### Safety Pharmacology

A total of 4 safety pharmacology studies were performed in Japan, an Organisation for Economic Cooperation and Development (OECD) country, in accordance with Good Laboratory Practice (GLP) and guidelines of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (Studies Project B-PT-0001, Project B-PT-0002, Project B-PT-0003 and Project B-PT-0004).

### Effects on CNS in Rats

PROJECT B was orally administered once to 6 male Sprague-Dawley rats at dose levels of 10, 30 and 100 mg/kg (Study Project B-PT-0001). Observations of general activity and behavior were performed using the modified Irwin’s method. PROJECT B did not affect the general activity or behavior at doses of ≤ 100 mg/kg.

### Effects on hERG Current

The effects of PROJECT B on hERG currents were studied in hERG transfected HEK293 cells by the whole-cell patch-clamp method (Study Project B-PT-0002). The compensated suppression rates of PROJECT B at 3, 10 and 30 μmol/L for tail peak currents were 9.78%, 11.00% and 25.78%, respectively. PROJECT B showed a statistically significant and concentration dependent inhibition of hERG currents at all concentrations in comparison with the negative control. Since the suppression rate did not reach 50% inhibition even at the highest concentration tested, an IC50 value could not be established and was estimated to be

> 30 μmol/L (13904 ng/mL).

### Cardiovascular System in Rats

In order to investigate the effects of PROJECT B on the cardiovascular system, PROJECT B was orally administered once to male Sprague-Dawley rats at dose levels of 10, 30 and 100 mg/kg under unanesthetized conditions (Study Project B-PT-0003). Using 3 to 4 catheterized animals/group, clinical signs were observed and blood pressure and heart rate were measured at predosing and 0.5, 1, 2, 4, 8 and 24 h postdosing. The heart was removed after measurements at 24 h postdose and examined histopathologically. In addition, using satellite animals, blood samples were collected for toxicokinetic analysis and cardiac troponin assays at predosing and 0.5, 1, 2, 4, 8 and 24 h postdosing from 3 animals/group. Similarly, blood samples were also collected for creatine kinase and LDH assays at predosing and 2, 4 and

24 h postdosing from the other 3 animals/group.

At doses of 10 and 30 mg/kg, there were no test article-related changes in any examinations. At doses of 100 mg/kg, a statistically significant increase in heart rate was noted at 0.5 and

1 h postdosing (maximum mean change in heart rate from predosing: 51 beats/min [13.6%]), though the changes were within the physiological range. No other test article-related changes were noted in blood pressure, assays of cardiac troponin, creatine kinase and LDH and histopathology.

### Cardiovascular and Respiratory Systems in Dogs

In order to investigate the effects on the cardiovascular and respiratory systems, a single oral dose of PROJECT B was orally administered to 4 male beagle dogs implanted with transmitters of a telemetry system at dose levels of 3, 10, 30 and 100 mg/kg under unanesthetized conditions (Study Project B-PT-0004). All animals received 5 doses in total at an interval of 7 or 14 days in a dose escalation manner under fasting conditions.

At doses of 3 mg/kg, no test article-related change was noted.

At doses of 10 mg/kg, vomiting and an increase in the heart rate 0.5 h after administration were noted in 1 animal for each finding (individual variation or percent change in heart rate from predosing: 32 beats/min or 36%).

At doses of 30 mg/kg, vomiting in 1 animal, decrease in blood pressure between 0.5 and 2 h after administration (maximum individual variation or percent change of mean blood pressure from predosing: −17 mmHg or −18%), and increase in the heart rate in 1 animal between 0.5 and 1 h after administration were noted (maximum individual variation or percent change in heart rate from predosing: 65 beats/min or 75%).

At doses of 100 mg/kg, vomiting in all animals between 14 min and 2 h 25 min after administration, loose stool in 1 animal 24 h after administration, decrease in blood pressure (maximum individual variation or percent change of mean blood pressure from predosing:

−24 mmHg or −26%), increase in heart rate (maximum individual variation or percent change in heart rate from predosing: 90 beats/min or 142%), and prolonged QTc interval (QT interval corrected for heart rate by Fridericia formula [QTcF]) between 0.5 and 4 h after administration (maximum mean variation or percent change of QTc interval from predosing: 25 msec or 11%), and decrease in the blood concentration of ionized calcium between 1 and 6 h after administration were noted (maximum mean decrease from predosing: 0.06 mmol/L).

