### Safety Pharmacology

Safety pharmacology and toxicokinetic studies have been performed in guinea pigs, monkeys, and cell lines derived from humans and Chinese hamsters [[Table 3](#_bookmark28) and [Table 4](#_bookmark29)].

### In vitro Effects on hERG Current

The effects of PROJECT V on the hERG current were studied in human embryonic kidney 293 cells transfected with hERG (hERG-transfected HEK293 cells) using the whole-cell patch-clamp technique (Study Project V-PT-0001). The test concentrations of PROJECT V were 3×10−7, 3×10−6 and 3×10−5 mol/L.

PROJECT V suppressed the hERG current by 18.4% at 3 × 10-5 mol/L (9912.6 ng/mL).

### In vitro Effects on Action Potential Duration

The effects of PROJECT V on action potential parameters in isolated guinea pig papillary muscle specimens were investigated using glass microelectrode techniques (Study Project V-PH- 0002). The test substance solutions at concentrations of 3×10−7, 3×10−6 or 3×10−5 mol/L were applied. No effects on APD30, APD90, resting membrane potential (RMP), action potential amplitude (APA), or dV/dt max were observed at any concentration. The change rates in APD30-90 were 9.7% at 3×10−7 mol/L, 1.0% at 3×10−6 mol/L and 9.4% at 3×10−5 mol/L. Although the APD30-90 value at 3 ×10-7 mol/L showed 9.7% prolongation, this was judged not to be an article related change because 3 ×10-7 mol/L and even 3 × 10-6 mol/L had no effect on hERG current (hERG current and APD30-90 are known to reflect changes in the Ikr current),

no dose dependence, no reproducibility of the APD30-90 at 3 × 10-7 mol/L in an additional study, and no effects on 6 ion channels (INa, ICa,L, Iks, Ik1, Ito, IkATP) expressed in mammalian cells except for a slight effect on IkATP by 22.3% at 3 × 10-5 mol/L. (Studies Project V-PT-0002, Project V-PT-0005 and Project V-PT-0006)

### In vivo Effects on Cardiovascular and Respiratory System in Monkeys

The effects of PROJECT V on the cardiovascular and respiratory systems were studied in 4 unanesthetized male cynomolgus monkeys using a telemetry system. PROJECT V was

administered orally at single doses of 0 (0.5% methylcellulose), 100, 300 and 1000 mg/kg. Dosing was conducted with intervals of 7 days using a 4 × 4 Latin square design. At 300 and 1000 mg/kg, a slight but lasting decrease in blood pressure was observed in all 4 animals (approximately 5% to 10% decreases from the predose averages). In addition, decreases in heart rate (20-35%, 1 to 6 hours after dosing) in 2 animals at both dose levels and decreases in intra-abdominal body temperature (0.5oC to 0.7oC) in 1 animal at 300 mg/kg and 2 animals at 1000 mg/kg were observed when compared to corresponding time points in the vehicle control. These changes were small, however, and were within the range of daily variation for the vehicle control. These changes were observed between 1 and 8 hours after dosing and all the parameters describe above returned to the vehicle control level by 24 hours after dosing. The magnitudes of the decreases were similar at both dose levels. There were no test article- related changes in any ECG parameter, respiratory parameter, electrolyte, or clinical signs at either dose level. (Study Project V-PT-0003)

The NOAEL was 100 mg/kg.

Mean Cmax values at 100, 300 and 1000 mg/kg were 24215.35, 40752.47 and

54995.63 ng/mL, respectively.

### In vivo Effects on Central Nervous Systems in Rats

PROJECT V was administered as a single oral dose at dose levels of 30, 100 and 300 mg/kg to 6 male and 6 female Crl:CD(Sprague Dawley) rats per group to evaluate its effect on the CNS. (Study Project V-PT-0004)

At 30 mg/kg, PROJECT V had no effect on general physical condition or behavior.

At 100 mg/kg, prone position and a decrease in locomotor activity were observed in males and females. Incomplete eyelid opening and a low level of arousal were observed in

1 female. These changes disappeared by 24 hours after dosing.

At 300 mg/kg, prone position, a decrease in locomotor activity, incomplete eyelid opening, slight flaccidity in abdominal tone, no resistance in limb tone, a low level of arousal, staggering gait, and absence/weak grip strength were observed in males and females. Tip-toe gait in 1 male and loss of righting reflex in females were observed. Bradypnea in females, piloerection, lacrimation, disappearance of visual placing and touch response and no struggle were observed in female. On the day following dosing, 2 females became moribund. Lateral position, bradypnea, hypothermia and no stool were observed in these animals before observation of general physical condition or behavior at 24 hours after dosing. Gross

pathological findings included black focus in the glandular mucosa of the stomach in

1 female. In other animals, except for those that became moribund, the changes observed after dosing disappeared by 24 hours after dosing.

The NOAEL was 30 mg/kg.

