### 4.1.3 Safety Pharmacology

The results of safety pharmacology studies are presented in End-of-Text Table 1.2. Project R dissolved in DMSO or suspended in 0.5% MC were used. The dose levels and concentrations are expressed as the free form.

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

PROJECT R at a concentration of up to 10 mcM concentration-dependently inhibited the hERG current in hERG-transfected human embryonic kidney (HEK293) cells using the whole cell clamp method (Project R-PT-0001). PROJECT R suppressed the hERG current with an IC50 of

2.8 mcM.

### Effects of PROJECT R on Action Potential Durations in Isolated Guinea Pig Papillary Muscles

PROJECT R at concentrations of up to 1 mcM did not affect action potential durations at APD30, APD90 or the difference between APD30 and APD90 (APD30-90) in guinea pig papillary muscles in a study using the glass microelectrode technique (Project R-PT-0002). PROJECT R at a concentration of 3 mcM caused prolongation of APD30 and APD90, without APD30-90 prolongation. No effect on resting membrane potential, action potential amplitude or (dV/dt)max was noted at any concentration tested.

### Effects of PROJECT R on Central Nervous System in Rats

The effects of a single oral dose of PROJECT R on the general activity and behavior in rats were determined using the modified Irwin’s method (Project R-PT-0004). PROJECT R (0, 100, 300 and 1000 mg/kg) did not affect the general activity or behavior in any of the rats up to 24 hours after administration. Decreased stool volume was observed at 1000 mg/kg.

### Effects on Central Nervous, Cardiovascular and Respiratory Systems in Cynomolgus Monkeys

A single oral dose of PROJECT R (0, 100, 300 and 2000 mg/kg) did not affect the general activity, behavior, body temperature, blood pressure, heart rate, electrocardiogram (ECG) parameters, respiration rates, blood gas parameters or blood electrolyte concentrations in monkeys (Project R-PT-0003). Yellowish-white stool color was observed at 2000 mg/kg.

## Toxicology

An overview of toxicity studies is shown End-of-Text Table 3.1. The current toxicological data package for PROJECT R includes 2 single oral dose toxicity studies, 2 exploratory 4-week oral dose toxicity studies, two 13-week oral dose toxicity studies with recovery assessment, 4 genotoxicity studies and 1 study of effects on male fertility. All pivotal toxicology studies were performed in accordance with Good Laboratory Practice (GLP). The nonclinical toxicity studies for PROJECT R were conducted using Project R as suspension in

0.5 % methylcellulose solution. All dose levels and plasma drug concentrations of PROJECT R are expressed as the free form, unless otherwise specified.

### Single-dose Toxicity

Single oral dose toxicity studies of PROJECT R were conducted in rats and monkeys [End-of-Text Table 3.4]. In rats, single oral doses of PROJECT R at 1000 or 2000 mg/kg

induced soft stool and decreased stool volume, and a tendency toward suppression of body weight gain; no gross pathological changes were observed (Project R-TX-0005). In monkeys, single oral doses of PROJECT R at 1000 or 2000 mg/kg induced soft stool, increased indirect, direct and total bilirubin as well as decreased neutrophil counts (Project R-TX-0006). No mortality was observed in rats or monkeys. Based on these results, the approximate lethal dose of PROJECT R when orally administered to rats and monkeys was estimated to be greater than 2000 mg/kg.

### Repeat-dose Toxicity

A total of two 4-week repeated-dose studies were performed in rats and monkeys to provide guidance for the selection of doses used in the definitive (GLP) studies (Project R-TX-0007 and Project R-TX-0008) [End-of-Text Table 3.5].