## Toxicology

A total of 10 toxicity studies were conducted (5 in rats, 2 in dogs and 3 in vitro). All pivotal studies were performed in Japan, an OECD country, in accordance with GLP and ICH guidelines. All dose levels and concentrations are expressed as PROJECT B free form. An overview of PROJECT B toxicology studies can be found in [End of-Text Table 3.1].

### Single-dose Toxicity

No single-dose toxicity studies have been performed with PROJECT B; however, there were no findings after a single dose of PROJECT B ≤ 300 mg/kg in the repeat-dose toxicity study in rats (Study Project B-TX-0001). In the repeat-dose toxicity studies in dogs, vomiting and soft stool were noted after a single dose of ≥ 10 mg/kg and cardiac troponin I was increased at a dose of 300 mg/kg (Study Project B-TX-0002).

### Repeat-dose Toxicity

Three preliminary 1-week oral toxicity studies in rats (2 studies) and dogs (1 study),

1 preliminary 4-week oral toxicity study in rats and two 4-week definitive oral toxicity studies in rats and dogs were conducted. Tabulated summaries of the 2 pivotal 4-week toxicity studies can be found in [End-of-Text Table 3.3].

### Preliminary 1-Week Repeat-dose Oral Toxicity in Rats

PROJECT B was orally administered once daily for 1 week at dose levels of 0, 10, 30, 100 and 300 mg/kg to 5 male and 5 female Sprague-Dawley rats (Study Project B-TX-0008). No test article-related changes were noted at doses of ≤ 30 mg/kg. At doses of ≥ 100 mg/kg per day, myocardial necrosis was observed. At a dose level of 300 mg/kg per day, effects on the male reproductive organs (seminiferous epithelial degeneration and necrosis in the testis, luminal cell debris in the epididymis, prostatitis) and other slight changes (a decrease in eosinophils, increases in blood urea nitrogen and chloride) were noted. The NOAEL was 30 mg/kg per day [End-of-Text Table 3.2].

### 4-Week Repeat-dose Oral Toxicity in Rats

PROJECT B was orally administered once daily for 4 weeks at dose levels of 0, 10, 30, 100 and 300 mg/kg per day to 10 male and 10 female Sprague-Dawley rats (Study Project B-TX-0001). To evaluate reversibility of the changes, an additional 5 animals/sex per group similarly received the vehicle (control) or PROJECT B at a dose level of 300 mg/kg and were subsequently assigned to a 4-week recovery period. No test article-related changes were noted at doses of ≤ 300 mg/kg. The NOAEL was 300 mg/kg per day [End-of-Text

Table 3.3.1].

### Preliminary 1-Week Repeat-dose Oral Toxicity in Dogs

PROJECT B was orally administered to 1 male and 1 female beagle dogs per group at doses of 1, 10 and 300 mg/kg once daily for 1 week to evaluate toxic changes (Study Project B-TX-0010). In the 1 and 10 mg/kg dose groups, no test article-related changes were observed. In the

300 mg/kg dose group, vomiting, salivation, watery feces, and increased heart rate were observed in the male and female, and soft feces, increased fibrinogen, white blood cell count, neutrophil count, and monocyte count, decreased red blood cell count, hemoglobin, hematocrit, and albumin, shortening of the RR, QT and QTc intervals (QT interval corrected for heart rate by Matsunaga correction [QTcM]), multiple dark red foci in the auricle and left ventricle, arteritis with medial and adventitial hemorrhage in the heart, myocardial degeneration and necrosis in the right atrium and right ventricle, and endocarditis in the left ventricle were also observed in the male.

The NOAEL was 10 mg/kg per day [End-of-Text Table 3.2].

### 4-Week Repeat-dose Oral Toxicity in Dogs

PROJECT B was orally administered once daily for 4 weeks at dose of 0, 3, 10, 30 and 300 mg/kg per day to 4 male and 4 female beagle dogs per group (Study Project B-TX-0002). Three males and 2 females were added to the 300 mg/kg dose group in order to assess the reversibility of toxicity during a subsequent 4-week recovery period.

One female in the 300 mg/kg dose group was sacrificed due to moribundity on day 11 of dosing, and 1 female in the 300 mg/kg dose group was found dead in the morning (before dosing) on day 13 of dosing. These animals showed multifocal arteritis, multifocal inflammatory cell infiltration with hemorrhage, multifocal myocardial necrosis in the heart, severe increases in cardiac troponin I and T (only measured in moribund animal), and lung edema accompanied by pleural fluid in 1 animal indicating that effects on the cardiovascular system possibly caused deterioration in general condition or death.