### Table 3 Summary Results of Safety Pharmacology Studies

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study Number and**  **Type** | **Species/ Strain** | **Concentration/ Doses** | **Group Size** | **Results** | | |
| Project V-PT-0001  Effects on the potassium current in cloned HEK293 cells expressing hERG | Cell line derived from human embryonic kidney cell line | 3×10−7,  3×10−6,  3×10−5  mol/L | 5 cells | Inhibition by 18.4% at 3×10−5 mol/L |  | |
| mol/L compensated inhibition rate (%)  3×10−7 1.4 ± 6.4 |
| 3×10−6 1.0 ± 3.2 |
| 3×10−5 18.4 ± 7.4\*\* |
|  |
| Project V-PT-0002  Effects on cardiac action potential parameters in isolated papillary muscles | Hartley guinea pigs | 3×10−7,  3×10−6,  3×10−5  mol/L | 5M | Marginal prolongation by 9.7% and 9.4% in APD30-90 at  3×10−7 mol/L and 3×10−5 mol/L , respectively | | |
| mol/L APD30 APD90 APD30-90  3×10−7 -1.2 ±0.7 2.9 ± 1.8 9.7 ± 4.8\* | |  |
| 3×10−6 0.2 ± 1.4 0.8 ± 2.3 1.0 ± 6.5 | |
| 3×10−5 -2.1 ± 4.9 2.3 ± 3.7 9.4 ± 4.5\* | |
|  | |
| Project V-PT-0005  Effects on cardiac action potential parameters in isolated papillary muscles (additional study) | Hartley guinea pigs | 3×10−8,  1×10−7,  3×10−7  mol/L | 5M | No effects on APD at up to 3×10−7 mol/L | |  |
| mol/L APD30 APD90 APD30-90  3×10−8 3.2 ± 5.0 2.7 ± 3.4 1.5 ± 2.1 | |
| 1×10−7 2.6 ± 3.6 1.2 ± 1.4 -1.0 ± 3.7 | |
| 3×10−7 3.6 ± 4.5 2.3 ± 3.8 0.6 ± 3.1 | |
|  | |
| Project V-PT-0006  Effects on 6 validated human ion channels expressed in cell lines | Cell line derived from HEK, CHO, or HEK293 | 1×10−5,  3×10−5  mol/L | 3 cells  (4 cells for IKATP) | Only marginal inhibition on IKATP by 22.3%  inhibition (%) n=3 1×10−5 3×10−5  mol/L mol/L  INa Nav1.5 0.5 ± 0.9% 1.6 ± 0.9%  ICa,L Cav1.2 0.6 ± 1.5% 2.6 ± 0.4%  Ito Kv4.3/KChiP2.2 4.8 ± 0.4% 7.9 ± 1.0%  Iks hKvLQT-hminK 4.8 ± 3.3% 14.5 ± 2.6%  Ik1 hKir2.1 1.9 ± 0.5% 1.6 ± 1.6%  IKATP Kir6.2/SUR2A 7.6 ± 1.8% 22.3 ± 2.3% (n=4) | | |
| Project V-PT-0004  Effects on central nervous system | Sprague  Dawley rats (Crl:CD[SD]) | 30, 100, 300  mg/kg | 6/M, 6/F |  100 mg/kg: prone position, decrease in locomotor activity,  incomplete eyelid opening, low level of arousal.  300 mg/kg: 2 females were moribund and sacrificed on the day following dosing. Bradypnea, piloerection, hypothermia, lacrimation, slight flaccidity in abdominal tone, no resistance in limb tone, slight staggered gait, severe staggered gait, disappearance of visual placing, disappearance of touch response, weak grip strength, no grip strength, no struggle held by foreleg, loss of righting reflex (land on side), tip-toe gait (hind limbs) | | |
| Project V-PT-0003  Effects on cardiovascular and respiratory systems | Cynomolgus monkeys | 100, 300, 1000  mg/kg | 4/M |  300 mg/kg: decreases in blood pressure, heart rate and intra-abdominal body temperature. These changes were observed between 1 and 8 hours after dosing and all parameters described above returned to the vehicle control level by 24 hours after dosing. | | |

APD: action potential duration; CHO: Chinese hamster ovary; HEK: human embryonic kidney; hERG: human Ether à-go-go ion channel

Significant difference at \* P < 0.05, \*\*P < 0.01 (Dunnett test)

Source: Studies Project V-PT-0001, Project V-PT-0002, Project V-PT-0003, Project V-PT-0004, Project V-PT-0005 and Project V-PT- 0006

### Table 4 Toxicokinetic Results of the Study of Cardiovascular and Respiratory System in Monkeys

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Study Number and Type** | **No. of Animals** | **Sex** | **Dose (mg/kg)** | **Unchanged drug** | | | |
| **Cmax (ng/mL)** | | **AUC24 (ng·h/mL)** | |
| **First**  **Dose** | **Last**  **Dose** | **First**  **Dose** | **Last**  **Dose** |
| Project V-PT-0003  Cynomolgus monkeys: Single dose Cardiovascular  and respiratory | 4 | M | 100 | 24215.35 | NA  NA | 206618 | NA  NA |
| 4 | M | 300 | 40752.47 | NA  NA | 508359 | NA  NA |
| 4 | M | 1000 | 54995.63 | NA  NA | 782872 | NA  NA |

NA: not applicable

Source: Study Project V-PT-0003

Cmax and AUC24 of PROJECT V increased with the dose.

