## Safety Pharmacology

A total of 3 safety pharmacology studies including 1 in vitro (Study Project A-PT-0006) and 2 in vivo (Studies Project A-PT-0004, Project A-PT-0005) were conducted in accordance with GLP standards, assessing potential effects on the cardiovascular and respiratory systems.

The effects on the CNS were assessed on 3 occasions (days -1, 1 and 22) as part of the GLP 4-week repeated oral dose toxicity study in rats (Project A-TX-0010).

## Effects on hERG Current

This study examined the in vitro effects of PROJECT A at near-physiological temperature on the hERG channel current, which is a surrogate for IKr, the rapidly activating delayed rectifier cardiac potassium current (Study Project A-PT-0006).

PROJECT A was tested at concentrations of 0 mM (vehicle control) and 10, 30 and 100 µM (approximately 4.6, 13.8 and 46 µg/mL, respectively).

PROJECT A inhibited the hERG current in a concentration-dependent manner by approximately 15% at 10 μM, 35% at 30 μM and 76% at 100 μM versus 3% in the control. hERG inhibition at 10, 30 and 100 µM was statistically significant when compared to vehicle control

values. The IC50 for the inhibitory effect of PROJECT A on the hERG current was 44.4 µM (20.4 µg/mL) (Hill coefficient: 1.3).

## Effects on Respiratory System in Rats

This study also evaluated the potential effects of PROJECT A on respiratory function in male rats given single oral doses of PROJECT A (Study Project A-PT-0004). To accomplish this objective, groups of 8 male rats were given single oral doses of PROJECT A at doses of

0 (vehicle), 3, 30 or 300 mg/kg via oral gavage at a dose volume of 10 mL/kg. Using a respiratory monitoring chamber, respiratory rate and tidal volume were recorded continuously for 1 h before dosing and for 5 h after dosing.

Male rats tolerated single oral doses of PROJECT A up to 300 mg/kg without clinical signs of toxicity or effects on respiratory function; consequently, the NOAEL for effects on respiratory function was considered to be 300 mg/kg.

## Effects on Cardiovascular System in Cynomolgus Monkeys

This study was conducted to evaluate the potential effects of the test article, PROJECT A, on cardiovascular function and plasma exposures in conscious, freely moving, male and female cynomolgus monkeys given single oral doses (Study Project A-PT-0005). To accomplish this objective, monkeys were given single oral doses of vehicle or PROJECT A (doses of 100, 300 or 1000 mg/kg via oral gavage at a dose volume of 5 mL/kg). Body temperature, blood pressure and ECG data were recorded by telemetry continuously from at least 2 h before through 24 h after dosing, and monkeys were observed for clinical signs of toxicity before and at approximately 3 and 24 h after dosing. For pharmacokinetics evaluation, blood samples were collected at specified intervals, processed to plasma and analyzed for plasma concentrations of PROJECT A.

Male and female cynomolgus monkeys tolerated single oral gavage doses of PROJECT A up to 1000 mg/kg without effects on body weight, body temperature, heart rate, pulse rate interval, QRS complex duration, uncorrected QT, corrected QT or qualitative aspects of the ECG. Administration of PROJECT A at 1000 mg/kg resulted in short-lived, slight increases in blood pressure (up to 15% above baseline), which were not considered to be adverse within the context of this safety evaluation.

In summary, with respect to the basic cardiovascular endpoints evaluated in this study, orally administered PROJECT A resulted in a no-observed-effect-level (NOEL) of 1000 mg/kg. At the NOEL, the mean Cmax value was approximately 7010 and 4690 ng/mL in male and female cynomolgus monkeys, respectively, and the mean AUC24 value was approximately

46100 and 33900 ng·h/mL in male and female cynomolgus monkeys, respectively.

## Effects on CNS in Rats

CNS parameters were measured in the GLP 4-week repeated oral dose toxicity study in rats (Study Project A-TX-0010).

PROJECT A (3, 30 or 300 mg/kg) or vehicle was administered to rats by oral gavage once daily for 4 weeks. Functional observational battery (FOB) evaluations were conducted without knowledge on the part of the testers of the treatment groups on designated main study animals predose (day -1), 2 to 4 h postdose on days 1 and 22 and once during the recovery period (day 56). FOB evaluations included those conducted in the home cage, during handling, in the open field and others. During open-field evaluations, each animal was placed in a black plexiglass box and observed for a minimum of 3 min. The parameters evaluated in the FOB were based on those outlined in [Moser & Ross, 1996; Moser et al, 1988]. The observations included, but were not limited to, evaluation of activity and arousal, posture, rearing, bizarre behavior, clonic and tonic movements, gait, mobility, stereotypy, righting reflex, response to stimulus (approach, click, tail pinch and touch), palpebral closure, pupil response, piloerection, exophthalmos, lacrimation, salivation and respiration.

Qualitative and/or quantitative measures of defecation and urination were also recorded. Forelimb and hindlimb grip strength were measured using the procedure described by [Meyer et al, 1979]. Hindlimb splay was quantitatively measured, as described by [Edwards & Parker, 1977]. Pain perception was assessed by measuring the latency of response to a nociceptive (thermal) stimulus when each animal was placed on a hot plate apparatus set to 52C (± 1°C) as described by [Ankier, 1974].

No effects of PROJECT A were reported at any of the doses tested for the categorical endpoint FOB findings or the activity/arousal, autonomic, neuromuscular or sensorimotor continuous endpoint FOB measurements in rats.

# Toxicology

The toxicology of PROJECT A has been evaluated in 17 completed studies, including 4 non- GLP repeated oral dose toxicity studies in rats (Project A-TX-0006, Project A-TX-0007,

Project A-TX-0008) and monkeys (Project A-TX-0009); 6 GLP repeated oral dose toxicity studies in rats (Project A-TX-0010, Project A-TX-0020 and Project A-TX-0023) and monkeys (Project A-TX-0011,

Project A-TX-0021 and Project A-TX-0024); 2 GLP in vitro genotoxicity studies with *Salmonella typhimurium* and *Escherichia coli* (Project A-TX-0013) and human peripheral blood lymphocytes (HPBLs) (Project A-TX-0012); 1 GLP in vivo genotoxicity toxicity study in rats

(Project A-TX-0014); 2 GLP toxicity studies in juvenile rats (Project A-TX-0018, Project A-TX-0019); 1 other non-GLP in vitro phototoxicity study with Balb/c 3T3 cells (Project A-TX-0015); and 1 GLP in vitro phototoxicity study with Balb/c 3T3 cells (Project A-TX-0022). The route of administration for all in vivo studies was oral. Overviews of the completed toxicology and

toxicokinetic studies of PROJECT A that have final reports are provided in [End-of-Text Tables

3.1 and 3.2, respectively].

## Single-dose Toxicity

No single-dose toxicity studies with PROJECT A have been conducted as of the preparation of this IB; however, rats and cynomolgus monkeys administered doses up to 300 or

1000 mg/kg, respectively, on day 1 of the GLP 4-week repeated oral dose toxicity studies (Project A-TX-0010 and Project A-TX-0011) and rats administered doses up to 2000 mg/kg on day 1 of the in vivo micronucleus test (Project A-TX-0014) showed no overt clinical signs of toxicity.

## Repeat-dose Toxicity

Ten repeated oral dose toxicity studies have been completed with PROJECT A. These included 4 non-GLP oral dose range-finding studies (two 14-day studies in male Wistar Han rats, one 4-week study in male and female Wistar Han rats and one 14-day study in male and female cynomolgus monkeys) and 6 GLP repeated oral dose toxicity studies (one 4-week study, one 13-week study and one 26-week study in male and female Wistar Han rats and one 4-week study, one 13-week study and one 52-week study in male and female cynomolgus monkeys).

The GLP repeated oral dose toxicity study data are presented below and in [End-of-Text Table 3.3].

## 4-week Repeated Oral Dose Toxicity Study in Rats

The objective of this study was to evaluate the toxicity and toxicokinetic profile following oral gavage administration of PROJECT A to male and female Wistar Han rats for

28 consecutive days (Study Project A-TX-0010). The reversibility of any test article-related effects was evaluated during a 4-week postdose recovery period.

PROJECT A or vehicle was administered once daily via oral gavage for 28 consecutive days to 10 male and 10 female rats per group at doses of 0 (control), 3, 30 or 300 mg/kg.