No test article-related changes were noted at doses of 3 mg/kg.

At doses of ≥ 10 mg/kg, soft stool and vomiting were observed in males and females.

At doses of 30 mg/kg, increased lung weight in males and focal cellular infiltration of inflammatory cell in lamina propria in the stomach in both sexes were found.

At doses of ≥ 30 mg/kg, diarrhea was observed in males and females. Focal peribronchiolar inflammatory cell infiltration in the lung in histopathology was observed in males at doses of 30 and 300 mg/kg, and in 1 female at a dose of 300 mg/kg. Hypertrophy of the bronchiole epithelium and foam cell accumulation in the lung in histopathology was observed in 1 male in both the 30 and 300 mg/kg dose groups.

In the survived animals at doses of 300 mg/kg, the following changes were noted in males and females: salivation, increased heart rate (maximum individual variation from time-matched value during acclimation period: 164 beats/min), shortened PR, QT and QTc interval (QTcM) in electrocardiography, decreased erythrocyte count, hematocrit value, hemoglobin concentration, eosinophil count, prolonged activated partial thromboplastin time in hematology, increased troponin I (from 1 day of dosing) and urea nitrogen in blood chemistry, red focus in the endocardium at the right atrium in the heart in gross pathology, multifocal arteritis, multifocal inflammatory cell infiltration with hemorrhage in the heart, and atrophy of the thymus in histopathology. In males in addition to the above changes, red focus in the lung in gross pathology, focal inflammatory cell infiltration in the bronchiolar lumen, and intraepithelium in the lung were observed in histopathology. And in females, increased fibrinogen, troponin T (only in 1 female, 8 h after administration on day 14), sodium, and chloride, and decreased potassium in hematology and blood chemistry, red focus in the stomach body serosa in gross pathology, increased heart weight and decreased thymus weight, multifocal myocardial necrosis, and thickening of the artery tunica intima in the heart, and perivascular hemorrhage in the muscle and serosa in the stomach were noted in histopathology.

After a 4-week recovery period, the only histopathological changes in the heart and lung were observed at 300 mg/kg as follows: thickening of the artery tunica intima, and lymph vessel dilatation in the heart accompanied by gross lesions, and fibrosis and brown pigment deposition in the lung. Thickening of the artery tunica intima observed in the heart was considered to be a state of the repair process of the arteritis observed at the end of the dosing period, and lymph vessel dilatation in the heart might be caused by the lymph vessel obstruction accompanied with arteritis or myocardial damage. Changes in the lung were considered to be scarring related to the change of focal peribronchiolar inflammatory cell infiltration in the lung at the end of the dosing period. Accordingly, all test article-related changes noted at the end of the dosing period, except for the histopathological change of the repair process or scarring in the heart and lung, showed full recovery during the 4-week recovery period.

The NOAEL was 3 mg/kg per day [End-of-Text Table 3.3.2].

### Genotoxicity

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

A reverse mutation test was performed with *Salmonella typhimurium* (TA100, TA1535, TA98, and TA1537) and *Escherichia coli* (WP2*uvrA*), using the preincubation method with and without metabolic activation (Study Project B-TX-0003).

Based on the results of the dose-finding test at 15 to 5000 μg/plate, the main test was performed at 156 to 5000 μg/plate.

In comparison with the negative control, the test article induced neither a 2-fold or greater nor a dose-dependent increase in the number of revertant colonies in any test strain with or without metabolic activation.

It was concluded that PROJECT B has no potential to induce gene mutation in bacteria.

### In Vitro Chromosomal Aberration

A chromosomal aberration test was performed with cultured mammalian (Chinese hamster lung [CHL/IU]) cells in short-term treatments for 6 h with and without metabolic activation, and continuous treatment for 24 h without metabolic activation (Study Project B-TX-0004).

Based on the results of the dose-finding test conducted at 7.81 to 500 μg/mL, the highest dose was set at 500 μg/mL, followed by 3 or 6 lower doses.

The cell proliferation ratio determined from the population doubling decreased dose-dependently in continuous treatment.

Based on the cell proliferation results, chromosomal aberrations were analyzed at the following doses: 125, 250 and 500 μg/mL in short-term treatments with and without metabolic activation, and 300, 350, 400 and 450 μg/mL in continuous treatment.

No significant increase in the number of cells with structural or numerical chromosomal aberrations was noted in any treatment group when compared with that in the negative control group.