The definitive 13-week repeated-dose studies (Project R-TX-0009 and Project R-TX-0010) indicated the NOAEL was 3 mg/kg/day for rats and 1 mg/kg/day in monkeys. In the 13-week study in rats, findings at the lowest-observed-adverse-effect level (LOAEL), 10 mg/kg/day, were increased lymphocyte count, total cholesterol, indirect bilirubin, BUN and creatinine, as well as histopathological changes in the spleen, adrenals, thymus and Harderian gland. In the

13-week toxicity study in monkeys, the main finding at the LOAEL, 3 mg/kg, was dilatation of the bile canaliculus. The minimum dose level with significant reduction of prostate weight was 3 mg/kg/day.

Target organs of toxicity were the erythrocytic and leukocytic system, liver, adrenals, thymus and pituitary.

### 13-week Oral Dose Toxicity Study in Rats with a 8-week Recovery Period

PROJECT R (0 [vehicle], 1, 3, 10 and 100 mg/kg) was administered orally once daily for

13 weeks to Crl:CD(SD) rats (10/sex/group) in Study Project R-TX-0009 [End-of-Text Table 3.6.1]. No test-article-related death or moribundity was observed in any group. No

test-article-related, toxicologically relevant changes were noted in the 1 and 3 mg/kg/day groups.

In the 10 mg/kg/day group and above, increased leukocyte count, basophil count and/or ratio, lymphocyte count and/or ratio, large unstained cell count, platelet count and reticulocyte ratio were noted as well as decreased neutrophil ratio and erythrocyte count. Increased total cholesterol, indirect bilirubin, blood urea nitrogen, creatinine and decreased sodium were noted. Increased thymus and adrenal weights were noted. Increased mitosis in the cortex in the thymus and vacuolation/hypertrophy of the cortical cell in the adrenal were observed as well as congestion in the red pulp of the spleen and hyperplasia of the lymphocyte in the white pulp of the spleen. An increased incidence and/or frequency of regeneration of the glandular epithelium in the Harderian gland and foamy cells in the alveolus in the lung were noted.

In the 100 mg/kg group, decreased spontaneous activity, nasal bleeding, blanching or pale skin, emaciation and decreased stool volume were observed in a few animals. Body weight and body weight gain and food consumption were decreased. Low total excretion of sodium, potassium and chloride were noted. High monocyte counts and prolongation of PT and APTT were noted. Increased ALT, gamma-globulin ratio, total bilirubin, alpha2-globulin ratio and beta-globulin ratio were observed as well as decreased triglyceride, calcium chloride, albumin, albumin ratio and albumin/globulin ratio. Enlargement of the adrenals and the spleen, and high absolute and/or relative spleen weights were noted. Cellular infiltration, degeneration/necrosis of the zona fasciculata/reticularis cells, depletion of the zona fasciculata cells and depletion of the zona reticularis cells were observed in the adrenal. An　increased incidence and/or frequency of yellowish-brown pigment in the lumen in the Harderian glands and foamy cell/cholesterol-like crystal in Rathke’s cleft in the pituitary was observed. Increased absolute and relative lung weights were observed as well as foamy cells in the alveolus.

Indicators of genital organ atrophy were observed upon gross pathology and histopathology at 3 mg/kg/day and above and increased serum testosterone concentration was noted in the 10 mg/kg/day group and above. These were considered to be related to the pharmacological effects of PROJECT R, which is an antiandrogen.

At the end of the recovery period in the 100 mg/kg group, increased leukocyte and lymphocyte count in males and decreased albumin ratio and albumin/globulin ratio in females persisted but showed a tendency toward recovery. Vacuolation in the zona fasciculata of the adrenal; and fibrosis in the zona reticularis of the adrenal was observed in males and hypertrophy of the interstitial cell in the ovary and increased yellowish-brown pigment in the lumen in the Harderian gland was observed in females after the recovery period; however, these changes showed a tendency toward recovery. Other changes noted during the dosing period were not observed or tended to recover. No tendencies toward recovery were noted in testicular or epididymal changes, which were considered to be related to the pharmacological effect of the test article.

Based on these results, it was concluded that the NOAEL of PROJECT R when administered orally to rats for 13 weeks was 3 mg/kg/day for males and females.