An additional 6 male and 6 female rats per group were treated at doses of 0, 30 or 300 mg/kg to assess the reversibility of any test article-related effects. Assessment of neurobehavioral effects and general toxicity were based on mortality, FOB evaluations, clinical observations, body weight and food consumption; ophthalmoscopic, examinations; and clinical and anatomic pathology. Toxicokinetic assessments were conducted for the test article.

The rats tolerated daily oral doses of PROJECT A at 3, 30 or 300 mg/kg for 4 weeks.

Drug-related adverse effects observed in the heart and skeletal muscle are explained below:

* Mild myocardial inflammation/necrosis with an increase in serum concentration of cTnI were observed in 2 male rats at 30 mg/kg per day and in 3 rats (2 males, 1 female) at 300 mg/kg per day. Increases in serum cardiac markers but no myocardial microscopic findings were noted in 2 male rats: an increase in serum activity of CK-MB was observed in 1 rat at 300 mg/kg per day and an increase in serum concentration of cTnI was observed in 1 rat at 3 mg/kg per day.
* Degeneration and/or mononuclear cell infiltrates were observed in the intercostal muscles of male rats at 30 mg/kg per day and of both sexes at 300 mg/kg per day and in the biceps femoris, quadriceps and gastrocnemius muscles of female rats at 300 mg/kg per day. The skeletal muscle changes, which were considered adverse at 300 mg/kg per　day only, were associated with increased serum activities of CK, CK-MM, aldolase, AST and/or LDH. Details on the severity of the skeletal muscle findings are provided in

[End-of-Text Table 3.3.1].

* At the end of the 4-week postdose recovery period, rats at 30 or 300 mg/kg per day of PROJECT A had only minimal findings in cardiac and skeletal muscle, the incidence of which was comparable to that seen in control animals. Therefore, the effects on cardiac and skeletal muscle were considered to be reversible.

Nonadverse findings included:

* Mildly reduced body weight and body weight gain and slightly lower average circulating red cell mass in both sexes
* Higher mean serum concentrations of triglycerides and low-density lipoprotein (LDL) and VLDL cholesterol in both sexes at 300 mg/kg per day
* Hepatocellular hypertrophy (visible microscopically or reflected in greater mean liver weight) and higher mean serum concentrations of total and high-density lipoprotein (HDL) cholesterol in both sexes at ≥ 30 mg/kg per day
* A greater incidence and/or grade of pancreatic acinar-cell apoptosis in male rats at 30 mg/kg per day and both sexes at 300 mg/kg per day
* Hypertrophy of thyroid follicular cells and chromophobe cells in the pituitary gland were observed in male rats at all dose levels. Both findings were likely secondary to hepatocellular hypertrophy [Hall et al, 2012] and/or a greater metabolic rate.
* A greater incidence of squamous cell hyperplasia in the nonglandular stomach mucosa at

≥ 30 mg/kg per day, most conspicuously as hyperkeratosis of the epithelium, accompanied by an increase in the number of Ki-67-positive nuclei

* A greater incidence or grade of protein droplets in renal tubular epithelial cells in the kidneys of male rats at ≥ 30 mg/kg per day
* Slightly higher mean serum concentrations of albumin, globulin, fibrinogen and total protein in male rats at 300 mg/kg per day
* Lower mean adrenal glands weight in female rats at 300 mg/kg per day, with no associated microscopic findings
* Adrenal cortical vacuolation in both sexes at 300 mg/kg per day

All PROJECT A-related adverse and nonadverse effects were reversible as at the end of the 4- week postdose recovery period, they were either absent or reduced in terms of incidence or intensity to the levels seen in control animals.

The Cmax and AUC24 generally increased more than dose-proportionally in both sexes on days 1 and 28. The Cmax and AUC24 for female rats were slightly higher than those for male rats in all cases. With repeated daily dosing, slight decreases in the Cmax and AUC24 were observed at doses of 30 and 300 mg/kg per day in both sexes.

Based on the observed cardiovascular findings, the NOAEL after repeated dosing of PROJECT A via oral gavage for 4 weeks was considered to be 3 mg/kg per day in male rats. At this dose level, mean Cmax and AUC24 values were 175 ng/mL and 1150 ng·h/mL, respectively. The 4-week NOAEL for PROJECT A was considered to be 30 mg/kg per day in　female rats, which corresponded to mean Cmax and AUC24 values of 8590 ng/mL and 20900 ng·h/mL, respectively.

## 13-week Repeated Oral Dose Toxicity Study in Rats

In the GLP 13-week oral dose toxicity study in rats (Study Project A-TX-0020), the dose levels were 0, 1, 3, 10 and 100 mg/kg per day, and the target organ of toxicity was the heart.

There were no PROJECT A-related effects on the following parameters: clinical or veterinary observations; ophthalmology; group mean body weight gains or food consumption; CK,

CK-MM, CK-MB or CK isoenzymes found in brain tissue (CK-BB) activities; bone marrow cytology endpoints; nor macroscopic findings at the terminal or recovery necropsy.

Group mean decreases in body weight (main study and/or toxicokinetic animals) were considered to be adverse at 100 mg/kg per day due to the decreases over consecutive intervals (main study and/or toxicokinetic animals) and the fact that group mean body weights remained decreased for main study males during the recovery phase (same magnitude as during the dosing phase).

PROJECT A-related changes in clinical pathology parameters included the following:

* Changes attributable to altered lipid metabolism in males at ≥ 3 mg/kg per day and females at ≥ 10 mg/kg per day included minimal to mild increases in triglyceride concentration with parallel increases in VLDL cholesterol in males at ≥ 3 mg/kg per day and females at 100 mg/kg per day, mild to moderate increases in total cholesterol and HDL cholesterol in both sexes at ≥ 10 mg/kg per day and mild increases in LDL cholesterol in both sexes at 100 mg/kg per day. These effects demonstrated partial resolution following a 4-week recovery period.
* Increased cTnI concentrations in a few individual animals of both sexes at ≥ 10 mg/kg per day, which reflected cardiomyocyte damage and correlated reasonably well with myocardial inflammation/necrosis seen microscopically.
* Increased urine protein in males at 100 mg/kg per day that correlated with the microscopic finding of chronic progressive nephropathy. This effect persisted following a 4-week recovery period.
* Reversible mild increases in aldolase activity in both sexes at 100 mg/kg per day that reflected a myofiber effect.
* Increased plasma and serum proteins in males at 100 mg/kg per day, including minimally increased total protein, fibrinogen, globulin and albumin concentrations that demonstrated partial or full (albumin) resolution following a 4-week recovery period.
* Reversible minimal decrease in alkaline phosphatase activity in males at 100 mg/kg per day.
* Reversible minimal decreases in eosinophil counts in males at ≥ 10 mg/kg per day and females at 100 mg/kg per day and minimal decreases in basophil counts in males at 100 mg/kg per day.
* Reversible minimal decreases in red cell mass in both sexes at 100 mg/kg per day that lacked correlative alterations in reticulocyte counts or in the bone marrow cytologically.
* Reversible minimal decreases in erythrocyte mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in males at 100 mg/kg per day that reflected an overall decrease in erythrocyte size (with a secondary decrease in hemoglobin content).

PROJECT A-related organ weight changes included increases in liver weights at 100 mg/kg per day (correlated to minimal to moderate hepatocellular vacuolation in males) and thyroid/parathyroid gland weights for 100 mg/kg per day males (correlated to minimal follicular cell hypertrophy/hyperplasia) and decreases in adrenal gland weights for 100 mg/kg per day males and in mandibular/salivary gland weights for 100 mg/kg per day females (no microscopic correlates for decreased weights). These changes persisted in the liver of males and females at the recovery necropsy.

PROJECT A-related microscopic findings were present in the heart, liver, kidney, tongue, esophagus, nonglandular stomach, pancreas, adrenal glands, thyroid gland, diaphragm and bone marrow (sternum).

In the heart, minimal or mild inflammation/necrosis (loss of myofibers with replacement of mononuclear cells and fewer neutrophils) was observed in all groups, including the control group. The incidence of this finding was greater in males at 100 mg/kg per day (10 of

10 males) than in control males (2 of 10 males), there was an increased severity of this finding in males at 100 mg/kg per day (relative to control males) and several affected males at 100 mg/kg per day had increased serum cTnI concentrations. It was concluded that the heart findings in males at 100 mg/kg per day should be considered adverse. Heart findings in males at 100 mg/kg per day showed evidence of resolution following a 4-week recovery period, and there were no males observed with increased cTnI.