It was concluded that, PROJECT B has no potential to induce chromosomal aberrations in CHL/IU cells, regardless of the presence or absence of metabolic activation, or treatment length.

### Carcinogenicity

No carcinogenicity studies have been conducted with PROJECT B as of the preparation of this investigator’s brochure.

### Reproductive and Developmental Toxicity

* + - 1. **Effects on Embryo-fetal Development**

**4.3.5.1.1 Effects on Embryo-fetal Development in Rats (Dose-range Findings)**

PROJECT B was orally administered once daily from day 7 to day 17 of gestation at dose levels of 0, 30, 100 and 1000 mg/kg per day to 6 pregnant rats (Study Project B-TX-0005).

In dams at doses of 1000 mg/kg, decreased food consumption and suppressed body weight gain were noted from 1 day after initiation of dosing (day 8 of gestation) and 2 of the 6 animals in this group had died by day 11 of gestation. Due to the deaths in the 1000 mg/kg dose group, this dose level was considered to be excessive and the surviving dams in this group were prematurely necropsied by day 14 of gestation.

In dams at doses of 30 and 100 mg/kg, no effects of treatment with the test article were noted in the clinical observations, body weights, food consumption, gross pathology, the number of corpora lutea and implantations or preimplantation loss (%).

In fetuses, incidence of thymic cord was increased at doses of 100 mg/kg, however it was of no statistical significance and since only a limited number of animals were available for evaluation in this preliminary study, an additional definitive study will be required to clarify the relationship to treatment with the test article. No effects of treatment with the test article were noted on the viability or development of embryos/fetuses or external or skeletal malformations of fetuses.

### Local Tolerance

No local tolerance studies have been conducted with PROJECT B as of the preparation of this investigator’s brochure.

### Other Toxicity Studies

**4.3.7.1 In Vitro Phototoxicity**

In order to investigate the potential phototoxicity of PROJECT B, a phototoxicity study was performed with cultured mammalian cells (Balb/c 3T3 cells) at 9.49, 13.3, 18.6, 26.0, 36.4, 51.0, 71.4 and 100 μg/mL in the presence and absence of UV-A irradiation

(Study Project B-TX-0006).

The phototoxic potential was judged from only the mean photo effect (MPE) because the IC50 for cell viability could not be determined in either the presence or absence of irradiation. The MPE was 0.002, which was lower than the threshold value of 0.15.

It was concluded that PROJECT B has no potential to induce phototoxicity in cultured mammalian cells (Balb/c 3T3 cells).

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

Biological signaling by NO is primarily mediated by cGMP, which is synthesized by

NO-cGMP and broken down by cyclic nucleotide PDEs. PDE9 is suggested to be involved in the degradation of cGMP in urethra. Inhibition of PDE9 therefore may stimulate NO availability in the urethra and promote urethral dilatation during the voiding reflex.

PROJECT B is an inhibitor of PDE9, and its inhibitory potency and selectivity over other PDE isozymes has been shown in studies using human isozymes. In isolated urethra strips and an in vivo study in rats, PROJECT B prolonged the duration of urethral relaxation elicited by EFS or micturition reflex. In animal model studies that mimicked UAB conditions, PROJECT B demonstrated the potential to improve voiding deficiencies. Based on the pharmacological profile and similarities between rat and man in the regulatory mechanism of the urethra via NO and cGMP, PROJECT B is expected to facilitate bladder emptying by prolonging the duration of urethral relaxation. PROJECT B thereby may alleviate symptoms associated with incomplete bladder emptying for those patients in whom urethral resistance is hindering uncomplicated voiding.

In the rat 4-week toxicokinetic study at doses of 10, 30, 100 and 300 mg/kg per day, PROJECT B Cmax and AUC24 values increased with dose increase in both sexes. The PROJECT B Cmax and AUC24 values in females were higher than those in males. There were no apparent changes in any parameter by repeated dosing. In the dog 4-week toxicokinetic study at doses of 3, 10, 30 and 300 mg/kg per day, PROJECT B Cmax and AUC24 values increased with dose increase in both sexes. There was no apparent sex difference and no apparent change by repeated dose. The in vitro plasma protein binding ratios of PROJECT B ranged from 28.2% to 53.2% in mice, rats, rabbits and dogs and ranged from 37.2% to 39.7% in humans. CYP2A6 and CYP3A4 were capable of metabolizing PROJECT B. The major metabolite in human hepatocytes was observed in hepatocytes from rats, dogs, and monkeys. It was estimated to be a dehydrogenated form, which was presumably mediated by CYP2A6 and CYP3A4. The PROJECT B IC50 values of direct and time-dependent inhibition were > 100 μmol/L for CYP1A2, CYP2B6, CYP2C8, CYP2C19, CYP2D6, CYP3A (midazolam and testosterone), and 16.7 and 18.1 μmol/L for CYP2C9. The PROJECT B IC50 values for OATP1B1 and OATP1B3-mediated transport were estimated to be > 100 μmol/L.