## Toxicology

### Single-dose Toxicity

Single dose toxicity and toxicokinetic studies have been performed in rats and monkeys [[Table 5](#_bookmark50) and [Table 6](#_bookmark51), respectively].

### Rats

PROJECT V was orally administered once at dose levels of 300 and 1000 mg/kg to 5 male and 5 female Crl:CD(Sprague Dawley) rats per group and observed for 14 days. At 1000 mg/kg, 2 of 5 males and 5 of 5 females were moribund and were sacrificed on the day after dosing and 8 hours after dosing, respectively. The approximate lethal dose level for male and female rats was 1000 mg/kg. The major symptoms were a decrease in spontaneous activity at 300

and 1000 mg/kg, and lateral position, bradypnea, hypothermia and lacrimation at 1000 mg/kg (Study Project V-TX-0004).

### Monkeys

PROJECT V was orally administered once at dose levels of 1000 and 2000 mg/kg to 1 male and 1 female cynomolgus monkey per group and they were observed for 14 days. There were no deaths or moribund sacrifices on the day of dosing (day 0) or during the observation period until 14 days after dosing (day 14). There were no test article-related effects in body weight, ECGs, gross pathological examination, organ weight or histopathological examination. The major symptoms at 2000 mg/kg were vomitus, high reticulocyte count, low red blood cell count, low hemoglobin concentration, low hematocrit and high neutrophil count (Study Project V- TX-0005).

Cmax and AUC24 values were comparable between the 100 mg/kg and 2000 mg/kg doses. The approximate lethal dose was greater than 2000 mg/kg.

### Table 5 Summary Results of Single Dose Toxicity Studies

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Study Number and**  **Type** | **Species/strain** | **Group Size** | **Doses (mg/kg)** | **Results** |
| Project V-TX-0004  Single dose in rats | Rat, Sprague Dawley | 5/M, 5/F | 300, 1000 | ALD: 1000 mg/kg  300 mg/kg: decrease in spontaneous activity, decrease in stool volume  1000 mg/kg: 2M and 5F were moribund sacrificed. Lateral position, bradypnea, hypothermia, lacrimation, reddish tear, no stool, and/or decrease in stool volume, black foci mucosa in glandular stomach,  erosion in glandular stomach, and decrease in body weight. |
| Project V-TX-0005  Single dose in monkeys | Monkey, cynomolgus | 1/M,1/F | 1000, 2000 | ALD: >2000 mg/kg  1000 mg/kg: whitish stool  1000 mg/kg: low white blood cell and lymphocyte counts, high monocyte count, high triglycerides.  2000 mg/kg: vomitus, high reticulocyte count, low red blood cell count, high neutrophil count. |

ALD: approximate lethal dose

Source: Studies Project V-TX-0004 and Project V-TX-0005

### Table 6 Toxicokinetic Results of the Single Dose Toxicity Study in Monkeys

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Study Number and Type** | **No. of Animals** | **Sex** | **Dose (mg/kg)** | **Unchanged Drug** | | | |
| **Cmax (ng/mL)** | | **AUC24 (ng·h/mL)** | |
| **First**  **Dose** | **Last**  **Dose** | **First**  **Dose** | **Last**  **Dose** |
| Project V-TX-0005  Cynomolgus monkeys: Single oral dose  toxicity study | 1  1 | M  F | 1000 | 106216.79  91374.48 | NA  NA | 1419717  1620633 | NA  NA |
| 1  1 | M F | 2000 | 83157.13  106169.62 | NA NA | 1406158  1522030 | NA NA |

NA: not applicable.

Source: Study Project V-TX-0005

Cmax and AUC24 values were comparable between the 1000 and 2000 mg/kg doses.

### Repeat-dose Toxicity

Repeat dose toxicity and toxicokinetic studies have been performed in rats and monkeys [[Table 7](#_bookmark55) and [Table 8](#_bookmark56), respectively].

### Rats

An exploratory 7-day oral dose range-finding study in rats is described in End-of-Text Table 3.5 (Study Project V-TX-0006).

A 13-week oral dose toxicity study in rats with 4-week recovery period was conducted. PROJECT V was orally administered once daily for 13 weeks at dose levels of 0 (vehicle

control), 1, 3, 10 and 100 mg/kg to 10 male and 10 female Crl:CD (Sprague Dawley) rats per group (Study Project V-TX-0008).

There were no test-article related changes on food consumption, clinical observation, ophthalmology, urinalysis, hematology, gross pathology or estradiol concentration at any dose level.

At 1, 3 and 10 mg/kg, no test article-related changes were noted.

At 100 mg/kg, high adrenal and liver weights, and hypertrophy in the zona fasciculata cells and increased lipids in the zona fasciculata of the adrenal were noted in males and females, disappearance of periportal hepatocellular microvacuolation and low testosterone concentration were noted in males, and transient suppression of body weight gain up to week 2 of dosing and low triglycerides were noted in females.