In addition to the heart findings described earlier, PROJECT A-related microscopic findings were present in the liver, kidney, tongue, esophagus, nonglandular stomach, pancreas, adrenal glands, thyroid gland, diaphragm and bone marrow (sternum). None of these microscopic findings observed was considered adverse.

* Hepatocellular vacuolation was noted in the liver at ≥ 10 mg/kg per day with increased Oil Red O staining present on hepatocytes of 3 males at 100 mg/kg per day.
* Chronic progressive nephropathy (basophilic tubules and/or dilated tubules with intraluminal hyaline casts) was present in the kidney at 100 mg/kg per day.
* There was also a minimal increase in hyaline droplets in the proximal renal tubular epithelial cells of males at 100 mg/kg per day.
* Hyperkeratosis (increased layers of lamellar keratin in the superficial squamous epithelial layer) was similar in the tongue (males at 100 mg/kg/day and females at

≥ 10 mg/kg/day), esophagus (males at ≥ 3 mg/kg/day and females at ≥ 10 mg/kg/day) and nonglandular stomach (males and females at 100 mg/kg/day). Additional PROJECT A-related findings in the nonglandular stomach included hyperplasia of the limiting ridge (males at ≥ 3 mg/kg/day and females at 1, 3 and 100 mg/kg/day); this finding was also present in 1 male and 1 female control animal. The limiting ridge hyperplasia was considered to be a PROJECT A-related exacerbation of this common background finding in rats.

* In the pancreas, acinar cell apoptosis was present in males at ≥ 10 mg/kg per day and females at ≥ 3 mg/kg per day.
* There was increased cortical vacuolation in the adrenal glands in males at all treated doses and 1 female at 100 mg/kg per day.
* Males at 100 mg/kg per day had hypertrophy/hyperplasia of the follicular epithelial cells in the thyroid gland.
* Increased Oil Red O staining was present in the diaphragm at 100 mg/kg per day. Increased hematopoietic cellularity was present in the bone marrow (sternum) at 100 mg/kg per day and in 1 male at 1 mg/kg per day.

Based on the above data, the 13-week NOAEL was identified as 10 mg/kg per day. On day 91 at 10 mg/kg per day, Cmax was 1960 and 6790 ng/mL (males and females,

respectively) and AUC24 was 7590 and 13400 ng·h/mL (males and females, respectively).

## 26-week Repeated Oral Dose Toxicity Study in Rats

In the GLP 26-week oral dose toxicity study in rats (Study Project A-TX-0023), dose levels were 0, 1, 3, 10 and 50 mg/kg per day.

There were no PROJECT A-related mortalities. Two females (1 mg/kg/day Animal No. 2510 and 10 mg/kg/day Animal No. 4505) were euthanized in extremis on days 145 and 90, respectively. There were no anatomic pathologic findings that explained the cause of moribundity in either animal. No test article-related effects were noted on mean

food consumption, ophthalmology or macroscopic findings, total CK, CK isoenzymes (CK-MM, CK-MB and CK-BB) or aldolase activities or bone marrow smears.

Possible PROJECT A-related clinical observations were limited to dry skin (tail) in males at 50 mg/kg per day beginning on day 91 and continuing through day 182. The toxicological

significance of this finding was unclear and this finding did not negatively impact the overall health status of these animals.

Mean body weight was lower among males and females at 50 mg/kg per day. This resulted in lower mean cumulative body weight change of -21% in males and -20% in females at this dose level. However, no observations of thin body condition or correlating decrements in mean food consumption were evident; therefore, these changes were not considered adverse at this magnitude.

PROJECT A-related changes in clinical pathology parameters included the following:

* Changes attributable to altered lipid metabolism in males at ≥ 3 mg/kg per day and females at ≥ 10 mg/kg per day that included minimal to mild increases in triglyceride concentration with parallel increases in VLDL cholesterol in males at ≥ 3 mg/kg per day, and mild to moderate increases in total cholesterol, HDL and LDL cholesterol in both sexes at ≥ 10 mg/kg per day that may have been related to microscopic hepatocellular lipid vacuolation.
* Higher individual cTnI concentrations were observed in one male each at 10 and 50 mg/kg per day, which correlated with test article-related myocardial necrosis/inflammatory cell infiltrate noted microscopically.
* Decreases in erythrocyte size in both sexes at 50 mg/kg per day that were reflected in minimal decreases in hemoglobin concentration, hematocrit, erythrocyte MCV and MCH, as well as a minimal increase in red cell distribution width (limited to males).
* Mildly higher urine protein (by dipstick) in males at 50 mg/kg per day correlated with test article-related chronic progressive nephropathy.

There were no PROJECT A-related macroscopic pathologic findings at the terminal necropsy, but PROJECT A-related differences in organ weights and/or microscopic findings were present in liver, heart, kidneys, nonglandular stomach, thyroid gland, bone marrow, salivary glands, uterus and diaphragm. None of these weight differences or microscopic findings was deemed to be adverse at any dose level.

In the liver, mean weight was greater in both sexes at ≥ 10 mg/kg per day. In males, this correlated with hepatocellular vacuolation at ≥ 10 mg/kg per day and increased Oil Red O staining in hepatocytes at 50 mg/kg per day. In females, the only microscopic finding was minimal hypertrophy of centrilobular hepatocytes in one female at 50 mg/kg per day.

Although the vacuolation in males was of moderate to marked severity at 50 mg/kg per day, it was not associated with degeneration/necrosis or a corresponding increase in liver enzymes or bilirubin levels, and, thus, considered nonadverse.

In the heart, the incidence of necrosis/inflammation, characterized by loss of myofibers with replacement by mononuclear cells and fewer neutrophils, was increased at ≥ 10 mg/kg per day in both sexes. This was considered to be an exacerbation of rodent cardiomyopathy, a spontaneous degenerative disease that is very common in males and infrequent in females of this age. This finding was graded as minimal in all rats except one male at 10 mg/kg per day.

In the kidneys, the incidence of chronic progressive nephropathy, characterized by basophilic tubules and/or dilated tubules with intraluminal hyaline casts, was increased in males at

≥ 3 mg/kg per day and severity was increased (mild) at 50 mg/kg per day; incidence was increased in females at 1, 3 and at 50 mg/kg per day. The increase in incidence and severity of chronic progressive nephropathy was correlated with increased urine protein levels identified. Collectively, these findings were considered test article-related and represented exacerbation/early onset of a common background finding.

In the nonglandular-stomach, potential PROJECT A-related findings included an increased incidence of minimal hyperkeratosis in males at ≥ 3 mg/kg per day and in females at

50 mg/kg per day, characterized by increased layers of lamellar keratin in the superficial squamous epithelial layer; an increased incidence of minimal hyperplasia of the limiting ridge in both sexes at 50 mg/kg per day, and an increased frequency of Ki-67-positive nuclei in the epithelium at ≥ 10 mg/kg per day in both sexes.

In the thyroid glands, minimal hypertrophy/hyperplasia of the follicular epithelial cells was present at all dose levels in males and at 50 mg/kg per day in females. In males, the incidence increased with dose level and this probably accounted for the greater mean weight of thyroid glands in males at 50 mg/kg per day.

In the bone marrow, increased hematopoietic cellularity was present at 50 mg/kg per day in both sexes. Both the incidence and average grade of this finding were greater in males than females.

In the salivary glands, mean weight was lower in males at ≥ 3 mg/kg per day in females and at 50 mg/kg per day. Microscopically, there were no associated findings.

In the uterus, mean weight was greater at ≥ 10 mg/kg per day. Microscopically, there were no associated findings.

In the diaphragm, Oil Red O staining was increased at ≥ 10 mg/kg per day in females and at 50 mg/kg per day in males.

In summary, daily oral gavage administration of PROJECT A in Wistar Han rats for 26 weeks at 1, 3, 10, or 50 mg/kg per day was generally well-tolerated and did not elicit any adversity in any parameter examined. As a result, the NOAEL for this study was 50 mg/kg per day, the highest dose level tested (equivalent to Cmax values of 15500 and 30400 ng/mL and AUC24 values of 41200 and 111000 ng·h/mL for males and females, respectively on day 182).