### 13-week Oral Dose Toxicity Study in Monkeys with an 8-week Recovery Period

PROJECT R (0, 1, 3, 10 and 100 mg/kg) was administered orally once daily for 13 weeks to cynomolgus monkeys (n = 4/sex/group) in Study Project R-TX-0010 [End-of-Text Table 3.6.2]. No toxic changes were noted in the 1 mg/kg/day group. Dilation of the bile canaliculus in the liver was observed in males and females at 3 mg/kg/day and above. Increased neutrophil count was observed in females at 10 mg/kg/day and above. Increased total, direct and indirect bilirubin levels were noted in males and females, and increased ALP was noted in females at 10 mg/kg/day and above. Increased liver weight was noted in males and/or females at 10 mg/kg/day and above. Hypertrophy and decreased lipid in the zona fasciculata cells of the adrenal were observed in males and females at 10 mg/kg/day and above.

Abnormal urine color, yellowish-brown urine and bilirubin positive urine were observed in 1 male in the 100 mg/kg/day group. Decreased body weights and sporadically decreased food consumption was observed at 100 mg/kg/day. Increased platelet counts and triglycerides and decreased total cholesterol were observed in males and/or females.

Increased liver and adrenal weights were noted in males and females. Histopathological examination of the liver revealed dilation of the bile canaliculus in all males and females, pigmentation suspected to be due to bile in the canaliculus in some females and hypertrophy of the centrilobular/midzonal hepatocytes in some males and females. Hypertrophy and decrease in lipid in the zona fasciculata cells were observed in the adrenal in all males and females. Cholestasis was diagnosed by findings of increased total, direct and indirect　bilirubin and ALP in plasma at ≥ 10 mg/kg and pigmentation of bile canaliculus at 100 mg/kg.

In 1 female at 100 mg/kg/day, swelling at the head, hydrothorax, ascites, pericardial fluid, subcutaneous edema at the neck and edema in the subcutaneous tissue were observed. This female also showed decreased total protein and albumin at weeks 7 and 13. The following observations in this animal were considered secondary to the findings mentioned above: decreased lymphocyte count and chloride, increased aspartate aminotransferase (AST), BUN, inorganic phosphorus and potassium at weeks 7 and/or 13, and decreased bone marrow nucleated cell count, atrophy of the myocardium and muscle fiber (femoral muscle and tongue), atrophy of the subepicardial adipocytes, atrophy of the white pulp in the spleen, lymphoid follicular atrophy in the lymph nodes (submandibular and mesenteric) and hypocellularity in the sternal bone marrow

Gross pathological indicators of genital organ atrophy and increased testosterone level and were observed at 1 mg/kg/day and above and were considered to be due to the pharmacological effect of PROJECT R.

All changes noted during or at the end of 13-week treatment period at 100 mg/kg had recovered by the end of the recovery period.

Under the conditions of this study, the NOAEL was 1 mg/kg/day for males and females.

### Toxicokinetics

A summary of toxicity studies including toxicokinetic parameters is presented in End-of-Text Table 3.2, with results presented in End-of-Text Table 3.3. The Cmax and area under the curve from time zero to 24 hours (AUC24) increased dose proportionally in rats between 1 and 10 mg/kg/day; however, increases in Cmax and AUC24 tended to be somewhat saturated from 100 mg/kg (Project R-TX-0009). Exposure (Cmax and AUC24) for females was slightly higher than that of males at 100 mg/kg [[Table 5](#_bookmark53)]. In males, repeated dosing for 49 and 91 days at 1 to 10 mg/kg resulted in increases in the Cmax and AUC24 compared to those on

day 1, but apparent increases were not observed at 100 mg/kg. In females, repeated dosing for 49 days resulted in increases in the Cmax and AUC24 at all doses tested. The systemic exposure level on day 91 was slightly higher than that on day 49.