At the end of the recovery period, a tendency toward high adrenal weight was noted in males and females, and hypertrophy in the zona fasciculata cells in the adrenal was noted in

1 female at 100 mg/kg. The overall degree and incidence of the changes diminished towards the end of the dosing period and they were therefore judged to be indications of a tendency toward recovery. For a list of changes noted, see End-of-Text Table 3.5.

The NOAEL was 10 mg/kg per day for males and females because effects on the adrenal and liver were noted in both sexes at 100 mg/kg. The changes noted during the dosing period either disappeared or tended to recover during the 4-week recovery period.

### Monkeys

An exploratory 7-day oral dose range-finding study in monkeys is described in End-of-Text Table 3.5 (Study Project V-TX-0007).

A 13-week oral dose toxicity study in monkeys with 4-week recovery period was conducted. PROJECT V was orally administered once daily for 13 weeks at dose levels of 0 (vehicle control), 10, 30, 100 or 1000 mg/kg per day to male and female cynomolgus monkeys (Study Project V-TX-0009).

Overall:

* + - * + No deaths occurred and there were no animals that were sacrificed due to moribund condition during the 13-week dosing period or the 4-week recovery period.
        + In clinical observation, abnormal stool color (black, negative occult blood reaction) was observed in males and females in the 100 and 1000 mg/kg per day groups and vomitus in females in the 1000 mg/kg per day group.
        + In blood chemistry, high triglycerides were recorded in females in the 1000 mg/kg per day group in week 13 of dosing, and high phospholipids in males and females in the 100 mg/kg group and females in the 1000 mg/kg per day group in weeks 7 and 13 or in week 13 of dosing.
        + There were no test article-related effects in body weight, food consumption, ophthalmological examination, electrocardiogram, urinalysis, hematological

examination, necropsy, organ weight, histopathological examination or hormone measurement.

* + - * + The clinical signs and the changes in blood chemistry examination that were observed during the dosing period were no longer observed during the 4-week recovery period, suggesting that the changes were reversible.

The NOAEL was 30 mg/kg per day for both males and females.

### Table 7 Summary of Repeated Dose Toxicity Studies

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Study Number and**  **Type** | **Species/strain** | **Group Size** | **Doses (mg/kg/day)** | **Results** |
| Project V-TX-0008 | Rat, Sprague | 10/M, | 0, 1, 3, 10, | NOAEL: 10 mg/kg/day. |
| 13-week dose in | Dawley | 10/F | 100 | 100 mg/kg/day: high adrenal and liver |
| rats |  |  |  | weights, and hypertrophy in the zona |
|  |  |  |  | fasciculata cells and increased lipids in |
|  |  |  |  | the zona fasciculata of the adrenal were |
|  |  |  |  | noted in males and females, |
|  |  |  |  | disappearance of periportal hepatocellular |
|  |  |  |  | microvacuolation and low testosterone |
|  |  |  |  | concentration were noted in males, and |
|  |  |  |  | transient suppression of body weight gain |
|  |  |  |  | in the initial stage of the dosing period |
|  |  |  |  | and low triglycerides were noted in |
|  |  |  |  | females. |
| Project V-TX-0009 | Monkey, | 3/M, 3/F | 0, 10, 30, | NOAEL: 30 mg/kg/day |
| 13-week dose in monkeys | cynomolgus | (6/M, 6/F  for 1000 mg/kg) | 100, 1000 | 100 mg/kg/day: abnormal stool color (black, negative occult blood reaction),  high values in phospholipids. |
|  |  |  |  | 1000 mg/kg/day: vomitus in females, |
|  |  |  |  | high values in triglycerides in females |
|  |  |  |  | No effects on circulating testosterone, |
|  |  |  |  | estradiol, ACTH or cortisol levels. |

ACTH: adrenocorticotropic hormone; NOAEL: no observable adverse effect level Source: Studies Project V-TX-0008 and Project V-TX-0009

### Table 8 Toxicokinetic Results of the Repeated Dose Toxicity Studies

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Study Number and Type** | **No. of Animals** | **Sex** | **Dose (mg/kg)** | **Unchanged drug** | | | |
| **Cmax (ng/mL)** | | **AUC24 (ng·h/mL)** | |
| **First**  **Dose** | **Last**  **Dose** | **First**  **Dose** | **Last**  **Dose** |
| Project V-TX-  0008  Sprague Dawley rats: 13-week oral dose toxicity study (definitive) | 6  6 | M  F | 1 | 20.58  42.56 | 67.15  122.50 | 32  83 | 95  267 |
| 6  6 | M  F | 3 | 193.42  583.63 | 151.99  768.96 | 269  877 | 450  2099 |
| 6  6 | M  F | 10 | 1643.49  3053.84 | 2225.54  3375.47 | 4728  8789 | 9307  14326 |
| 6  6 | M  F | 100 | 22127.13  32049.06 | 17822.66  26665.45 | 165367  466207 | 207851  380404 |
| Project V-TX-  0009  Cynomolgus monkeys:  13-week oral dose toxicity study (definitive) | 3  3 | M  F | 10 | 4733.75  5202.11 | 6057.61  5087.00 | 18796  24645 | 25200  26898 |
| 3  3 | M  F | 30 | 12573.31  9861.63 | 15598.47  13601.22 | 85122  71364 | 107459  90856 |
| 3  3 | M  F | 100 | 28688.74  39138.51 | 42842.83  30697.16 | 254543  338901 | 336699  306295 |
| 6  6 | M  F | 1000 | 61888.40  60263.57 | 65985.23  74328.83 | 733805  799919 | 762377  981571 |