## 4-week Repeated Oral Dose Toxicity Study in Cynomolgus Monkeys

The objective of this study was to investigate the preliminary toxicity of PROJECT A in cynomolgus monkeys when administered daily via oral gavage for 4 weeks, to assess plasma exposure to the test article after the first and last doses and to evaluate the reversibility of any test article-related effects during a 4-week postdose recovery period (Study Project A-TX-0011).

PROJECT A or vehicle was administered once daily via oral gavage for 28 consecutive days at doses of 0, 10, 100 or 1000 mg/kg. The 10 mg/kg group consisted of 3 male and 3 female monkeys. All other treatment groups consisted of 5 male and 5 female monkeys. After the final dose, 3 male and 3 female animals in each group were euthanized and necropsied. The remaining animals were allowed to recover for 4 weeks and then were euthanized and necropsied to evaluate the reversibility of any effects.

Animals were monitored for clinical signs of toxicity; findings detectable by ophthalmoscopic, physical and electrocardiographic examinations; and effects on body weight and hematology, coagulation, clinical chemistry and urinalysis parameters. Clinical chemistry parameters included cTnI, aldolase, CK (total and isozymes), LDH and cholesterol (total, HDL, LDL and VLDL). Plasma samples were taken at intervals after the first and last doses to measure PROJECT A concentration and evaluate plasma exposure.

At necropsy, macroscopic pathologic findings were recorded; the whole animal and selected organs were weighed; bone marrow smears were made and tissue samples were taken and fixed. Bone marrow smears were stained and examined for cytologic findings. All fixed tissue samples from all monkeys were processed and examined for histopathologic findings.

Monkeys tolerated daily oral doses of PROJECT A for 4 weeks at all dose levels. The only findings considered to be related to PROJECT A were a greater incidence of soft and/or watery feces, slight decreases in red cell mass and hepatocellular hypertrophy at 1000 mg/kg per day. None of these findings were considered adverse. These findings were reversible when dosing stopped.

The relationship between dose level and plasma exposure was similar in both sexes and did not change meaningfully with repeated daily dosing. Based on mean Cmax and AUC24 values for sexes combined, plasma exposure to PROJECT A tended to increase dose-proportionally from 10 to 100 mg/kg per day but less than dose-proportionally from 100 to 1000 mg/kg per day.

There were no adverse findings observed up to the highest dose tested (1000 mg/kg), and the 4-week NOAEL for PROJECT A in cynomolgus monkeys was considered to be 1000 mg/kg per day. At this dose level, mean Cmax and AUC24 values were 4710 ng/mL and 22700 ng·h/mL, respectively, in male cynomolgus monkeys and 5120 ng/mL and 28200 ng·h/mL, respectively, in female cynomolgus monkeys.

## 13-week Repeated Oral Dose Toxicity Study in Cynomolgus Monkeys

In the GLP 13-week study in cynomolgus monkeys (Study Project A-TX-0021), initial dose levels were 0, 10, 100 and 1000 mg/kg per day, but the high-dose level was reduced to 500 mg/kg per day on day 24 after 3 weeks of dosing because of clinical signs of ill health that included an increased frequency of diarrhea in the group as a whole. Clinical findings (including hunched posture, unkempt appearance, decreased activity, lying on bottom of cage, neurological weakness, eyelids partially closed) at 1000 mg/kg per day led to the decision to put 2 monkeys on a dosing holiday (from day 22 to day 27; dosing at

500 mg/kg/day resumed on day 28) and to euthanize a third monkey for humane reasons on day 16. The monkey that was euthanized had diarrhea throughout the dosing period and had become cachexic and severely dehydrated by day 14. Prior to day 16, clinical signs in this animal were limited to fecal changes (soft/watery feces) and/or sparse hair. Clinical observations prior to euthanasia included decreased activity and reactivity, hunched posture, inappetence, thin body condition, dilated pupils, skin-cold-to-the–touch, decreased body temperature, both eyes partially closed, pink tacky mucous membranes, dehydration and red discolored feces. The animal had adverse changes in functional renal parameters including increased urea nitrogen and creatinine concentrations and decreased sodium and chloride concentrations. Histopathological findings of bilateral kidney injury (tubular degeneration/ necrosis, tubular regeneration, tubular casts and mixed inflammation) were observed in this animal. The acute renal tubular necrosis was most likely secondary to PROJECT A-related diarrhea and dehydration, rather than a direct effect of PROJECT A. No similar kidney microscopic findings were observed in any other animal in this study. Functional renal parameters were normal in all other animals.

Other findings in the 13-week cynomolgus monkey study included: PROJECT A-related fecal changes; decreases in group mean body weight (males) at 1000 and 1000/500 mg/kg per day; decreases in red cell mass (males; erythrocyte counts, hemoglobin concentration and/or hematocrit) at 1000/500 mg/kg per day and increases in triglyceride and VLDL concentrations in both sexes at ≥ 100 mg/kg per day at day 49 and at ≥ 10 mg/kg per day at day 89 (no microscopic correlates). These findings were not deemed to be adverse. Based on the available data, the NOAEL is 500 mg/kg per day. At this dose level on day 91, Cmax was 1740 and 2870 ng/mL (males and females, respectively) and AUC24 was 10500 and 17100 ng·h/mL (males and females, respectively). No cardiac findings were observed in any monkey at daily doses up to 500 mg/kg per day; however, day 91 AUC values were substantially lower (approximately 7- to 11-fold at 1000 mg/kg/day) than those seen for day 91 values in male rats.

## 52-week Repeated Oral Dose Toxicity Study in Cynomolgus Monkeys

In the GLP 52-week study in cynomolgus monkeys (Project A-TX-0024), the dose levels were 0, 10, 100 and 500 mg/kg per day.

There was no mortality and no PROJECT A-related effects on veterinary observations, ophthalmology findings, ECG parameters, group mean body weights, coagulation or urinalysis endpoints, Troponin I, CK, CK isoenzymes (MM, MB and BB) or aldolase through week 52. PROJECT A-related clinical signs were limited to fecal changes (soft/watery) for both sexes at ≥ 100 mg/kg per day, although the incidence was higher among males. These changes were not deemed adverse due to the lack of correlating changes in body weight and minimal requirement for veterinary treatment for a few animals.

Alterations in lipid metabolism were seen in both sexes and consisted of minimal decreases in LDL cholesterol in both sexes (males at 500 mg/kg/day and females at all dose levels), minimal to mild increases in VLDL cholesterol and triglyceride concentrations in males at

≥ 10 mg/kg per day. None of these changes were considered clinically meaningful.

There were also minimal decreases in red cell mass parameters (erythrocyte count, hemoglobin concentration and hematocrit) in males at ≥ 100 mg/kg per day and females at

≥ 10 mg/kg per day that lacked corresponding alterations in reticulocyte counts.

In summary, daily oral gavage administration of PROJECT A (at dose levels of 10, 100, and 500 mg/kg/day) to cynomolgus monkeys for 52 weeks was well-tolerated. There was no mortality, and none of the observed clinical pathology changes were considered adverse.

## Genotoxicity

## In Vitro Reverse Mutation Test in Bacteria

The mutagenic potential of PROJECT A was evaluated by measuring its ability to induce reverse mutations at selected loci of several strains of *S. typhimurium* and at the tryptophan locus of *E. coli* strain WP2 uvrA in the presence and absence of exogenous metabolic activation (Study Project A-TX-0013).

In the mutagenicity assay, the concentration levels tested were 0 (vehicle), 50, 150, 500, 1500 and 5000 μg per plate. Precipitate was observed beginning at 500 μg per plate under all conditions. Toxicity was defined as a reduction in revertant count. No definitive background lawn toxicity was observed; however, toxicity was observed at 5000 μg per plate under

1 condition. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation.

These results indicate that PROJECT A was not mutagenic at selected loci of several tester strains of *S. typhimurium* or at the tryptophan locus of *E. coli* strain WP2 uvrA in the presence and absence of exogenous metabolic activation.

## In Vitro Chromosome Aberration Test in HPBLs

The potential of PROJECT A to induce structural chromosomal aberrations was evaluated using HPBLs in both the absence and presence of exogenous metabolic activation (Study

Project A-TX-0012). HPBLs were treated for 4 h in the absence and presence of S9 and for 20 h in the absence of S9.