The Cmax and AUC24 increased almost dose proportionally in monkeys up to 10 mg/kg/day, but less than dose proportionally at 100 mg/kg (Project R-TX-0010) [[Table 5](#_bookmark53)]. The Cmax and AUC24 were increased 2- to 5-fold on day 49 and then remained constant on day 91. There was no clear sex difference in any parameter during the dosing period.

### Table 5 Repeat-dose Toxicokinetics after Oral Administration in Rats and Monkeys

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Type of Study Study No.** | **Dose (mg/kg)** | **Sex** | **Total Plasma Concentration**  **[Unbound Plasma Concentration†]** | | | |
| **Cmax (ng/mL)** | | **AUC24**  **(ng·h/mL)** | |
| **First**  **Dose** | **Last**  **Dose** | **First**  **Dose** | **Last**  **Dose** |
| Rat 13-week oral dose toxicity study (definitive)  (Project R-TX-0009) | 1 | M  F | 243.786  [2.32]  256.353  [2.44] | 687.556  [6.53]  848.822  [8.06] | 3298  [31.3]  3889  [36.9] | 9261  [88.0]  13147  [125] |
| 3 | M  F | 1115.679  [10.6]  1170.261  [11.1] | 2860.929  [27.2]  2917.744  [27.7] | 17014  [162]  19232  [183] | 44229  [420]  56808  [540] |
| 10 | M  F | 3381.532  [32.1]  4037.331  [38.4] | 7389.695  [70.2]  11433.586  [109] | 55112  [524]  70443  [669] | 143203  [1360]  234040  [2223] |
| 100 | M  F | 17979.239  [171]  22015.630  [209] | 20711.679  [197]  46269.593  [440] | 351182  [3336]  412178  [3916] | 412104  [3915]  849778  [8073] |
| Monkey 13-week oral dose toxicity study (definitive)  (Project R-TX-0010) | 1 | M  F | 164.617  [3.95]  255.032  [6.12] | 742.918  [17.8]  547.580  [13.1] | 3351  [80.4]  3959  [95.0] | 14328  [344]  9931  [238] |
| 3 | M  F | 459.332  [11.0]  721.407  [17.3] | 1796.377  [43.1]  1731.793  [41.6] | 8429  [202]  11179  [268] | 33939  [815]  28057  [673] |
| 10 | M  F | 1172.404  [28.1]  1341.510  [32.2] | 5139.860  [123]  4943.954  [119] | 23154  [556]  23821  [572] | 102526  [2461]  95616  [2295] |
| 100 | M  F | 3548.723  [85.2]  3381.809  [81.2] | 10453.157  [251]  9082.809  [218] | 56234  [1350]  62993  [1512] | 207920  [4990]  176081  [4226] |

No-observed-adverse-effect levels (NOAELs) for general toxicity are underlined.

† Unbound plasma concentrations were calculated based on data from Project R-ME-008. Source: Project R-TX-0009 (rat) and Project R-TX-0010 (monkey)

### Genotoxicity

PROJECT R at concentrations up to 5000 mcg/plate showed no mutagenic activity in the bacterial reverse mutation assay (Project R-TX-0001) [End-of-Text Table 3.7.1]. PROJECT R at concentrations ranging from 5 to 80 mcg/mL induced structural chromosomal aberrations in CHL/IU cells in short-term treatment without and with metabolic activation (Project R-TX-0002) [End-of-Text Table 3.7.2]. PROJECT R at doses up to 2000 mg/kg did not induce

micronucleated polychromatic erythrocytes in murine bone marrow cells (Project R-TX-0003) [End-of-Text Table 3.8.1]. PROJECT R at doses up to 2000 mg/kg did not induce DNA damage in rat hepatocytes (Project R-TX-0004) [End-of-Text Table 3.8.2].

### Carcinogenicity

No carcinogenicity studies have been conducted to date.

### Reproductive and Developmental Toxicity

PROJECT R (0.1, 1, 10 and 100 mg/kg/day) was administered orally to male rats (20/group) from 4 weeks before mating and through the mating period (2 weeks), followed by an 8-week recovery period, to assess reversibility of the effects on male fertility (Project R-TX-0011)

[End-of-Text Table 3.9].