Source: Studies Project V-TX-0008 and Project V-TX-0009

In rats, Cmax increased with the dose and AUC24 increased more than dose-proportionally. In monkeys, both Cmax and AUC24 increased with the dose and were comparable on day 1 and week 7 and week 13 of administration.

### Genotoxicity

A standard panel of genotoxicity and toxicokinetic studies have been performed [[Table 9](#_bookmark61) and [Table 10](#_bookmark62), respectively].

### In vitro reverse mutation

Mutagenicity was evaluated by exposing 4 histidine auxotrophs of *Salmonella typhimurium* and a tryptophan auxotroph of *E. coli* (WP2 uvrA) to PROJECT V with or without metabolic activation (Study Project V-TX-0001).

At doses of 39.1 to 5000 mcg/plate (as free form), PROJECT V did not double the mean number of revertant colonies compared to the mean number in the vehicle control group for any bacterial strain tested, regardless of the presence or absence of metabolic activation.

These results indicate that PROJECT V has no mutagenic potential.

### In vitro chromosome aberration

PROJECT V was evaluated for potential to induce chromosomal aberrations under 3 treatment conditions: 6-hour treatment in the presence of S9 Mix (with metabolic activation) and 6- and 24-hour treatments in the absence of S9 Mix (without metabolic activation), using a CHL fibroblast cell line. In the 6-hour treatment groups with and without metabolic activation, chromosomal aberrations were analyzed over a concentration range of 100 to 200 mcg/mL.

In the 24-hour treatment group without metabolic activation, chromosomal aberrations were analyzed over a concentration range of 50 to 100 mcg/mL (Study Project V-TX-0002).

PROJECT V did not significantly increase the number of chromosomally aberrant cells compared to the vehicle control.

The results indicate that PROJECT V has no potential to induce chromosomal aberrations

### In vivo micronucleus test in rats

A micronucleus study was conducted in Sprague-Dawley strain male and female rats (Crl:CD[Sprague Dawley]) to examine whether PROJECT V has a potential to induce micronucleus formation in rats in vivo (Study Project V-TX-0003).

PROJECT V was orally administered twice, with an interval of approximately 24 hours, via oral gavage to groups of rats, each consisting of 5 males and 5 females at 8 weeks of age, at dose levels of 0 (vehicle control group, 0.5 % methylcellulose solution), 125, 250 and 500 mg/kg per day. Bone marrow specimens were prepared approximately 24 hours after the second dosing.

There were no statistically significant increases in the incidence of micronucleated polychromatic erythrocytes in any test article dosing group as compared with that in the vehicle control group. There were no statistically significant decreases in the incidence of polychromatic erythrocytes among the 125, 250 or 500 mg/kg per day groups compared to the vehicle control group.

In conclusion, PROJECT V has no potential to induce chromosomal aberrations in bone marrow cells of Crl:CD(Sprague Dawley) strain SPF male and female rats under the conditions of this study.

### Table 9 Summary Results of Genotoxicity Studies

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Study Number and**  **Type** | **Species/strain** | **Group Size** | **Dose** | **Results** |
| Project V-TX-0001  Reverse mutation | *S. typhimurium,* and  *E.coli* | in vitro | 39.1-5000  mcg/plate | Negative |
| Project V-TX-0002  Chromosome aberration | CHL/IU cell | in vitro | 50-275 mcg/mL | Negative |
| Project V-TX-0003  Micronucleus | Rat, Sprague Dawley | 5/M, 5/F | 0, 125, 250, 500  mg/kg/day | Negative |

CHL/IU: Chinese hamster lung

Source: Studies Project V-TX-0001, Project V-TX-0002 and Project V-TX-0003

### Table 10 Toxicokinetic Results of the Micronucleus Study in Rats

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Study Number and Type** | **Number of Animals** | **Sex** | **Dose (mg/kg)** | **Unchanged drug** | | | |
| **Cmax (ng/mL)** | | **AUC24 (ng·h/mL)** | |
| **First**  **Dose** | **Last Dose** | **First**  **Dose** | **Last**  **Dose** |
| Project V-TX-0003  Sprague Dawley rats:  Oral dose, micronucleus test | 3  3 | M F | 500 | NA NA | 65470.37  126866.84 | NA NA | 1246517  2566690 |

NA: not applicable.