In the chromosomal aberration assay, cytotoxicity (≥ 45% reduction in mitotic index relative to the vehicle control) was observed at concentrations ≥ 200 μg/mL in the nonactivated 4-h exposure group, ≥ 70 μg/mL in the S9-activated 4-h exposure group and ≥ 80 μg/mL in the nonactivated 20-h exposure group. The concentrations selected for evaluation of chromosomal aberrations were 100, 175 and 200 μg/mL in the nonactivated 4-h exposure group; 25, 50 and 70 μg/mL in the S9-activated 4-h exposure group; and 50, 70 and

80 μg/mL in the nonactivated 20-h exposure group.

No significant or concentration-dependent increases in structural or numerical (polyploid or endoreduplicated cells) aberrations were observed in treatment groups either in the presence or absence of the S9 hepatic microsomal fraction.

These results indicate that PROJECT A was negative for the induction of structural and numerical chromosomal aberrations in the presence and absence of the exogenous metabolic activation system.

## In Vivo Micronucleus Test

Groups of 10 rats (5 of each sex) were given a single dose of cyclophosphamide (positive control) or daily oral doses of vehicle or PROJECT A at 500, 1000 or 2000 mg/kg per day for 2 days (Study Project A-TX-0014). Two days after the last dose, the peripheral blood was

collected from the rats and they were subsequently euthanized. In life, rats were observed for clinical signs of toxicity and effects on body weight. Blood samples were analyzed for the relative number of reticulocytes to evaluate hematopoietic toxicity and for the relative number of micronucleated reticulocytes to evaluate clastogenic effects or effects on mitotic apparatus. This study was conducted in 2 phases that ran in parallel: a Micronucleus Phase to evaluate clastogenic potential and a Toxicokinetic Phase to evaluate plasma exposure to PROJECT A.

PROJECT A at doses up to 2000 mg/kg per day did not induce mortality or clinical signs of toxicity nor did it affect body weight gain (growth). It also did not increase the relative number of micronucleated reticulocytes at any dose level.

In conclusion, in an in vivo peripheral blood micronucleus assay in rats, PROJECT A at oral doses up to 2000 mg/kg per day was considered to be negative for both clastogenic activity and disruption of the mitotic apparatus.

## Carcinogenicity

No carcinogenicity studies of PROJECT A have been conducted to date. In the repeated oral dose toxicity studies conducted to date, no preneoplastic or neoplastic findings were noted.

## Reproductive and Developmental Toxicity

No reproductive and developmental toxicity studies of PROJECT A have been conducted to date. However, no findings indicative of toxicity to male or female reproductive organs have been seen in rats given daily doses of PROJECT A at up to the maximum tolerated dose level for 26 weeks or in cynomolgus monkeys given daily doses of PROJECT A at up to the maximum tolerated dose level for 52 weeks.

## Studies in Juvenile Animals

Juvenile toxicity studies with PROJECT A included 1 GLP 4-week dose range-finding study in rats and 1 GLP 11-week definitive study in rats.

In the GLP juvenile rat study (Study Project A-TX-0019), dose levels were 0, 1, 3, 10 and

30 mg/kg per day, and rats were 21 days of age at first dose. Animals were evaluated over an 11-week dosing period (during the period of puberty and through sexual maturation), followed by a 4-week recovery period.

No adverse effects of PROJECT A were observed on survival, mean body weight, food consumption, motor activity, passive avoidance, bone length and macroscopic findings at any dose level of PROJECT A. An increased occurrence of dry skin of the tail was noted in several animals at 30 mg/kg per day but was not seen in the controls. This finding was considered PROJECT A-related, but not adverse, as there was no overall impact on the health of affected animals. Sexual maturation was unaffected in females at all dose levels evaluated and in males at 1, 3 and 10 mg/kg per day. At 30 mg/kg per day, preputial separation was statistically delayed (46.9 days); however, the delay was slight in magnitude when compared to controls (44.0 days) and, while considered related to PROJECT A, this finding was not considered adverse.

An increased incidence of minimal inflammation/necrosis (loss of myofibers with infiltration of mononuclear cells) of the myocardium that correlated in part with increases in serum concentrations of cTnI (at ≥ 10 mg/kg/day), was observed in males at doses of ≥ 3 mg/kg per day (6 of 10 males in each of these dose groups versus 1 of 10 males in the concurrent control group). Increases in cTnI and minimal cardiac inflammation/necrosis were resolved following a 4-week recovery period. Minimal cardiac inflammation/necrosis is a background finding that has been reported in several strains of rats including Wistar Han [Blankenship et al, 2013; Berridge et al, 2016], but the increased incidence of this finding in PROJECT A-treated males at ≥ 3 mg/kg per day, relative to concurrent controls, was considered to be

PROJECT A-related. Because the observed cardiac inflammation/necrosis remained minimal in severity, because the incidence was within the range of historical control values for Wistar Han rats of similar age, and because the finding was reversible when dosing stopped, the PROJECT A-related increase in incidence was not considered to be adverse.

Evidence of altered lipid metabolism in both sexes at ≥ 3 mg/kg per day was indicated by dose-related increases in total and HDL cholesterol, with concurrent increases in VLDL and/or LDL cholesterol in both sexes at 30 mg/kg per day and increases in triglycerides in males at ≥ 10 mg/kg per day. These effects were resolved at 30 mg/kg per day following a 4-week recovery period. Minimal decreases in red cell mass (mean erythrocyte counts, hemoglobin concentrations and/or hematocrit) were noted in males at 30 mg/kg per day, which lacked correlative findings and were resolved following a 4-week recovery period.

PROJECT A-related organ weight changes included increases in mean liver weights (absolute and/or relative to body and/or brain weight) at ≥ 3 mg/kg per day in males and ≥ 10 mg/kg per day in females; however, there were no microscopic correlates for the liver weight changes. Mean thyroid/parathyroid gland weights in males (absolute and relative to brain and/or body weight) were increased at ≥ 10 mg/kg per day. Microscopically, the increased thyroid/parathyroid gland weights correlated to follicular cell hypertrophy/hyperplasia of the thyroid gland. At recovery necropsy, PROJECT A-related organ weight differences were present in the liver, kidney and thyroid/parathyroid gland of males and the adrenal gland of females. There was an increase in mean absolute and relative (to body weight and brain weight) weights in the liver and kidney of males at 30 mg/kg per day and an increase in mean absolute thyroid/parathyroid gland weights at 30 mg/kg per day. In females, there was a decrease in mean absolute and relative (to brain weight) adrenal gland weights at 30 mg/kg per day. There were no microscopic correlates for the liver, kidney, thyroid/parathyroid gland or adrenal gland weight differences.

In addition to the heart findings described earlier, PROJECT A-related microscopic findings were present in the kidney and adrenal glands of males only and in the tongue, esophagus, thyroid gland, pancreas and diaphragm of males and females. None of the microscopic findings observed was considered adverse.

* In the kidneys, there was a dose dependent accumulation of hyaline droplets (minimal) within the cytoplasm of epithelial cells lining the proximal tubules in all treated groups of males (≥ 1 mg/kg/day). Male rat-specific hyaline droplets are often associated with α2u-globulin (an adult male rat-specific protein) accumulation in the renal proximal tubular epithelium and can occur spontaneously or be increased by the administration of certain chemicals [Hamamura et al, 2017; Hard & Snowden, 1991].
* In the adrenal glands, there was increased incidence and severity of minimal to mild diffuse cortical vacuolation at 30 mg/kg per day.
* Minimal hyperkeratosis (increased layers of lamellar keratin in the superficial squamous epithelial layer) was present in the tongue of males at all treated doses (≥ 1 mg/kg/day) and females at ≥ 10 mg/kg per day; minimal hyperkeratosis was also present in the esophagus of males and females at 30 mg/kg per day. A single female at 30 mg/kg per day had mild hyperkeratosis in the nonglandular stomach.
* Hypertrophy/hyperplasia of the follicular epithelial cells in the thyroid gland was present in the 3 males and 2 females at 30 mg/kg per day, 1 male and 1 female at 10 mg/kg per day and 1 male at 1 mg/kg per day.
* In the pancreas, there was an increased incidence of minimal to mild acinar cell apoptosis in males at ≥ 1 mg/kg per day and females at 3 and 30 mg/kg per day, compared to concurrent study controls.
* There was a minimal increase in Oil Red O staining in the diaphragm of 6 males and 1 female at 30 mg/kg per day.