No males died in any group. In the 1 and 0.1 mg/kg/day groups, no test-article-related changes were noted in clinical signs, body weight or food consumption. Emaciation was observed in 1 male in the 100 mg/kg/day group. Significant suppression of body weight gain and low body weight were noted during the dosing period in the 100 mg/kg/day group and during the early phase of the dosing period in the 10 mg/kg/day group. Low food consumption was also noted during the dosing period in the 100 mg/kg group. In the

10 mg/kg/day recovery group, suppression of body weight gain and low body weight were noted during the dosing period; however, effects on body weight recovered by the end of recovery period.

No test-article-related changes were noted in the copulation rate, mean copulatory interval, fertility rate or sperm examination parameters in the 1 or 0.1 mg/kg group, or in reproductive organ weight in the 0.1 mg/kg/day group. The copulation rate was slightly low at mating during the dosing period in the 10 mg/kg/day group; however, no test-article-related changes were noted in the mean copulatory interval or fertility rate in this group. Of the males in the 100 mg/kg/day group, 17 of 20 did not copulate with nontreated females, and the remaining 3 males who copulated failed to impregnate the females.

Gross pathological examination at the end of the dosing period revealed reduced size and weight of the seminal vesicle, prostate and epididymis in the 1, 10 and

100 mg/kg/day groups. A low sperm count was noted in the 10 and 100 mg/kg/day groups. Increased testis weight, reduced sperm activity and viability rate were also noted in the

100 mg/kg/day group. At the end of the recovery period, reduced seminal vesicle and prostate weights were noted in recovery group treated with 10 mg/kg compared with the control group; however, these changes were less severe than those at the end of the dosing period. All copulated males impregnated females at mating and no test-article-related changes were noted in sperm examination parameters after the 8-week recovery period.

No test-article-related changes were noted in the preimplantation loss rate, number of live embryos, number of postimplantation losses or postimplantation loss rate in the 0.1, 1 or 10 mg/kg/day groups.

Based on these results the NOAEL of PROJECT R was 1 mg/kg/day for general toxicity in males, 0.1 mg/kg/day for reproductive function in males and 10 mg/kg/day for early embryonic development.

### Local Tolerance

No local tolerance studies have been conducted with PROJECT R.

### Other Toxicity Studies

No other toxicity studies have been conducted with PROJECT R.

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

PROJECT R is a potent antagonist of human AR. PROJECT R has antagonistic activity on both T877A- and W741C-mutated AR, but it does not have any agonistic activities. In a 5-week repeated-dose study, PROJECT R dose dependently reduced the ventral prostate weight in mature male rats with an ED50 value of 0.56 mg/kg/day.

Repeated oral administration of PROJECT R to SCID mice suppressed the tumor volume of KUCaP xenograft expressing the W741C-mutated AR. Pharmacology studies performed to date provide proof of mechanism for the pharmacological activity of PROJECT R and also provide nonclinical proof of concept for reduction of tumor growth by antagonizing W741C-mutated AR.

In pharmacokinetic studies, dose-dependent accumulation of PROJECT R occurred in both rats and monkeys. Plasma protein binding (mainly to HSA) was highest in humans (99.12%).

No clear human-specific PROJECT R metabolites were formed in the liver microsomes or hepatocyte incubations. CYP3A4/5 was the major CYP isozyme involved in the PROJECT R metabolism in human liver microsomes. PROJECT R showed weak direct inhibition of CYP2C8, 2C9, 2C19 and 2D6 and a weak time-dependent irreversible inhibition of CYP3A4. While CYP1A and 3A were induced dose dependently, CYP2B was not induced. PROJECT R was mainly excreted via feces and to a lesser extent via urine. Enterohepatic circulation was substantial (34.6%). PROJECT R inhibited P-gp-mediated transport and also proved to be a substrate for P-gp transport. Based on these results, PROJECT R may have clinically relevant drug-drug interactions with drugs that are metabolized and/or excreted by CYP2C9 and P-gp. Concomitant medications that are CYP2C9 or P-gp substrates with a narrow therapeutic/safety range should be administered with caution.