Source: Study Project V-TX-0003

### Carcinogenicity

No carcinogenicity studies have been conducted with PROJECT V. There are no findings suggesting carcinogenic potential, such as genotoxicity or hyperplasia findings, in the toxicity studies of PROJECT V conducted to date.

### Reproductive and Developmental Toxicity

A single reproductive study has been performed in rats [[Table 11](#_bookmark66)].

### Effects on Male Fertility

PROJECT V at 0, 10, 30, and 100 mg/kg per day was administered via oral gavage to males daily for a total of 42 to 45 days beginning 28 days before the start of pairing and throughout the mating period. Females were untreated and necropsied on day 13 of gestation. A group treated with 0.5 % methylcellulose solution was employed as the control group. Each test group consisted of 20 animals of each sex (Study Project V-TX-0014).

No dead males or moribund males occurred in any group. No adverse effects of the test article were noted in the general signs, body weight, body weight gain, food consumption, necropsy findings, organ weights or sperm analysis in any PROJECT V group.

No adverse effects of the test article were noted in the number of days until copulation after the start of pairing, copulation index, fertility index, number of corpora lutea, number of implantation sites, implantation rate, number of pre-implantation losses, pre-implantation loss rate, number of embryonic deaths, index of embryonic deaths, incidence of females having embryonic death or number of live embryos in any PROJECT V group.

The NOAEL was 100 mg/kg per day in males for general toxicity and reproductive functions

### Table 11 Summary Result of Reproduction Toxicity Study

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Study Number**  **and Type** | **Species/strain** | **Group**  **Size** | **Doses**  **(mg/kg/day)** | **Results** |
| Project V-TX-0014  ICH1 male fertility | Rat, Sprague  Dawley | 20/M,  20/F | 0, 10, 30, 100 | NOAEL: 100 mg/kg/day for male general  toxicity and male reproductive function |

NOAEL: no observable adverse effect level. Source: Study Project V-TX-0014

### Phototoxicity Studies

Phototoxicity and toxicokinetic studies have been performed in rats and mice [[Table 12](#_bookmark70) and [Table 13](#_bookmark71), respectively].

### Phototoxicity Study in Balb/c 3T3 Cells

A phototoxicity study of PROJECT V was performed with cultured mammalian cells (Balb/c 3T3 Cells). A main test was performed in the presence and absence of UV-A irradiation, and two 96-well plates were used for both the test article and positive control groups (total

4 plates). The dose levels of PROJECT V were set at 40.4, 48.5, 58.1, 69.8, 83.7, 100, 121 and

145 mcg/mL in the presence of irradiation, and at 83.7, 100, 121, 145, 174, 208, 250 and 300 mcg/mL in the absence of irradiation (Study Project V-TX-0030).

The IC50 of the test article groups was determined to be 69.494 mcg/mL in the presence of irradiation and 215.819 mcg/mL in the absence of irradiation. The photo irritation factor (PIF) and mean photo effect were calculated to be 3.111 and 0.325, respectively. The PIF result, from which it was possible to determine the IC50 for both the irradiated and nonirradiated plates, was evaluated. The judgment criterion was probable phototoxicity:

2 ≤ PIF < 5. Accordingly, it was judged probable that PROJECT V has the potential to induce phototoxicity.

It was concluded from these results that, under the conditions of this study, PROJECT V was predicted to be probably phototoxic to humans.

### Single Oral Dose Phototoxicity Study in Hairless Mice

A single dosing of the vehicle or 10 or 100 mg/kg PROJECT V to female Crl:SKH1-*hr* hairless albino mice followed approximately 30 (10 mg/kg PROJECT V) or 60 (vehicle and 100 mg/kg PROJECT V) minutes later by a single exposure to solar-simulated UV radiation resulted in no skin reactions indicative of phototoxicity. A single dosing of 600 mg/kg PROJECT V followed by approximately 30 minutes later to a single exposure to solar-simulated UV radiation resulted in skin reactions indicative of a mild phototoxic reaction (erythema, edema, flaking). There were no adverse clinical observations or body weight effects in the phototoxicity phase. All mice in all phases survived to scheduled sacrifice. A single dosing of comparator article 8-methoxypsoralen followed approximately 60 minutes after dosing by a single exposure to solar-simulated ultraviolet radiation resulted in skin reactions indicative of phototoxicity with greater incidence and severity than that of PROJECT V at 600 mg/kg and validated the assay (Study Project V-TX-0023).

### Table 12 Summary Results of Phototoxicity Studies

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Study Number and**  **Type** | **Species/strain** | **Group Size** | **Doses (mg/kg)** | **Results** |
| Project V-TX-0030  in vitro 3T3 NRU | Balb/c 3T3 cell | in vitro | 40.4-300  mcg/mL | Positive (PIF: 3.111) Categorized in probable phototoxicity:  2 ≤ PIF < 5. |
| Project V-TX-0023 | Mouse, | 10/F | 0, 10, 100, | Positive |
| Single dose in | SKH1-*hr* |  | 600 | 600 mg/kg: erythema (7/10), edema |
| mice |  |  |  | (1/10) and flaking (1/10) |
|  |  |  |  | 100 mg/kg: No skin reaction |

PIF: photo irradiation factor.

