rate, electrocardiogram, respiration rate, blood gases or blood-electrolyte concentrations in beagle dogs. The tmax was between 0.4 and 0.5 hours for all groups. Mean Cmax and AUC24 increased less than dose-proportionally up to 1000 mg/kg.

## Pharmacodynamic Drug Interactions

No pharmacodynamic drug interaction studies with PROJECT U have been conducted to date.

## Other Pharmacology Studies

No other pharmacology studies with PROJECT U have been conducted to date.

# Toxicology

Single and repeat dose toxicity studies are summarized in End-of-Text Table 3.1. The formulations of PROJECT U used in the toxicology studies are shown below.

|  |  |  |
| --- | --- | --- |
| **PROJECT U Formulation** | **Type of Study** | **Study Number** |
| PROJECT U monohydrate | Reverse mutation, bacteria | Project U-TX-0005 |
| Chromosome aberration | Project U-TX-0006 |
| Preliminary 1-week oral dose toxicity, dogs | Project U-TX-0019 |
| PROJECT U solid dispersion | Single oral dose toxicity, rats | Project U-TX-0001 |
| Single oral dose toxicity, dogs | Project U-TX-0002 |
| 4-week oral dose toxicity, rats | Project U-TX-0003 |
| 4-week oral dose toxicity, dogs | Project U-TX-0004 |
| Micronucleus, mice | Project U-TX-0008 |
| Fertility and early embryonic development rats | Project U-TX-0009 |
| Embryo-fetal development dose range-finding, rats | Project U-TX-0010 |
| Embryo-fetal development, rats | Project U-TX-0011 |
| Embryo-fetal development dose range-finding, rabbits | Project U-TX-0012 |
| Embryo-fetal development, rabbits | Project U-TX-0013 |
| Preliminary 1-week oral dose toxicity, dogs | Project U-TX-0017 |
| PROJECT U dipotassium | Preliminary 1-week oral dose toxicity, rats | Project U-TX-0018 |
| Preliminary single intravenous dose toxicity, rats and dogs | Project U-TX-0014 |
| Preliminary 1-week intravenous dose toxicity, rats | Project U-TX-0015 |
| Preliminary 1-week intravenous dose toxicity, dogs | Project U-TX-0016 |

## Single-dose Toxicity

Non-GLP single intravenous dose range-finding studies in rats indicated the maximum tolerable dose (MTD) of PROJECT U to be between 30 and 60 mg/kg in rats and >100 mg/kg in dogs.

GLP single oral dose toxicity studies of PROJECT U were conducted in rats and dogs [see End-of-Text Table 3.4]. No mortality was observed after a single oral dose of PROJECT U solid dispersion 0 (vehicle), 500, 1000 and 2000 mg/kg to SD rats (n=5/sex/group) [Project U-TX-0001]. At 2000 mg/kg, soiled fur around the anus was observed in one male. At 1000 mg/kg, salivation and soiled fur around the anus were observed in 1 or more animals. Based on these results, the approximate lethal dose was estimated to be > 2000 mg/kg in rats. No mortality was observed after single oral doses of PROJECT U solid dispersion (0 [vehicle], 500 and 1500 mg/kg) to dogs (1/sex/group) [Project U-TX- 0002]. At 500 mg/kg, vomitus was noted in the female and at 1500 mg/kg, watery stools were noted in both animals. No other treatment-related changes were observed; therefore, the approximate lethal dose was estimated to be > 1500 mg/kg in dogs.

## Repeat-dose Toxicity

Five exploratory repeat dose studies were conducted in rats and dogs to provide guidance for the selection of doses used in the definitive (GLP) studies [oral: Project U-TX-0018; Project U-TX-0019; Project U- TX-0017; iv: Project U-TX-0015 and Project U-TX-0016; see End-of-Text Table 3.5]. The definitive

4-week repeat oral dose toxicity studies as well as 1-week iv toxicity studies were conducted in rats and dogs.

The definitive 4-week repeat oral dose toxicity studies were conducted in rats and dogs [Project U-TX- 0003 and Project U-TX-0004]. Overall, the definitive studies indicated the NOAEL in animals treated orally for 4 weeks was 1000 mg/kg/day for males and 100 mg/kg/day for females for rats and 100 mg/kg/day for both sexes for dogs. The lethal dose was 1000 mg/kg/day for rats (females only); no mortality was noted at up to 1000 mg/kg/day in dogs. Erythropoiesis in rats and the gastrointestinal tract in dogs were identified as target organs of toxicity. Findings consisted of decreased

hematocrit, hemoglobin and red blood cells, and increased extramedullary hematopoiesis in the spleen in rats and vomiting and loose stool in dogs, all of which recovered fully after discontinuation of the drug.