In safety pharmacology studies, PROJECT B did not have an effect on the CNS in rats at doses of ≤ 100 mg/kg. PROJECT B showed statistically significant inhibition of hERG current at doses of ≥ 3 μmol/L (1390 ng/mL) (up to a maximum of 25.78% inhibition as compensated rate at 30 μmol/L [13904 ng/mL]); although an IC50 value could not be established, it was estimated to be > 30 μmol/L. In the dog cardiovascular and respiratory study (Study

Project B-PT-0004), QTcF prolongation was observed at doses of 100 mg/kg (Cmax,u = 8896 to 9190 ng/mL, based on plasma binding protein ratio of 28.2 to 30.5% in dog), and considered to be possibly related to the hERG inhibitory effect of PROJECT B. However, the exposure levels are well above the intended clinical exposure, and therefore not regarded as relevant. In contrast, shortenings of PR, QT and QTcM intervals found at doses of 300 mg/kg in the dog 4-week study (Study Project B-TX-0002) are considered as secondary changes to increased heart rate considering that the heart rate reached excessively high values at doses of 300 mg/kg. This study was not specifically designed to examine the effect of PROJECT B on heart rate; therefore, increases in heart rate may occur at lower doses. In dogs, moreover, an increase in heart rate and a decrease in blood pressure were observed. In the rat cardiovascular study, heart rate was increased at doses of 100 mg/kg. In vitro pharmacological study (Study Project B-PH-0001) suggested that at concentrations higher than the expected therapeutic concentration, PROJECT B has inhibitory effects on other PDE subtypes in addition to PDE9. Since it is well known that inhibitory effects on PDE3 or PDE5 can cause reflex tachycardia due to vasodilation via increases in cGMP or cyclic adenosine monophosphate (cAMP) [Kaumann et al, 2009; Kloner, 2004; Brunkhorst et al, 1989], changes in heart rate and blood pressure by PROJECT B are considered to be possibly related to the inhibitory effects of PROJECT B on other PDE subfamilies.

Histopathological findings including arteritis and myocardial necrosis in the heart were observed in dogs at doses of 300 mg/kg after 1 and 4 weeks of dosing and such effects on the cardiovascular system were considered to cause the deterioration in general condition or death. Myocardial necrosis was not seen in the 4-week study in rats but found in a preliminary 1-week study in rats (Study Project B-TX-0008). In dogs, cardiac troponin I increased a few hours after dosing on day 1, indicating that the effect is acute in nature. As described above, PROJECT B has the potential to induce tachycardia and increased heart rate at doses of 300 mg/kg as noted in the dog 4-week study where the heart rate reached excessively high values (over 200 beats/min in some animals). There have been many reports of cardiac lesions induced by vasodilator agents in preclinical studies [Clemo et al, 2003; Mesfin et al, 1995; Mesfin et al, 1987]. Considering that heart rate change and pathologic characteristics induced by these agents are similar to pathological changes seen with PROJECT B (e.g., hemorrhagic lesion indicated by red focus in the right atrium, coronary arteritis, inflammation with hemorrhage), effects on hemodynamics may have played a role in the development of the myocardial necrosis. Although the precise etiology of myocardial necrosis induction by PROJECT B is unclear, it is probable that cardiac damage was secondary to hemodynamic changes. The majority of vasodilator drugs have established clinical safety profiles and are not known to cause any heart damage in man [Bendjama et al, 2014; Mikaelian et al, 2014]. Cardiovascular biomarkers are considered to be necessary to monitor cardiac condition, and include troponins and creatine kinase isozymes for myocardial necrosis, and heart rate and blood pressure for cardiac function. It is well accepted that heart rate and blood pressure can be used as biomarkers of potential vascular injuries caused by vasodilatory agents, such as the ones leading to heart lesions in animals [Bendjama et al, 2014; Mikaelian et al, 2014].