In safety pharmacology studies, PROJECT R was shown to inhibit the hERG current, slightly prolonged APD30 and APD90 in isolated guinea pig muscles at approximately 3 mcM, which is approximately 500 times higher than the assumed pharmacologically effective plasma concentration. However, the in vivo monkey cardiovascular safety study and the 13-week repeated-dose study in monkeys did not show effects on ECG, QTc or heart rate. Although the cardiovascular risk for PROJECT R is low, cardiac monitoring will be performed in human studies, as 1 female monkey in the exploratory 4-week study displayed a decreased heart rate.

No animals died in the single-dose toxicity studies and therefore the minimum lethal dose was greater than 2000 mg/kg in both rats and monkeys. In the repeated-dose toxicity studies in rats and monkeys, the targets of PROJECT R-induced toxicity included the liver (cholestasis), hematopoietic system (slight anemia, increased lymphocytes) and cardiovascular system (decreased heart rate). In addition, histopathological changes in the pituitary, adrenals and thymus were observed; and PROJECT R was shown to increase cholesterol levels.

The NOAEL in the 13-week rat study was 3 mg/kg/day, based on hematological changes and effects on thymus, adrenals and cholesterol levels at 10 mg/kg. At this NOAEL, AUCu is 4.5 to 14.9 times higher than the pharmacologically effective AUC. In the 13-week monkey study, the NOAEL was 1 mg/kg, as dilation of bile canaliculi occurred at 3 mg/kg. At this NOAEL, AUCu is 2.2 to 9.5 times higher than the pharmacologically effective AUC. It is important to note that the dilation of bile canaliculi at 3 mg/kg may be an adaptive response to slightly increased bile viscosity, as no bilirubin or ALP changes occurred. Evident cholestasis occurred at 10 mg/kg, but was mild in nature (no signs of necrosis) and reversible.

In the fertility study, male rats dosed with 100 mg/kg failed to impregnate females, while the copulation rate was slightly lowered at 10 mg/kg, accompanied by histopathological changes in the male reproductive organs. No changes were observed in early embryonic development. These results were consistent with the pharmacological activity of PROJECT R.

Findings of potential concern for clinical trials include changes in liver function (cholestasis), hematological findings (slight anemia, increased lymphocytes), cardiovascular events (decreased heart rate, elevated cholesterol levels) and histopathological changes in the adrenals. In addition, class-related antiandrogen effects can be expected.

### Effects on the Liver

Cholestasis was observed in the repeated-dose toxicity studies in monkeys, as characterized by dilatation of bile canaliculus, pigmentation in the centrilobular bile canaliculi and high bile viscosity, which are all suggestive of bile flow obstruction. Increase in serum bilirubin and bilirubinurina and increased ALP, indicative of cholestasis, were also found. In contrast, little damage to hepatocytes in histopathology and minor changes in serum AST or ALT were observed. These findings were absent after an 8-week recovery period.

PROJECT R or its metabolites are thought to affect on bile flow directly, as increased total, indirect and direct bilirubin was observed after a single dose of 1000 mg/kg. The type of cholestasis observed in monkeys resembles the bland cholestasis (cholestasis without hepatitis) induced by anabolic steroids, estrogens and various drugs such as cyclosporine, and is consistent with an inhibitory effect on hepatic bile acid transporters.

Direct bilirubin and ALP were shown to be suitable biomarkers of cholestasis in the 13-week toxicity in monkeys. In addition, gamma-glutamyl transpeptidase (GGT) and serum bile acids can be used to monitor cholestasis in clinical trials.