The results of the 1-week iv toxicity studies indicated a NOAEL of 10 mg/kg/day for both sexes in rats, and 30 mg/kg/day for the male and <10 mg/kg/day for the female in dogs [Project U-TX-015 and Project U-TX-016]. Findings included local irritation at the injection site and changes related to intravascular hemolysis in rats and necrosis of the perivascular tissue/subcutitis at the injection site was observed for both sexes in dogs.

## 4-week Oral Dose Toxicity Study in Rats with a 4-week Recovery Period (Project U-TX- 0003)

PROJECT U solid dispersion was administered to SD rats (10/sex/group) for 4 weeks at oral doses of 0 (control), 1, 10, 100, and 1000 mg/kg/day [Project U-TX-0003; End-of-Text-Table 3.6.1]. To investigate the reversibility of any toxicological changes observed during treatment period, two groups of rats (5/sex/group) were added: one as a reversibility control and the other one treated with 1000 mg/kg/day for 4 weeks followed by a 4-week recovery period.

No changes considered to be related to the test article were noted in the 1, 10 and 100 mg/kg/day groups. At 1000 mg/kg/day, decreased hematocrit, hemoglobin and red blood cells, and increased extramedullary hematopoiesis in the spleen were observed in females. These changes were not noted after the 4-week recovery period, suggesting reversibility. One female was found dead on day 29. Histopathological examination of this animal revealed extramedullary hematopoiesis in the spleen, localized focal fibrosis in the base of heart, and congestion in the lungs. Since there were no obvious findings that could have directly contributed to the death, the cause of death remained unclear. Focal fibrosis in the heart was the only noteworthy finding in this animal. However, no similar change was observed in other rats in this dose group nor were changes like these noted in the 1-week intravenous dosing toxicity study with PROJECT U dipotassium in which much higher exposure was achieved [Project U-TX-0015]. Therefore, this change was not considered directly related to PROJECT U exposure. One female in the satellite group at 1000 mg/kg/day was found dead on day 18; however, no laboratory examination of the satellite animal was available and the relationship to the treatment was unknown.

Based on these findings, the NOAEL for PROJECT U in rats treated for 4 weeks was 1000 mg/kg/day for males and 100 mg/kg/day for females.

## 1-week Intravenous Dose Toxicity Study in Rats (Project U-TX-0015)

PROJECT U dipotassium was administered intravenously to SD rats (4/sex/group) at doses of 0 (control), 3, 10 and 60 mg/kg/day PROJECT U for 1 week [Project U-TX-0015; End-of-Text-Table 3.5].

No deaths occurred. On the first day of dosing for males, irritation at the injection site was observed in all animals at the dose level of 60 mg/kg/day. Therefore, on day 2 of dosing for males (the first day of dosing for females), the highest dose level was decreased to 30 mg/kg/day. In males in the high dose group (60/30 mg/kg/day), clinical signs indicative of severe irritation, such as discoloration (light red, dark purple or black), swelling and wounds at the injection site, were observed. Decreases in body weights, body weight gain and food consumption, hematology and

clinical chemistry findings suggestive of inflammatory changes such as increases in the platelet and white blood cell counts, chloride and globulin and decreases in prothrombin time, albumin and albumin/globulin ratio were noted. In addition, changes suggestive of hemolytic effects such as chromaturia, decreases in the red blood cell count, hemoglobin concentration, hematocrit, increases in the reticulocyte count and ratio, an increase in total bilirubin, and brownish yellow urine with protein-positive (2+) and occult blood-positive (3+) responses were noted. In histopathology, thrombus formation, perivascular inflammatory cell infiltration and necrosis were observed at the injection site. Increased granulopoiesis and megakaryocytes in the sternal bone marrow and/or spleen were observed. These findings were considered to be related to the inflammation at the injection site. A slight cell debris with hyaline casts in the proximal tubules were noted in the kidneys in the animal in which dosing was discontinued. In females, irritant effects were limited to discoloration of the tail (light red or dark purple) in the high dose (30 mg/kg/day) group. No treatment-related changes were noted in any male or female at 10 mg/kg/day or 3 mg/kg/day.