Effects on the gastrointestinal system were indicated as shown by findings in dogs such as vomiting, salivation, abnormal feces and inflammatory cell infiltration in the lamina propria of the stomach (in addition, only at lethal dose, perivascular hemorrhage in the muscle and serosa in the stomach were found). Vomiting and abnormal feces are known to be induced by several drugs which have inhibitory effects on PDE3, 4 or 5 ([Tenor et al, 2011] FDA approval packages of milrinone, rolipram, tadalafil, sildenafil, and avanafil). In addition, indigestion and reflux are known as adverse events of PDE5 inhibitors (sildenafil, tadalafil and vardenafil), and reported to be caused by relaxation of smooth muscle [Rezvanfar et al, 2012].

Focal peribronchiolar inflammatory cell infiltration, focal inflammatory cell infiltration in bronchiolar lumen and intraepithelium, hypertrophy of the bronchiole epithelium and foam cell accumulation in the lung at doses of 30 mg/kg were found in dogs. Findings were possibly caused by aspiration of vomit which was noted dose-dependently; however, the detailed mechanism is unclear.

In the preliminary embryo-fetal development study in rats, thymic cord in fetus was found at doses of 100 mg/kg. Thymic cord is incidentally seen in rats and generally classified as not being an abnormality but a variation. Therefore, this finding is not considered to affect fetal mortality or physiological function (not regarded as risk of teratogenicity).

PROJECT B revealed neither in vitro genotoxic nor phototoxic potential.

Mean plasma exposure levels of PROJECT B in animals are shown in [[Table 3](#_bookmark51)].

### Table 3 Mean Plasma Exposure Levels of Safety Studies

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Study No.** | **Species/Study Duration/Route** | **Dose (mg/kg/day)** | | **Sex** | | **Steady State Cmax (ng/mL)** | **Steady State AUC24**  **(ng.h/mL)** |
| Project B-TX-0001 | Rat, 4-week, po | 10 | | M | | 288 | 773 |
| F | | 793 | 1330 |
| 30 | | M | | 1270 | 3020 |
| F | | 3760 | 6650 |
| 100 | | M | | 3020 | 10400 |
|  |  |  | F | 6800 | 19200 |
| 300 (NOAE | L) |  | M | 5190 | 21000 |
|  | F | 10300 | 46600 |
| Project B-PT-0003 | Rat, cardiovascular, po | 10 | | M | | 229 | 652 |
| 30 (NOAEL) | | M | | 1330 | 2830 |
| 100 (LOAEL) | | M | | 2310 | 7950 |
| Project B-TX-0002 | Dog, 4-week, po | 3 (NOAEL) | | M | | 323 | 1250 |
| F | | 276 | 849 |
| 10 (LOAEL) | | M | | 1710 | 5630 |
| F | | 1400 | 4200 |
| 30 | | M | | 7330 | 16900 |
| F | | 9910 | 22900 |
| 300 | | M | | 21800 | 127000 |
| F | | 25700 | 149000 |
| Project B-PT-0004 | Dog, cardiovascular, po | 3 (NOAEL) | | M | | 376 | 1930 |
| 10 (LOAEL) | | M | | 1540 | 7180 |
| 30 | | M | | 7700 | 30600 |
| 100 | | M | | 12800 | 75400 |

NOAEL: no observed adverse-effect level; LOAEL: lowest observed adverse-effect level.

Overall, target organ toxicities were identified in the cardiovascular system, lung and gastrointestinal tract. Nonclinical key observations and their relevance to human usage are summarized in [[Table 4](#_bookmark52)].

### Table 4 Potential Safety Concerns of PROJECT B for Clinical Studies

|  |  |  |
| --- | --- | --- |
| **Target organs/systems** | **Potential Safety Concern from nonclinical studies** | **Relevance to Human Usage and Risk Mitigation Actions** |
| Cardiovascular System | Increased heart rate, decreased blood pressure Arteritis, inflammatory cell infiltration, myocardial necrosis | Exposure limit, measurement of myocardial necrosis biomarkers (including CK isozymes, LDH, troponin I and T). Standard vital signs and ECG monitoring (including continuous cardiac  monitoring) |
| Gastrointestinal Tract | Salivation, vomiting, soft stool, diarrhea Inflammatory cell infiltration in lamina propria,  perivascular hemorrhage in muscle and serosa in stomach | Standard monitoring of clinical symptoms |
| Lungs | Inflammatory cell infiltration in peribronchiolar, bronchiolar lumen and intraepithelium,  hypertrophy of the bronchiole epithelium, foam cell accumulation | Standard monitoring of clinical symptoms (lung related and general) |

CK: creatine kinase; ECG: electrocardiogram; LDH: lactate dehydrogenase.

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