### Cardiovascular Effects

The IC50 for hERG current inhibition by PROJECT R was 2.8 mcM and in isolated guinea pig papillary muscles, and slight prolongation of APD30 and APD90 were observed at 3 mcM. Decreased heart rate was noted in 1 of 2 females dosed with 300 mg/kg after dosing at week 4 in the 4-week monkey study. However, the in vivo monkey cardiovascular safety study and the 13-week repeated-dose study in monkeys did not show effects on ECG, QTc or heart rate. Standard cardiac monitoring will be conducted in clinical trials.

Total cholesterol levels were increased by PROJECT R. In patients receiving hormone deprivation therapy, hypercholesterolemia is frequently observed. As it is a risk factor for cardiovascular disease, elevation of cholesterol levels should be monitored in clinical trials of PROJECT R.

### Hematological Effects

In the 13-week rat study, slight decreases in hematocrit, hemoglobin and/or red blood cells were noted at 3 mg/kg and above, which were judged to be of little toxicological concern because of their magnitude (approximately 4%) and the observed reversibility after cessation of treatment. Reticulocytes increased in response to decreasing erythrocyte counts and increased extramedullary hematopoiesis in the spleen was also found. Normocytic normochromic anemia is commonly encountered in men receiving hormonal deprivation therapy.

Increased neutrophil counts and increased platelets, but no changes in the erythrocyte lineage were observed in the 13-week monkey study. In rats, increased lymphocytes and decreased neutrophil ratio as well as hyperplasia in white pulp of the spleen were found. Increased lymphocyte counts are considered to be due to the pharmacological activity of PROJECT R. In clinical studies, differential white blood cells (WBCs) should be measured.

### Adrenal Effects

In the 13-week rat study, increased adrenal weights and vacuolation/hypertrophy of the cortical cells, and at higher dose, enlargement, cellular infiltration, degeneration and necrosis in the zona fasciculata/reticularis were observed. Furthermore, in the 13-week monkey study, hypertrophy and decreased lipids in the zona fasciculata were observed. As the

14C-PROJECT R distribution study showed high tissue concentration in the adrenals, it is likely

that the adrenal findings at 100 mg/kg are caused by a direct effect of PROJECT R on the adrenals. Adrenal function will be monitored in early clinical trials using cortisol and adrenocorticotropic hormone (ACTH) as biomarkers.

### Other Class-related Effects

As an AR antagonist, class-related endocrine side effects (hot flushes, gynecomastia, breast pain) and gastrointestinal effects (abdominal pain, diarrhea, constipation, nausea) may occur. Decreased libido and impotence are also frequently reported. It is currently not known what the effects of single-dose administration of PROJECT R are on the libido of healthy volunteers.

### Risk Minimization Actions

Potential safety concerns associated with PROJECT R are summarized in [Table 6](#_bookmark60) along with the corresponding risk minimization actions.

### Table 6 Potential Safety Concerns of PROJECT R and Risk Minimization Actions

|  |  |  |
| --- | --- | --- |
| **Target Organs** | **Potential Risks** | **Risk Minimization Action** |
| Adrenals | Adrenal hypertrophy, and at higher dose, depletion of cells in the zona  fasciculata and reticularis | Monitoring of ACTH and cortisol levels |
| Liver | Dilatation of bile canaliculus, increases in indirect, direct and total bilirubin and ALP | Standard monitoring of liver function parameters (ALT, AST, ALP, GGT, total bilirubin);  in addition, direct bilirubin and serum bile acids should be measured |
| Cardiovascular system | Decrease in heart rate  Increase total cholesterol levels | Cardiac monitoring should be  performed during the first 12 hours after dosing in the FIH study  Monitoring of total cholesterol, HDL and LDL |
| Hematopoietic system | Slight anemic changes, increase in lymphocyte count | Monitoring of hematological parameters  (hematocrit, hemoglobin, erythrocytes, reticulocytes, differential WBC) |
| Endocrine system | High-circulating testosterone level | Testosterone, LH and FSH should be  monitored |
| Reproductive system | Lack of information on female reproductive and embryo-fetal toxicity | Healthy volunteers should employ 2 forms of contraception, one of which is a barrier method, to avoid pregnancy and exposure of females to PROJECT R  through male ejaculate |

ACTH: adrenocorticotropic hormone; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP; alkaline phosphatase; GGT: gamma-glutamyl transpeptidase; FIH: first in human; HDL: high density lipoprotein; LDL: low density lipoprotein; WBC: white blood cells; LH: luteinizing hormone; FSH: follicle stimulating hormone.