Based on these findings, the NOAEL in rats after intravenous administration of PROJECT U for 1 week was 10 mg/kg/day.

## 4-week Oral Dose Toxicity Study in Dogs with a 4-week Recovery Period (Project U-TX- 0004)

PROJECT U solid dispersion was administered orally to beagle dogs (4/sex/group) at doses of 0 (control), 1, 10, 100 and 1000 mg/kg/day for 4 weeks [Project U-TX-0004; see End-of-Text Table 3.6.2]. A recovery period of 4 weeks was established for the high-dose group (3 animals/sex) following completion of the administration period to investigate reversibility of changes observed during treatment period.

No deaths occurred. In the 1000 mg/kg/day group, loose stool, watery stool and mucous stool were frequently observed in both sexes throughout the administration period. These changes were observed mainly at 4 or 8 hours post dose. In addition, vomitus was frequently observed in both sexes in this group at 4 hours post dose. No test article-related changes were detected in body weight or food consumption, ophthalmology, electrocardiography, urinalysis, hematology analysis, blood chemistry analysis, necropsy, organ weight measurement, or histopathological examination. After the recovery period, none of the changes noted during the administration period were observed, suggesting their reversibility.

Based on these findings, the NOAEL was 100 mg/kg/day for both male and female dogs treated with PROJECT U for 4 weeks.

## 1-week Intravenous Dose Toxicity Study in Dogs (Project U-TX-0016)

PROJECT U dipotassium dissolved in physiological saline solution was administered intravenously to beagle dogs (1/sex/group) at doses of 0 (control), 10, 30 and 100 mg/kg/day PROJECT U for 1 week.

No deaths occurred. Vomiting/vomitus was observed for females in the 10, 30 and 100 mg/kg/day groups and for the male in the 100 mg/kg/day group immediately after dosing or at 1 hour postdose. Salivation was observed in the female in the 100 mg/kg/day group immediately after dosing.

Retching was observed in the female in the 10 mg/kg/day group immediately after dosing.

Decreased movement was also observed in the female in the 100 mg/kg/day group immediately after dosing but was not observed at 1 hour postdose.

The following findings were noted at the injection site in the male and the female in the

100 mg/kg/day group: swelling in the female from day 7 until the day of necropsy; thrombus formation in the male and the female and dark reddish thickening of the subcutis in the female; thrombus formation, necrosis of the perivascular tissue/subcutis, fibrosis of the vascular wall/perivascular tissue and edema in the perivascular tissue/subcutis in the male and the female and inflammatory cell infiltration in the dermis and perivascular tissue/subcutis in females.

Thrombus in the lung, increased germinal center of the spleen and increased white blood cell count, mainly due to increased neutrophils, were noted in the female in the 100 mg/kg/day group. These findings were considered to be associated with thrombus formation or inflammation at the injection site.

The NOAEL in dogs was 30 mg/kg/day for males and <10 mg/kg/day for females treated daily with intravenous PROJECT U for 1 week.

## Toxicokinetics

The toxicokinetic parameters of PROJECT U after repeated oral and iv administration to rats and dogs are summarized in [Table 5](#_bookmark70) and End-of-Text Table 3.3. In the 4-week rat study, mean Cmax and AUC24 values for both males and females increased with increasing dose between 1 and

1000 mg/kg/day for all sampling periods [Project U-TX-0003; [Table 5](#_bookmark70)]. However, the increases were less than dose proportional. No appreciable difference between males and females was noted. At doses of 1 to 1000 mg/kg/day PROJECT U, Tmax ranged from 0.25 to 1 hour on day 1; from 0.25 to 6

hours in week 2; and from 0.25 to 1 hour in week 4.

In the 1-week iv toxicity study in rats, plasma PROJECT U concentrations decreased rapidly after dosing on day 1, with t1/2 values tending to increase in females at 30 mg/kg/day [Project U-TX-0015 [Table 5](#_bookmark70)]. The AUC24 values increased with increasing doses. On day 7, there was no obvious accumulation.