Source: Studies Project V-TX-0030 and Project V-TX-0023

Cmax and AUC24 increased with the dose.

### Table 13 Toxicokinetic Results of Single Oral Dose Phototoxicity Study in Hairless Mice

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Study Number and Type** | **Number of Animals†** | **Sex** | **Dose (mg/kg)** | **Unchanged drug** | |
| **Cmax (ng/mL)** | **AUC24 (ng·h/mL)** |
| Project V-TX-0023 SKH1-*hr*  mouse: Phototoxicity  study | 3 | F | 10 | 1274.73 | 1167 |
| 3 | F | 100 | 17204.14 | 59349 |
| 3 | F | 600 | 44808.69 | 393189 |

†Number of mice per time point. Source: Study Project V-TX-0023

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

The identified target organs in the non-clinical package were the adrenals, liver, lipid metabolism and gastrointestinal (GI) tract. In addition, the most serious findings were the CNS effects which were noted in rats in the safety pharmacology study and in the 7-day exploratory rat toxicity study (in which animals became moribund at 300 mg/kg). Lastly, PROJECT V was classified as possibly phototoxic. The interpretation and consequences of the various target organ toxicities are described below:

Major pharmacological effect-related findings

As for effects on circulating testosterone level, 17-beta-HSD5 converts AD, synthesized in the adrenal gland, to T. PROJECT V inhibits T synthesis in the prostate by inhibiting prostate

17-beta-HSD5. Since PROJECT V does not affect gonadal T synthesis, plasma testosterone level cannot be reduced to castration level by PROJECT V. Actually, the 13-week oral dose toxicity study in monkeys demonstrated no effects on circulating T level even at 1000 mg/kg and no test article-related findings were observed in the male reproductive organs.

In the 13-week oral dose toxicity study in rats, slightly low plasma T level with no histopathological changes in reproductive organs was recorded at 100 mg/kg. However, in the male fertility study, oral dosing of 100 mg/kg for 42 to 45 days did not affect sperm analysis and male fertility. Since rat 17-beta-HSD5 was not inhibited by PROJECT V even at 10-5 mol/L, low T levels may be a secondary change induced by deteriorated general

condition or may indicate that an enzyme more upstream in androgen synthesis is inhibited in rats.

CNS

Prone position, decreased locomotor activity, incomplete eyelid opening, and low level of arousal occurred in rats at 100 mg/kg in the safety CNS pharmacology study. At higher doses, various other effects on neuromuscular, lower motor neuron, and spinocerebellar functions were observed. No CNS effects were recorded at 100 mg/kg per day in the 13-week study in rats throughout the dosing period, which indicates that repeated dosing does not increase the effects on the CNS. No CNS effects were observed in any of the monkey studies, however the Cmax,unbound value in monkeys (1495.88 ng/mL at 1000 mg/kg) was lower than that which was associated with abnormal CNS findings in rats (5005.3 ng/mL).

Pharmacokinetic studies in rats suggest brain penetration of PROJECT V. Potential CNS related adverse events in humans at high dose cannot be excluded. Cmax values in monkeys did not reach the Cmax values seen in rats.

GI tract

GI events in the repeated toxicity studies included histopathological findings in stomach and duodenum and basophilic changes in mucosal epithelial cells in rats and vomiting and black stool color (positive occult blood in the 1-week exploratory study, negative occult blood in the 13-week study) in monkeys. Although there seems to be an appropriate safety margin, GI-tract related adverse events, such as nausea, vomiting and abnormal stool should be monitored in the clinical studies.

Adrenal effects

PROJECT V inhibits the monkey 17-beta-HSD5 homologue and 17-beta-HSD5 localizes in the adrenals. In the 13-week toxicity study in monkeys, there were no changes in the adrenals in terms of organ weight, histopathological examination, and adrenal hormone levels of cortisol and ACTH in all PROJECT V dosing groups, indicating that PROJECT V did not affect the function of the adrenals.

The adrenal effects in rats are currently not fully understood [Yap et al, 2008]. Increased adrenal weight and hypertrophy in the zona fasciculata cells and increased lipids in the zona fasciculata of the adrenals, which are considered to be indicative of adrenal hyperfunction, were noted in the repeated dose studies in rats. These findings are considered to be unrelated to 17-beta-HSD5 inhibition due to the lack of pharmacological activity of PROJECT V in rats.

Although PROJECT V has no adverse effect on the adrenals in monkeys, monitoring of markers of adrenal function, such as ACTH and cortisol, are recommended during the early phase clinical trials.

Liver

Increased liver weights and histopathological changes, which suggest slight enzyme induction, were observed in rats; however, the nonclinical evaluations did not reveal signs of hepatotoxicity. The standard panel of liver transaminases is recommended for monitoring potential adverse liver effects in clinical trials.