PROJECT A-related microscopic findings that were present at the terminal necropsy in the heart, kidney, adrenal gland, tongue, esophagus, nonglandular stomach, thyroid gland and diaphragm were not present at the recovery necropsy, thus indicating a full recovery. In the pancreas, an increased incidence and/or severity (minimal to mild) of apoptosis, relative to concurrent controls, were observed at the end of the 4-week recovery period in 4 males and

1 female at 30 mg/kg per day. These pancreatic findings were not considered to be adverse because of low severity and low incidence.

Because there were no adverse findings observed in this study, the NOAEL for juvenile rats was considered to be 30 mg/kg per day in males and females. At the NOAEL, day 79 mean Cmax values were 3960 and 7840 ng/mL for males and females, respectively, and AUC24 values were 22500 and 27700 ng·h/mL for males and females, respectively. The findings observed in this juvenile rat study (including clinical pathology and histopathology findings) were generally similar to findings observed in the 13-week adult rat study (Study

Project A-TX-0020).

## Local Tolerance

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

## Other Toxicity Studies

An in vitro GLP study (Study Project A-TX-0022) was performed using cultured mammalian cells to determine the phototoxicity potential of PROJECT A. Cultured Balb/c 3T3 cells were treated with test article for 1 h followed by UV-A irradiation (5 J/cm2) while incubation continued. For the comparator, irradiation was not conducted. Cell viability was determined by Neutral Red extraction from cells (measurement of absorbance at 540 nm).

The main test was performed at 9.49, 13.3, 18.6, 26.0, 36.4, 51.0, 71.4 and 100 μg/mL as PROJECT A (free form of PROJECT A) in the absence and presence of UV-A irradiation because cytotoxicity and test article precipitation were not observed at up to 100 μg/mL in the dose range-finding test. Vehicle (dimethyl sulfoxide)-treated and a chlorpromazine hydrochloride (CPZ)-treated cells served as the negative and positive controls, respectively. Concentrations of CPZ were 0.391, 0.781, 1.56, 3.13, 6.25, 12.5, 25.0 and 50.0 μg/mL in the absence and presence of irradiation. An ethanol-treated group was set as the vehicle control for the positive control groups.

For CPZ, the photo irritation factor (PIF) and mean photo effect were 17.0 and 0.324, respectively; however, for the PROJECT A groups, the IC50 values for cell viability in the absence and presence of irradiation were determined to be 73.7 and 73.2 μg/mL, respectively, and the PIF was 1.01 and less than 5. Therefore, PROJECT A was categorized as having no phototoxicity.

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

# 4.4 Integrated Nonclinical Overview and Conclusion: Potential Clinical Relevance

PROJECT A is a low-molecular-weight PPARδ modulator that is being developed for the treatment of DMD and MM. PROJECT A increased mRNA expression of PPARδ target genes and activated FAO in mouse C2C12 myotubes and myoblasts and in muscle cells isolated from a patient with DMD. In *mdx* mice, PROJECT A showed a tendency to increase endurance (as measured in a treadmill exhaustion test) while decreasing inflammation and necrosis in skeletal muscle and diaphragm fibrosis and preserving cardiac function in older *mdx* mice.

PROJECT A also increased the expression of PPARδ target genes and activated FAO in cells in cells from patients with mitochondrial mutation disorders. These included LHON/Leigh Syndrome, KSS, MELAS and MERRF cells. Although there are no animal models of PMM, the muscle dysfunction and fatigue in both the *mdx* mouse and aged DIO mouse are accompanied by mitochondrial dysfunction; in both models, PROJECT A improved animal endurance. These data from patient cells with mitochondrial mutations combined with animal data from different muscle disorders with mitochondrial dysfunctions suggest that PROJECT A could improve muscle function and endurance in varied muscle diseases including DMD and PMM.

Nonclinical safety pharmacology studies of PROJECT A revealed no effects on the CNS and respiratory system in rats at doses up to 300 mg/kg. Although PROJECT A inhibited the hERG channel current in hERG transfected HEK293 cells, no QT prolongation or other

PROJECT A-related effects on the cardiovascular system were observed in cynomolgus monkeys at doses up to 1000 mg/kg.

There was no evidence of genotoxic potential with PROJECT A based on the findings from a standard battery of genotoxicity assays. PROJECT A showed no phototoxic potential in an in vitro phototoxicity study in cultured Balb/c 3T3 cells.

No mortality was observed in the rat up to 26 weeks of treatment at doses up to 100 mg/kg per day. In the 26-week rat study, 2 females (1 from the 1 mg/kg/day group and 1 from the 10 mg/kg/day group), were found moribund at approximately 20 h post dosing on either day 90 (10 mg/kg/day) or day 145 (1 mg/kg/day). There were no anatomic pathology findings that explained the cause of moribundity in either animal.

In the 4-week monkey study, no morbidity was noted at any dose tested (up to

1000 mg/kg/day). However, in the 13-week monkey study, 1 female from the 1000 mg/kg per day group was euthanized in a moribund state on day 16. At necropsy, the animal had adverse changes in functional renal parameters (including increased urea nitrogen and creatinine concentrations and decreased sodium and chloride concentrations), as well as histopathological findings of bilateral kidney injury (tubular degeneration/ necrosis, tubular regeneration, tubular casts and mixed inflammation). These findings were considered to be secondary to PROJECT A-related diarrhea, dehydration and shock, rather than a direct effect of PROJECT A. Two additional monkeys from this dose group showed similar clinical findings and were placed on a dosing holiday (from day 22 through day 27; dosing was resumed on day 28). The high dose was subsequently lowered to 500 mg/kg on day 24, and this dose was well-tolerated for the remainder of the study. No similar kidney microscopic findings were observed for any other animal in this study. Renal function safety markers were normal in all other animals. Comparison of day 1 toxicokinetic data (Cmax and AUC) in cynomolgus monkeys from the 4-week and 13-week toxicity studies at the same dose levels revealed substantially higher exposures (3- to 5-fold higher) in the 13-week monkey study, which may, in part, explain the difference in toxic response between the 2 studies. The higher day 1 exposures seen in the 13-week cynomolgus monkey study may be related to the fact that the drug substance used in each study came from different manufacturers.

Major target organ toxicities in the rat were seen in the heart and/or skeletal muscles (only at the highest dose tested, 300 mg/kg/day after 4 weeks of dosing). With subchronic (13 weeks) and chronic daily dosing (26 weeks) in the rat, changes in the heart and skeletal muscle were either minimal or absent, and the NOAEL improved from 3 mg/kg per day (4 weeks of dosing) to 10 and 50 mg/kg per day, respectively. No target organs of toxicity were observed in the cynomolgus monkey. Systemic exposures of PROJECT A in humans and animals are shown in [[Table 1](#_bookmark61)].

Mild inflammation/necrosis of cardiac muscle, that was considered adverse, was observed in rats at doses of 30 (males only) and 300 mg/kg per day (males and females) in the 4-week study and at 100 mg/kg per day (males) in the 13-week study, but not in the 26-week adult rat (doses up to 50 mg/kg/day) or 11-week juvenile rat (doses up to 30 mg/kg/day) studies.

These cardiac findings were generally associated to some extent with increased serum levels of cTnI, while baseline levels of cTnI were often observed in animals without histological cardiac change.

Minimal inflammation/necrosis of the myocardium was also seen in both control- and PROJECT A-treated animals. This finding at least in control animals (predominantly males) was likely related to spontaneous cardiomyopathy, a commonly encountered age-related background change in male rats characterized by minimal degeneration/necrosis or degeneration/inflammation of the myocardium and a lack of correlation with elevations in cTnI levels [Chanut et al, 2013]. The relevance of the observed minimal cardiac finding in the rat studies to treatment with PROJECT A was determined on a study by study basis taking into consideration the incidence of the finding and cTnI levels. For instance, in the 4-week study, the incidence of minimal inflammation/necrosis of the myocardium was similar to that seen in both historical and concurrent control animals; however, in the 11-week juvenile rat study, the observed minimal inflammation/necrosis was considered PROJECT A-related because the incidence was substantially higher in males at dose levels of ≥ 3 mg/kg per day than in concurrent controls and correlated in part with increases in serum concentrations of cTnI (at ≥ 10 mg/kg/day). However, because in the 11-week juvenile study cardiac inflammation/necrosis remained minimal in severity, was within the range of historical control values for Wistar Han rats of similar age and was reversible when dosing stopped, this finding was not considered to be adverse.