In the 4-week dog study, Cmax and AUC24 increased almost dose proportionally up to

1000 mg/kg/day [Project U-TX-004; [Table 5](#_bookmark70)]. Tmax values showed a tendency to be constant regardless of the dose. During the dosing period, no consistent difference between males and females was observed for Cmax and AUC24 at any dose. There was no obvious accumulation following repeated dosing.

In the 1-week iv toxicity study in dogs, C0 values increased more than dose proportionally in both sexes on days 1 and 7, excluding the values in males on day 7, and AUC24 values also increased more than dose proportionally up to 100 mg/kg/day [Project U-TX-[0016; Table 5](#_bookmark70)]. C0 values in males on day 7 increased less than dose proportionally. Since all plasma concentrations at 24 hours post dosing in the 10 mg/kg/day group were below the lower limit of quantification, accurate t1/2 values could not be obtained in this group, while t1/2 in the 30 and 100 mg/kg/day groups were similar and constant. No remarkable differences between males and females were observed in the C0 or AUC24 at any dose level.

## Table 5 Toxicokinetic Parameters of PROJECT U After Oral and Intravenous Administration in Rats and Dogs

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study type** | **n per time point** | **Sex** | **Dose (mg/kg)** | **Unchanged drug** | | | | **Study No.** |
| **Cmax (ng/mL) ‡** | | **AUC24 (ng**･**h/mL)** | |
| **First dose** | **Last dose** | **First dose** | **Last dose** |
| **SD rats:**  **4-week oral dose toxicity study** | 3 | M | 1 | 2.490 | 1.096 | 17.439 | 17.396 | Project U-TX-  0003 |
| 3 | F | 1 | 2.005 | 4.660 | 9.899 | 15.392 |
| 3 | M | 10 | 23.037 | 9.006 | 44.750 | 27.266 |
| 3 | F | 10 | 23.672 | 3.513 | 23.504 | 17.422 |
| 3 | M | 100 | 55.599 | 51.740 | 183.213 | 137.455 |
| 3 | F | 100 | 64.808 | 59.628 | 182.545 | 138.812 |
| 3 | M | 1000 | 715.421 | 1958.816 | 1295.659 | 1724.924 |
| 3 | F | 1000 | 484.423 | 303.849 | 1311.113 | 662.802 |
| **SD rats:**  **1-week iv dose toxicity study (exploratory)** | 3 | M | 3 | 4846.596 | 4915.977 | 549.144 | 547.819 | Project U-TX-  0015 |
| 3 | F | 3 | 5193.287 | 2294.949 | 488.984 | 242.926 |
| 3 | M | 10 | 15813.689 | 20551.051 | 1963.355 | 2350.271 |
| 3 | F | 10 | 15519.226 | 8617.400 | 1691.001 | 1080.628 |
| 3 | M | 30 | - | 67907.596 | - | 15128.889 |
| 3 | F | 30 | 92159.029 | 130968.989 | 10329.489 | 13150.912 |
| 3 | M | 60 | 258016.793 | - | 54135.275 | - |
| **Beagle dogs: 4-week oral dose toxicity study** | 4 | M | 1 | 0.749 | 0.431 | 0.752 | 0.365 | Project U-TX-  0004 |
| 4 | F | 1 | 0.172 | 0.170 | 0.140 | 0.173 |
| 4 | M | 10 | 4.799 | 3.099 | 8.936 | 5.794 |
| 4 | F | 10 | 15.615 | 6.825 | 34.157 | 22.080 |
| 4 | M | 100 | 23.269 | 15.957 | 36.648 | 27.567 |
| 4 | F | 100 | 42.884 | 22.043 | 48.244 | 28.627 |
| 7 | M | 1000 | 609.694 | 353.625 | 580.333 | 514.444 |
| 7 | F | 1000 | 1201.578 | 419.968 | 692.298 | 396.607 |
| **Beagle dogs: 1-week iv dose toxicity study (exploratory)** | 1 | M | 10 | 53946.052 | 63846.859 | 6604.429 | 7498.963 | Project U-TX-  0016 |
| 1 | F | 10† | 45552.881 | 30432.232 | 5486.683 | 4328.430 |
| 1 | M | 30 | 183710.598 | 144266.396 | 40551.076 | 32283.933 |
| 1 | F | 30 | 174526.461 | 158527.63 | 38993.351 | 38175.349 |
| 1 | M | 100 | 1018657.668 | 529393.424 | 295165.031 | 334745.598 |
| 1 | F | 100 | 671717.658 | 882107.432 | 415483.607 | 496985.618 |

-: Toxicokinetic parameters not available. NOAEL is underlined. †NOAEL was 30 mg/kg/day for males and

<10 mg/kg/day for females.