Cardiovascular system

PROJECT V slightly suppressed hERG current in vitro and slightly prolonged APD30-90 in guinea-pig papillary muscles both at 3 × 10-5 mole/L. An in vitro study on 6 individual ion channels showed a marginal inhibition of IKATP. ECGs were monitored in all 3 oral dose toxicity studies in monkeys but no abnormal variations were observed at the high dose level in any of these studies. PROJECT V did, however, induce statistically significant but biologically irrelevant (nearly all values remained within the predose variation of the individual animals) decreases in heart rate and blood pressure at ≥300 mg/kg compared to the control group.

The intended patient population consists mainly of elderly men with high incidence of prior cardiovascular problems and frequent use of concomitant medications that carry potential cardiovascular risks. While the risk for cardiovascular events in humans as a result of PROJECT V administration is small, close ECG monitoring is recommended in phase 1 and phase 2 clinical trials.

Phototoxicity

PROJECT V absorbs light in the UV-B region of the electromagnetic spectrum and shows minor accumulation in rat skin and eyeball. The in vitro 3T3 fibroblast neutral red uptake (3T3- NRU) assay indicated a probable phototoxicity and, in addition, the in vivo test in hairless mice showed a phototoxic response at the highest dose level (600 mg/kg).

Due to this phototoxicity potential, appropriate protective measures should be taken for patients. Men participating in the PROJECT V clinical trials should be asked to minimize the exposure of the skin to sunlight or use sunscreen and sunglasses when outdoors.

The plasma exposure and Cmax levels at which findings were observed in the safety pharmacology and toxicity studies surpass the expected human plasma levels that were derived from the exposure at the potential pharmacologically active dose in mice [[Table 14](#_bookmark73)]. The anticipated Cmax and AUCinf in humans were calculated using an allometric two- compartment model that described both the rat and monkey PK data. Using this model, safety margins at a human dose of 30 mg/day were derived. Minimal safety margins for Cmax of 48 towards the lowest rat NOAEL and 46 towards the lowest monkey NOAEL were obtained.

For AUCinf, the safety margins were 40 and 61 for the lowest NOAELs in rat and monkey

non-clinical safety studies. All safety margins were calculated using the plasma unbound fractions.

### Table 14 Compilation of Systemic Exposure Data of PROJECT V at No Observed Adverse Effect Level and Lowest Observable Adverse Effect Level

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study Number** | **Species/ Study Duration** | **Dose (mg/kg)** | **Sex (M/F)** | **Cmax (ng/mL)** | | **AUC0-24**  **(ng·h/mL)** | | **Remarks** |
| **First**  **Dose** | **Last**  **Dose** | **First**  **Dose** | **Last**  **Dose** |
| Project V- PH-5003 | Mouse, single, po | 3 | M | 771.8 | NA | 355.5 | NA | Pharmacologically effective dose † |
| Project V- TX-0008 | Rat, 13-week,  po | 10  (NOAEL) | M | 1643.49 | 2225.54 | 4728 | 9307 | NOAEL based on changes in adrenal, liver, and blood chemistry‡ |
| F | 3053.84 | 3375.47 | 8789 | 14326 |
| 100  (LOAEL) | M | 22127.13 | 17822.66 | 165367 | 207851 |
| F | 32049.06 | 26665.45 | 466207 | 380404 |
| Project V- TX-0009 | Monkey, 13-week, po | 30  (NOAEL) | M | 12573.31 | 15598.47 | 85122 | 107459 | NOAEL based on changes in stool color, and blood chemistry |
| F | 9861.63 | 13601.22 | 71364 | 90856 |
| 100  (LOAEL) | M | 28688.74 | 42842.83 | 254543 | 336699 |
| F | 39138.51 | 30697.16 | 338901 | 306295 |
| Project V- PT-0003 | Monkey Cardio- vascular study single, po | 100 (NOAEL) | M | 24215.35 | NA | 206618 | NA | NOAEL based on decreases in heart rate, blood pressure and body temperature |
| 300  (LOAEL) | M | 40752.47 | NA | 508359 | NA |
| Project V- TX-0023 | Mouse Photo- toxicity  single, po | 100  (NOAEL) | F | 17204.14 | NA | 59349 | NA | NOAEL based on skin reaction |
| 600  (LOAEL) | F | 44808.69 | NA | 393189 | NA |

CNS: central nervous system; CV: cardiovascular; LOAEL: lowest observable adverse effect level; NA: not applicable; NOAEL: no observable adverse effect level.

† Inhibition of intratumoral testosterone production in prostate cancer xenograft on nude mice at 3 mg/kg

‡ CNS study in rats (Project V-PT-0004), prone position, decreased locomotor activity, incomplete eyelid opening, and a low level of arousal were observed at 100 mg/kg.

Source: Studies Project V-PH-5003, Project V-TX-0008, Project V-TX-0009, Project V-PT-0003 and Project V-TX-0023

#### List of References

Yap TA, Carden CP, Attard G, de Bono JS. Targeting CYP17: established and novel approaches in prostate cancer. Curr Opin Pharmacol. 2008:8;449-57.