No cardiac findings were observed in cynomolgus monkeys at doses up to 1000 mg/kg per day for 4 weeks or 13 weeks (up to 500/1000 mg/kg/day). No functional cardiac findings (ECG examination) were noted during the first 26 weeks of the 52-week monkey study (at doses up to 500 mg/kg/day). AUC values seen in male monkeys after 4 weeks of dosing at 1000 mg/kg per day were comparable to AUC values in male rats after 4 weeks of dosing at a dose where cardiac effects were observed (30 mg/kg/day), suggesting that the observed cardiac findings in rats may be rodent specific.

Taken together, these data show that while repeated daily dosing with PROJECT A resulted in minimal to mild cardiomyocyte necrosis at doses of ≥ 3 mg/kg per day, the finding was found to resolve after a 4-week postdose recovery period. A similar finding in humans cannot be ruled out. However, this finding appears to be monitorable by the measurement of serum concentrations of cTnI. Thus, appropriate measures can be included in clinical studies to minimize risk to human subjects.

Adverse skeletal muscle findings in the rat were observed in the 4-week study at 300 mg/kg per day but not in the 13- and 26-week adult studies or 11-week juvenile study up to the highest doses tested (100, 50 and 30 mg/kg/day, respectively). Additionally, skeletal muscle findings were not seen in the 4-week or 13-week repeated oral dose toxicity studies in cynomolgus monkeys.

The occurrence of similar skeletal muscle findings in humans cannot be ruled out; however, these findings appear to be monitorable by measuring serum activities of total CK, CK-MM and aldolase. Thus, appropriate measures can be included in the clinical study to minimize risk to human subjects.

PROJECT A at ≥ 30 mg/kg per day for 4 weeks resulted in increased liver weights in rats, which were associated with histopathologic changes of hepatocellular hypertrophy. Increased liver weights were also observed in the 13-week (100 mg/kg/day) and 26-week (≥ 10 mg/kg/day) adult rat studies, as well as in the 11-week oral toxicity study in juvenile rats

(≥ 3 mg/kg/day). Microscopic correlates of minimal to moderate hepatocellular vacuolation were noted in the 13-week and 26-week studies at ≥ 10 mg/kg per day, but not in the

11-week juvenile rat study.

Liver samples were taken at the end of dosing phase of the 4-week rat and monkey studies to determine liver enzyme activities (Project A-TX-0016, Project A-TX-0017, respectively). In the monkey, CYP4A activity in liver microsomes and carnitine acetyltransferase (CAT) activity in 10% liver homogenates were not affected at doses up to 1000 mg/kg per day. However, in the rat increased hepatic protein concentration (male rats) and slight (30 mg/kg/day females only) to substantial (both sexes) induction of hepatic CAT activity, as well as substantial induction of hepatic CYP4A (both sexes) and hepatic AcylCoA oxidase activities (male rats), were observed at 300 mg/kg per day, the highest dose tested.

Similar liver findings were absent in the cynomolgus monkey up to 1000/500 mg/kg per day after 13-weeks of dosing. In the 4-week and 13-week rat studies, the observed hepatic effects were reversible upon discontinuation of dosing and were not considered adverse. Therefore,

it is concluded that hepatic changes are not adverse and may be rodent-specific, suggesting that the risk to human subjects is small. Even if this is not the case, the findings are monitorable and reversible and, as such, the risk to human subjects in clinical studies can be minimized.

Evidence of PROJECT A-related altered lipid metabolism, as indicated by increases in total and HDL cholesterol, with concurrent increases in VLDL and/or LDL cholesterol and increases in triglycerides, was seen in both rats and cynomolgus monkeys. In rats, lipid changes were seen at 300 mg/kg after 4 weeks of dosing and at much lower dose levels (≥ 3 mg/kg/day) after longer durations of dosing (11, 13 and 26 weeks). In monkeys, lipid changes were noted after 13 weeks (1000/500 mg/kg/day) and after 26 weeks (≥ 10 mg/kg/day) of dosing. PROJECT A-induced alterations in lipid metabolism may be explained, at least in part, by the fact that target activation of PPARδ in adipose tissue specifically induces expression of genes required for FAO and energy dissipation, which in turn leads to improved lipid profiles and reduced adiposity [Wang et al, 2003]. Effects on lipid metabolism have also been reported with the PPARδ agonist GW501516 [Cox 2017]. PROJECT A-related lipid findings were found to be reversible when dosing stopped and are monitorable, and as such, the risk to human subjects in clinical studies can be minimized.

PROJECT A-related dry skin of the tail area was observed in rat studies of 11 to 26 weeks in duration at doses of ≥ 30 mg/kg per day, which showed signs of resolution soon after scheduled dosing was stopped. This finding may be related to the pharmacology of PROJECT A, as PPARð activation has been demonstrated to stimulate keratinocyte activation in vivo [Mao-Qiang et al, 2004] and in vitro in a disease model of epidermal hyperproliferation [Schmuth et al, 2004]. Similar findings were not seen in the monkeys in studies of up to

13 weeks duration.

In the GLP 4-week repeated oral dose toxicity study in rats administered PROJECT A at

≥ 30 mg/kg per day, gastrointestinal findings of squamous cell hyperplasia in the nonglandular stomach mucosa were noted, most conspicuously as hyperkeratosis of the epithelium, accompanied by an increase in the number of Ki-67-labeled nuclei. These findings were also observed in the 13-week (males at ≥ 3 mg/kg/day and females at 1, 3, and 100 mg/kg/day) and 26-week (≥ 10 mg/kg/day) studies and suggest that PROJECT A may induce a proliferative response in the nonglandular stomach mucosa in rats. However, the nonglandular stomach is a rodent-specific anatomical structure with no counterpart in monkeys or humans, and thus these findings are of limited clinical relevance. In both the

13-week and 11-week juvenile toxicity studies in rats, nonadverse minimal to mild hyperkeratosis was observed in the tongue and esophagus. These findings were not seen in the cynomolgus monkeys up to 13 weeks of dosing at doses at ≥ 500 mg/kg per day and higher. The clinical relevance of the tongue and esophageal findings observed in the rat is unknown.

To date, no carcinogenicity studies have been conducted with PROJECT A. PROJECT A is considered to pose no genotoxic carcinogenic hazard to human subjects based on negative results in a 3 genotoxicity studies (2 in vitro, 1 in vivo). However, it should be noted that some peroxisome proliferating chemicals have been found to be nongenotoxic animal carcinogens [Klaunig et al, 2003].

PPARδ modulation has been associated with embryonic stem cell proliferation [Lee et al, 2009], blastocyst implantation [Lim & Dey, 2000] and placental malformation [Nishimura et al, 2013]. While no reproductive toxicity studies of PROJECT A have been performed at this time, no findings indicative of toxicity to male or female reproductive organs of either rats or cynomolgus monkeys have been observed after dosing with PROJECT A for up to 13 weeks of repeated daily dosing.