‡ C0 values are reported for Project U-TX-0015 and Project U-TX-0016

Source:Project U-TX-0003 and Project U-TX-0004 (oral); Project U-TX-00015 and Project U-TX-0016 (iv)

## Genotoxicity

PROJECT U at concentrations up to 5000 mcg/plate showed no mutagenic activity in the bacterial reverse mutation assay [Project U-TX-0005]. PROJECT U at concentrations up to 3067 mcg/mL did not induce chromosomal aberrations in Chinese hamster lung (CHL/IU) cells, regardless of the presence or absence of metabolic activation [Project U-TX-0006]. PROJECT U at doses up to 2000 mg/kg did not induce micronucleated polychromatic erythrocytes (MNPCE) in murine bone marrow cells [Project U-TX-0008].

## Carcinogenicity

No carcinogenicity studies have been conducted to date.

## Reproductive and Developmental Toxicity

A study of effects on fertility and early embryonic development was conducted in rats. Studies to assess the teratogenic potential of PROJECT U were conducted in rats and rabbits.

## Effects on Fertility and Early Embryonic Development to Implantation

PROJECT U solid dispersion suspended in water for injection was administered orally to SD male and female rats (20/sex/group, 8 and 10 weeks old, respectively) at 0 (control) 100, 300 and

1000 mg/kg/day for 4 weeks for males (up to a day before necropsy) and 2 weeks for females (prior to mating up to gestation day 7) [Project U-TX-0009]. No death occurred and no adverse effects were observed in parent animals or their reproductive function. Likewise, no effects of PROJECT U were observed on early embryonic development. Based these results, the NOAEL was considered to be 1000 mg/kg/day for general toxicity, reproductive function, fertility and early embryonic development.

## Effects on Embryo-Fetal Development

* + - * 1. **Definitive Study of the Effects on Embryo-Fetal Development in Rats**

PROJECT U solid dispersion was suspended in water for injection and administered orally to pregnant SD rats (20/group) at doses of 0 (control), 100, 300, and 1000 mg/kg/day from gestation day 7 to 17 to investigate effects on dams and embryo-fetal development [Project U-TX-0011]. No deaths occurred and no adverse effects of PROJECT U were observed in dams. Likewise, no effects of PROJECT U were observed on embryo-fetal development. Based on these results, the NOAEL was considered to be 1000 mg/kg as PROJECT U for general toxicity in dams and embryofetal development.

## Definitive Study of the Effects on Embryo-Fetal Development in Rabbits

PROJECT U solid dispersion was administered orally to pregnant NZW rabbits (18 or 20/group) at doses of 0 (control), 10, 30 or 60 mg/kg/day for 13 days (gestation day 6 to 18) [Project U-TX-0013]. No test article-related deaths were noted and no toxicologically significant findings were observed for dams or embryofetal development. Based on these results, the NOAEL was 30 mg/kg as PROJECT U for general toxicity in dams and 60 mg/kg as PROJECT U for embryofetal development.

## Local Tolerance

No local tolerance studies have been conducted with PROJECT U.

## Other Toxicity Studies

No other toxicity studies have been conducted with PROJECT U.

# 4.4 Integrated Nonclinical Overview and Conclusion: Potential Clinical Relevance

In the definitive single dose toxicity studies, no mortality was observed after oral doses of PROJECT U solid dispersion were administered to rats and dogs; therefore, the approximate lethal dose was estimated to be > 2000 mg/kg in rats and > 1500 mg/kg in dogs. Overall, the definitive 4-week toxicity studies indicated the NOAEL in rats was 1000 mg/kg/day for males and 100 mg/kg/day for females and the NOAEL in dogs was 100 mg/kg/day for both sexes. The lethal dose was 1000 mg/kg/day for rats (females only); no mortality was noted at up to 1000 mg/kg/day in dogs. Erythropoiesis in rats and the gastrointestinal tract in dogs were identified as target organs of toxicity. Findings consisted of decreased hematocrit, hemoglobin and red blood cells, and increased extramedullary hematopoiesis in the spleen in rats and vomiting and loose stool in dogs, all of which recovered fully after discontinuation of the drug; focal fibrosis in the base of heart was also observed at necropsy in 1 female rat. Based on the results from the entire battery of mutagenicity assays, it was concluded that PROJECT U did not exhibit genotoxicity potential. No toxicological effects were observed in reproductive function of parent rats and early embryonic development up to 1000 mg/kg/day and no teratogenic potential was indicated in rats and rabbits.