### Safety Margin

The human equivalent dose of the NOAEL observed in rats was calculated as 0.484 mg/kg and in monkeys was calculated as 0.322 mg/kg. The maximum recommended starting dose (MRSD) based on the monkey NOAEL, using a safety factor of 10, is 1.93 mg/day for a 60 kg human. The monkey NOAEL was based on the occurrence of cholestasis (without hepatocellular damage), which is a monitorable event in clinical trials. A dose of

1 mg/day was recommended as the starting dose in the planned first-in-human (FIH) study. At the recommended starting dose, the exposures based on the plasma unbound fraction are approximately 44 to 172-fold and 41 to 166-fold for Cmax and AUC24, respectively, lower than those at the LOAEL for dilatation of bile canaliculi in the monkey 13-week study [[Table 7](#_bookmark61)].

### Table 7 Compilation of Doses and Systemic Exposure Data of PROJECT R at PAD/NOAEL/HED and the First-in-human (FIH) Dose

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Species/ Route of**  **Administration Test category** | **Sex** | **Dose (mg/kg/day)** | **HED†**  **(mg/kg)** | **Total Plasma PROJECT R Concentration**  **[Unbound Plasma Concentration]** | | | |
| **Cmax (ng/mL)** | | **AUC24 (ng**･**h/mL)** | |
| **First dose** | **Last dose** | **First dose** | **Last dose** |
| Rat, SD;  oral, pharmacology | M | 0.56 (PAD) | 0.090 | \_ | \_ | \_ | \_ |
| Rat, SD;  oral  13-week toxicity | M | 3 (NOAEL) | 0.484 | 1115.679  [10.6] | 2860.929  [27.2] | 17014  [162] | 44229  [420] |
| F | 3 (NOAEL) | 0.484 | 1170.261  [11.1] | 2917.744  [27.7] | 19232  [183] | 56808  [540] |
| M | 10  (LOAEL） | 1.613 | 7390  [70.2] | 143203  [1360] | 7390  [70.2] | 143203  [1360] |
| F | 10 (LOAEL) | 1.613 | 11434  [108.6] | 234040  [2223] | 11434  [108.6] | 234040  [2223] |
| Monkey, Cynomolgous. oral  13-week toxicity | M | 1 (NOAEL) | 0.322 | 164.617  [3.95] | 742.918  [17.8] | 3351  [80.4] | 14328  [344] |
| F | 1 (NOAEL) | 0.322 | 255.032  [6.12] | 547.580  [13.1] | 3959  [95.0] | 9931  [238] |
| M | 3 (LOAEL） | 0.968 | 459.3  [11.0] | 1796  [43.1] | 8429  [202] | 33939  [815] |
| F | 3 (LOAEL) | 0.968 | 721.4  [17.3] | 1732  [41.6] | 11179  [268] | 28057  [673.4] |
| Human  FIH | M | 0.017 | １mg/man/day | 28.0‡  [0.25] | | 557.6‡  [4.9] | |

–: Toxicokinetic parameters not available; HED: human equivalent dose;

LOAEL: lowest-observed-adverse-effect level; MRSD: maximum recommended starting dose; NOAEL: no-observed-adverse-effect level; PAD: pharmacologically active dose.

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

‡The predicted systemic exposure in humans at a dose of 1 mg/day, based on physiologically based pharmacokinetic modeling using total drug concentrations in rats and monkeys.

Sources: Project R-TX-0009 and Project R-PH-0006 (rat) and Project R-TX-0010 (monkey)