## Table 1 Comparison of Systemic Exposures of PROJECT A Between Humans and Animals

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Species/ Study Duration, Route (Study Number)** | **Dose (mg/kg/day)** | **Sex (M/F)** | **Steady- state Cmax† (ng/mL)** | **Steady-state AUC†**  **(ng·h/mL)** | **Ratios Between**  **Human and Animal Exposures**†‡ | |
| **Cmax Ratio** | **AUC**  **Ratio** |
| Rat/ 4-week, oral (Project A-TX-0010) | 3 | M | 175 | 1150 | 1.2 | 1.4 |
| F | 452 | 1540 | 3.0 | 1.9 |
| 30 | M | 3310 | 12800 | 22.2 | 15.6 |
| F | 8590 | 20900 | 57.7 | 25.4 |
| 300 | M | 50700 | 252000 | 340.3 | 306.6 |
| F | 56200 | 295000 | 377.2 | 358.9 |
| Rat/ 13-week, oral  (Project A-TX-0020) | 1 | M | 93.4 | 541 | 0.6 | 0.7 |
| F | 203 | 676 | 1.4 | 0.8 |
| 3 | M | 371 | 1360 | 2.5 | 1.7 |
| F | 999 | 2670 | 6.7 | 3.2 |
| 10 | M | 1960 | 7590 | 13.2 | 9.2 |
| F | 6790 | 13400 | 45.6 | 16.3 |
| 100 | M | 30900 | 120000 | 207.4 | 146.0 |
| F | 43300 | 168000 | 290.6 | 204.4 |
| Rat/ 26-week, oral (Project A-TX- 0023) | 1 | M | 46.4 | 286 | 0.3 | 0.3 |
| F | 150 | 633 | 1.0 | 0.8 |
| 3 | M | 380 | 1030 | 2.6 | 1.3 |
| F | 619 | 2190 | 4.2 | 2.7 |
| 10 | M | 1110 | 4020 | 7.4 | 4.9 |
| F | 3590 | 9090 | 24.1 | 11.1 |
| 50 | M | 15500 | 41200 | 104.0 | 50.1 |
| F | 30400 | 111000 | 204.0 | 135.0 |
| Cynomolgus monkey/ 4-week, oral  (Project A-TX-0011) | 10 | M | 53.4 | 529 | 0.4 | 0.6 |
| F | 114 | 742 | 0.8 | 0.9 |
| 100 | M | 1020 | 6340 | 6.8 | 7.7 |
| F | 934 | 7340 | 6.3 | 8.9 |
| 1000 | M | 4710 | 22700 | 31.6 | 27.6 |
| F | 5120 | 28200 | 34.4 | 34.3 |
| Cynomolgus monkey/ 13- week, oral (Project A-TX-0021) | 10 | M | 96.1 | 934 | 0.6 | 1.1 |
| F | 415 | 1610 | 2.8 | 2.0 |
| 100 | M | 633 | 4770 | 4.2 | 5.8 |
| F | 798 | 7120 | 5.4 | 8.7 |
| 1000/500 | M | 1740 | 10500 | 11.7 | 12.8 |
| F | 2870 | 17100 | 19.3 | 20.8 |
| Cynomolgus monkey/ 52- week, oral (Project A-TX-0024) | 10 | M | 149 | 1430 | 1.0 | 1.7 |
| F | 203 | 1640 | 1.4 | 2.0 |
| 100 | M | 849 | 4750 | 5.7 | 5.8 |
| F | 336 | 2370 | 2.3 | 2.9 |
| 500 | M | 1480 | 8400 | 9.9 | 10.2 |
| F | 523 | 4090 | 3.5 | 5.0 |

*Table continued on next page*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Species/ Study Duration, Route (Study Number)** | **Dose (mg/kg/day)** | **Sex (M/F)** | **Steady- state Cmax† (ng/mL)** | **Steady-state AUC†**  **(ng·h/mL)** | **Ratios Between**  **Human and Animal Exposures**†‡ | |
| **Cmax Ratio** | **AUC**  **Ratio** |
| Juvenile rat/ 11- week, oral (Project A-TX-0019) | 1 | M | 92.2 | 441 | 0.6 | 0.5 |
| F | 227 | 675 | 1.5 | 0.8 |
| 3 | M | 381 | 2520 | 2.6 | 3.1 |
| F | 603 | 2270 | 4.0 | 2.8 |
| 10 | M | 1220 | 5350 | 8.2 | 6.5 |
| F | 2000 | 5580 | 13.4 | 6.8 |
| 30 | M | 3960 | 22500 | 26.6 | 27.4 |
| F | 7840 | 27700 | 52.6 | 33.7 |
| Human/ 14-day, oral  (Project A-CL-0001) | 75 mg | M/F | 149 | 822 | NA | |

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

† Exposures and exposure ratios at the NOAEL were underlined.

‡ The exposure ratios are calculated as animal exposures divided by human exposures.

PROJECT A has shown favorable effects on muscle cells based on in vitro findings and in vivo disease models and is expected to address muscle weakness and wasting in DMD and other muscle diseases. Nonclinical studies have identified the target organs of potential toxicity as the heart and skeletal muscle, although these findings were observed only in rats but not monkeys. In clinical studies, cardiac and muscle biomarkers indicative of damage will be closely monitored to mitigate the potential risks. Monitoring plans for cardiac and skeletal muscle toxicity have been implemented in the protocol for Study Project A-CL-0102 in accordance with recommendations made in the type C meeting FDA written response document dated 21 Nov 2018 and FDA meeting minutes on 28 Feb 2020. Similar monitoring plans are intended for the protocol for Study Project A-CL-1201. These plans include assessment of cTnI and cTnT levels, CK-MB, ECGs and echocardiograms (if indicated) at protocol specified intervals or more frequently if new symptoms arise. For skeletal muscle specific toxicity, these plans include monitoring the levels of enzymes, which are indicators of muscle damage (aldolase, total CK, CK-MM, transaminases and LDH).

Potential safety concerns and relevance to human usage for PROJECT A are summarized in [[Table 2](#_bookmark62)].

## Table 2 Potential Safety Concerns of PROJECT A

|  |  |
| --- | --- |
| **Key Nonclinical Observations** | **Relevance to Human Usage** |
| **Myocardium**   * After 28 daily doses, mild inflammation/necrosis was seen in 2 of 16 male rats at 30 mg/kg/day and in 2 of 16 male rats and 1of 16 female rats at   300 mg/kg/day. All 5 rats also had increased serum of cTnI concentrations.   * Minimal inflammation/necrosis was seen in a few male rats in all groups, including the control group, and 1 of 16 female rats at 30 mg/kg/day. One of 10 males at 3 mg/kg/day had an increased serum cTnI concentration but no inflammation/necrosis. These findings were considered to represent spontaneous rodent cardiomyopathy, a common incidental background disease in rats. * Myocardial effects were considered reversible as neither inflammation/necrosis nor increased serum concentration of cTnI was present at the end of the 4-week postdose recovery period. * In the 13-week study in rats, minimal (7 of 10 male rats) to mild (3 of 10 male rats) inflammation/necrosis of the myocardium, which correlated for the most part, with increases in cTnI concentrations were seen in male rats at 100 mg/kg/day after 13 weeks of dosing. Increases in cTnI and cardiac histopathology findings resolved at 100 mg/kg/day following a 4-week recovery period. These cardiac findings were considered adverse. * In the 26-week study in rats, higher individual cTnI concentrations were observed in 1 male each at 10 and 50 mg/kg/day (1 of 12 males in each dose group), which correlated microscopically with test article-related myocardial necrosis/inflammatory cell infiltrate. The incidence of necrosis/inflammation, characterized by loss of myofibers with replacement by mononuclear cells and fewer neutrophils, was increased at ≥ 10 mg/kg/day in both sexes. This was considered to be an exacerbation of rodent cardiomyopathy, a spontaneous degenerative disease that is very common in males and infrequent in females of this age. This finding was graded as minimal in all rats except 1 male at   10 mg/kg/day.   * In the 11-week juvenile toxicity study in rats, an increased incidence of minimal inflammation/necrosis of the myocardium that correlated in part with increases in serum concentrations of cTnI (at ≥ 10 mg/kg/day) was seen in males at dose levels of ≥ 3 mg/kg/day (6 of 10 males in each dose group) than in control males (1 of 10). This finding was considered to be an   PROJECT A-related exacerbation of this common background finding. Because cardiac inflammation/necrosis findings remained minimal, stayed within the range of historical control values for Wistar Han rats and were reversible, these  findings were not considered to be adverse. | * PROJECT A may cause myocardial inflammation/necrosis in human subjects. |
| **Skeletal muscle**   * Skeletal muscle degeneration and/or mononuclear cell infiltrates in both male and female rats at 300 mg/kg/day after 28 daily doses. * Increased serum activities of total CK, CK-MM, aldolase, AST and/or LDH (in both male and female rats at 300 mg/kg/day). * Skeletal muscle degeneration was considered reversible because at the end of the 4-week postdose recovery period, these findings were generally of minimal intensity and their incidence was comparable between the control and treated animals. | * PROJECT A may cause skeletal muscle degeneration in human subjects. |

AST: aspartate aminotransferase; CK: creatine kinase; CK-MM: CK isoenzymes found in skeletal and heart muscle; cTnI: cardiac troponin I; LDH: lactate dehydrogenase.

Source: Study Project A-TX-0010; Study Project A-TX-0020; Study Project A-TX-0019; Study Project A-TX-0023.

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