Based on these results, findings of potential concern for clinical trials are discussed below.

*Potential Cardiac Effects*

In the 4-week oral dose toxicity study in rats, one female rat at the 1000 mg/kg/day dose was found dead on day 29; focal fibrosis in the heart was the only noteworthy finding in this animal. No similar change was observed in other rats in this dose group nor were changes like these noted in the 1-week intravenous dosing toxicity study with PROJECT U dipotassium in which much higher exposure was achieved (Project U-TX-0015). Mortality was also observed in 1 female rat in the toxicokinetic satellite group at the same dose of 1000 mg/kg/day on day 18; however, no histopathological examination was available and the relationship to the treatment was unknown.

It has been reported that BB2 receptors are not expressed in the heart [Jensen RT et al, 2008], and BB2 receptor-knockout mice also did not exhibit abnormal cardiac findings [Hampton et al, 1998; Wada et al, 1997]. In addition, there is no information readily available in the literature that would suggest a relationship between BB2 receptor inhibition and cardiac changes. The cause of death in the 2 female rats in the 4 week toxicology studies is unclear. One animal had a small focal area of cardiac fibrosis which is not believed to have been the cause of death, but a relationship cannot be completely excluded. The absence of cardiac fibrosis in other animals dosed at 1000 mg/kg/day and lack of BB2 receptors in the heart does not support PROJECT U having a causative role in the development of focal cardiac fibrosis. A relationship of PROJECT U to the single case of focal cardiac fibrosis will require further investigation in the planned 13-week rat toxicology study.

Dose escalation with careful monitoring of the cardiovascular system will be performed in the phase 1 clinical trial.

*Potential Hematological Effects*

In the preliminary 1-week oral repeated-dose study in rats, a decrease in the number of reticulocytes (approximately 72% of the control value) was noted in females at 100 mg/kg/day or more; however, no other changes suggesting anemia except for reticulocytopenia were observed in animals receiving up to 1000 mg/kg/day. In the 4-week oral repeated-dose study in rats, decreases in hematocrit, hemoglobin and red blood cell count, and increased extramedullary hematopoiesis in the spleen were noted in females at 1000 mg/kg/day. These changes were mild (approximately 95%

of the control value) and no changes in reticulocyte ratio or bone marrow were noted. Considering the results of the 1-week study, anemia and related changes observed after the 4 weeks of treatment could be attributed to a decrease in reticulocytes and poor RBC production early in the treatment period. These changes were not observed after a 4-week recovery period. Though statistically insignificant, slight but similar changes were also observed in the male rat. No changes suggestive of anemia were detected up to 1000 mg/kg/day in the 4-week dog study.

PROJECT U exhibited a potential to cause anemia, as shown in the 4-week oral repeated-dose toxicity study in rats; however, the observed changes were mild and occurred only at the highest dose; likewise, their reversibility was confirmed. At the doses that caused anemia, AUC24 was more than 700 times higher than that at the pharmacologically active dose (PAD; 0.1 mg/kg) in the rat model [see [Table 6](#_bookmark81)].

Dose escalation with careful monitoring of the hematological parameters will be performed in the phase 1 clinical trial.

*Potential Gastrointestinal Effects*

In the safety pharmacology study, and single dose and preliminary 1-week repeated oral dose general toxicity studies in dogs, changes in stool (i.e., soft stool and liquid stool) were observed at 1000 mg/kg or more, and vomiting was observed at 500 mg/kg or more. In the 4-week oral dose toxicity study in dogs, soft and mucous stools were observed at 1000 mg/kg/day throughout the dosing period, but not during the recovery period. The change in stool occurred only at the highest dose (1000 mg/kg/day) which is 10000 times higher than the PAD (0.1 mg/kg) in animal models; additionally, AUC24 at 1000 mg/kg was more than 400 times higher than that at the PAD in animal models. There were no changes in body weight, food consumption, serum electrolyte, or histopathology of the digestive tract.

Dose escalation with careful monitoring of the gastrointestinal system will be performed in the phase 1 clinical trial.

## Safety Margin

The human equivalent doses of the NOAEL observed in rats was calculated as 960 mg/day and in dogs was calculated as 3240 mg/day. Therefore, a dose of 3 mg/day was recommended as the starting dose in the planned first-in-man (FIM) study as this will provide an approximately 320-fold safety margin to the NOAEL in rats and an approximately 1080-fold safety margin to the NOAEL in dogs.

## Table 6 Compilation of Doses and Systemic Exposure Data of PROJECT U at NOAEL/PAD/HCD

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Species/ Route of  Admini- stration | Sex | Dose (mg/kg/day) | HED†  (mg/60kg) | Cmax (ng/mL) | | AUC24 (ng･h/mL) | | Study No. |
| First dose | Last dose | First dose | Last dose |
| Rat, SD;  oral | M | 1000 (NOAEL) | 9600 | 715.4 | 1958.8 | 1295.7 | 1724.9 | Project U-TX-  0003 |
| F | 100 (NOAEL) | 960 | 64.8 | 59.6 | 182.5 | 138.8 |
| M | >1000 (LOAEL） | >9600 | ≥715.4 | ≥1958.8 | ≥1295.7 | ≥1724.9 |
| F | 1000 (LOAEL) | 9600 | 484.4 | 303.8 | 1311.1 | 662.8 |
| Rat, SD;  iv ¶ | M | 10 (NOAEL) | 96 | 15814 | 20551 | 1963 | 2350 | Project U-TX-  0015 |
| F | 10 (NOAEL) | 96 | 15519 | 8617 | 1691 | 1081 |
| M | 30 (LOAEL） | 288 | – | 67908 | – | 15129 |
| F | 30 (LOAEL） | 288 | 92159 | 130969 | 10329 | 13151 |
| Dog, Beagle; oral | M | 100 (NOAEL) | 3240 | 23.3 | 16.0 | 36.6 | 27.6 | Project U-TX-  0004 |
| F | 100 (NOAEL) | 3240 | 42.9 | 22.0 | 48.2 | 28.6 |
| M | 1000 (LOAEL) | 32400 | 609.7 | 353.6 | 580.3 | 514.4 |
| F | 1000 (LOAEL) | 32400 | 1201.6 | 420.0 | 692.3 | 396.6 |
| Dog, Beagle; iv ¶ | M | 30 (NOAEL) | 972 | 183711 | 144266 | 40551 | 32284 | Project U-TX-  0016 |
| F | <10 (NOAEL) | <324 | ≤45553 | ≤30432 | ≤5487 | ≤4328 |
| M | 100 (LOAEL） | 3240 | 1018658 | 529393 | 295165 | 334746 |
| F | 10 (LOAEL） | 324 | 45553 | 30432 | 5487 | 4328 |
| Rat, Wistar; oral | M | 0.1 (PAD) | 1 | 0.35 | | 0.93 | | Project U-PH-  0008  Project U-PH-  0010  Project U-PH-  0011  Project U-ME-  0009 |
| Monkey, Cyno-  molgous. oral | M | 0.091 (PAD) | 1.7 | - | | - | | Project U-PH-  0009 |
| Human |  | 0.3 | 18  (Estimated human clinical dose  0.3 mg/kg x 60 kg) | 0.75 ‡  0.18 § | | 2.0 ‡  0.5 § | | Project U-ME-  0009  Project U-ME-  0010  Project U-ME-  0011 |

HED: human equivalent dose; NOAEL: no-observed-adverse-effect level; LOAEL: lowest-observed-adverse-effect level; PAD: pharmacologically active dose; HCD: human clinical dose; –: Toxicokinetic parameters not available.

† The human equivalent dose levels were calculated by using the body surface area conversion factors ( rat: 0.16, dog: 0.54, monkey: 0.32) and the human body weight of 60 kg

‡ The predicted systemic exposure in humans, calculated by extrapolation from rat pharmacokinetic data (Project U-ME- 0009) corrected by plasma protein binding ratio of humans to rats (Project U-ME-0011)

§ The predicted systemic exposure in humans, calculated by extrapolation from dog pharmacokinetic data (Project U-ME- 0010) corrected by plasma protein binding ratio of humans to dogs (Project U-ME-0011)

¶ Preliminary non GLP study.

### List of References

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