### Safety Pharmacology

PROJECT X has been evaluated in 3 core safety pharmacology studies. A detailed description of the studies and their results can be found in End-of-Text Table 1.2. Key findings are discussed below.

### Effects on Central Nervous System

The effects of PROJECT X on the central nervous system were evaluated in rats. PROJECT X was administered to 6 male Sprague-Dawley (SD) rats per group at dose levels of 0 (0.5% MC), 0.1, 1 and 10 mg/kg per day (Study Project X-PT-0001). The rats were observed for general physical condition and behavior using a modified Irwin’s method. Observations of general physical condition and behavior were conducted before dosing and at 1, 2, 4, 6, 8, 10 and 24 hours after dosing.

PROJECT X at doses up to 10 mg/kg per day had no effects on general physical condition or behavior in rats; therefore no effects were observed on the central nervous system.

### Effects on the Cardiovascular and/or Respiratory System

* + - * 1. **In Vitro Effects on the hERG Current**

The effects of PROJECT X on the hERG current were investigated in hERG-transfected human embryonic kidney (HEK) 293 cells using the whole cell clamp method

(Study Project X-PT-0003). The test cells (5 cells/group) were perfused with the application solutions containing PROJECT X, the vehicle control substance (dimethyl sulfoxide, 0.3 vol%), and the positive control substance (E-4031, a specific IKr blocker, 0.1 mcmol/L). The peak amplitudes of the tail current were measured before and after perfusion for 10 minutes, and the inhibition rate (% inhibition) was calculated for each cell. Moreover, the compensated inhibition rate (compensated % inhibition) was calculated for each cell using the mean inhibition rate of the vehicle control group to eliminate the effect of the vehicle. The expected concentrations in perfusate of PROJECT X were set at 0.03, 0.3 and 3 mcmol/L as PROJECT X; however the concentrations were adjusted at 0.0375, 0.375 and 3.75 mcmol/L because it had been revealed that PROJECT X adsorbs to the chamber. The actual concentrations were 0.0142, 0.153 and 3.39 mcmol/L, so that the actual concentrations were used for the following description.

The hERG-current-suppression rates of PROJECT X, compensated by the mean suppression rate of the vehicle control group at the concentrations of 0.0142, 0.153 and 3.39 mcmol/L were

-0.9 ± 6.1%, 6.9 ± 6.4%, respectively. At 3.39 mcmol/L, the compensated suppression rate was 13.8 ± 11.1% and the rate was statistically significant (Williams’ test, P<0.05) when compared to that of the vehicle control group.

The results described above indicate that PROJECT X does not affect the hERG current in hERG-transfected HEK293 cells up to the concentration of 0.153 mcmol/L. PROJECT X suppressed the hERG current by 13.8% at the concentration of 3.39 mcmol/L.

### In Vivo Effects on Cardiovascular and Respiratory System

The effects of PROJECT X on cardiovascular and respiratory parameters were evaluated in the cynomolgus monkey. Four unanesthetized males were administered PROJECT X at doses of 0 (0.5% MC), 0.1, 1 and 10 mg/kg per day (Study Project X-PT-0002). Dosing of PROJECT X was performed in an escalating single-dose design on days 1, 8, 15 and 22. Astemizole

(10 mg/kg) was administered as a positive control for QT prolongation on Day 36. PROJECT X concentrations in plasma were measured to determine systemic exposure.

There were no test article-related changes in the following items at doses up to 10 mg/kg per day: blood pressure (systolic, diastolic and mean), heart rate, electrocardiogram (ECG) parameters (PR interval, QRS duration, QT interval and QTcF [corrected with Fridericia’s formula]), respiratory rate, blood gas parameters (arterial blood pH, arterial oxygen and carbon dioxide tensions and hemoglobin oxygen saturation), electrolytes (ionized calcium, sodium, potassium and chloride), or intra-abdominal body temperature.

Vomiting was observed once in one animal approximately 8 hours after dosing at 1 mg/kg per day. This was not dose-dependent, occurred infrequently and was therefore considered to be of little toxicological significance. No other abnormalities related to the test article were observed in any animal at any dose level.

The Cmax and AUC24 increased nearly dose proportionally between 0.1 and 1 mg/kg, but the increase between 1 and 10 mg/kg per day was less than the dose ratio. Mean Cmax and AUC24 values at 10 mg/kg were 509 ng/mL and 7030 ng·h/mL, respectively.

In conclusion, PROJECT X at doses up to 10 mg/kg per day had no effects on the cardiovascular and respiratory systems, electrolytes, intra-abdominal body temperature or clinical signs.

### 4.1.4 Other Pharmacology Studies

AS1920697 (FTY720 phosphate), is a S1P receptor agonist that was used as an active comparator to PROJECT X. AS1920697 was manufactured by Astellas and referred to as FTY720 phosphate. AS1920697 (FTY720 phosphate) was administered intravenously to conscious Lewis rats as a 10 minute continuous infusion at doses of 0.01, 0.03 and 0.1 mg/kg (Study Project X-PH-0013). AS1920697 (FTY720 phosphate) affected heart rate and blood pressure at doses of 0.03 mg/kg or more, indicating that AS1920697 (FTY720 phosphate) affects the cardiovascular system.

## Toxicology

Tabulated overviews of toxicology studies can be found in End-of-Text Table 3.1. An overview of toxicokinetic (TK) studies can be found in End-of-Text Table 3.2. A summary of TK data can be found in End-of-Text Table 3.3. The pivotal studies included single dose and 4-week repeated-dose (with and without recovery assessment) in both rats and monkeys, 2 in vitro genotoxic studies (bacterial reverse mutation and chromosomal aberration tests) and reproductive studies for embryo-fetal development in both rats and rabbits. All pivotal toxicology studies were conducted in accordance with the standards of Japanese Good Laboratory Practices (GLP) (Notifications and Ordinances, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare). These standards are also in accordance with United States Food and Drug Administration (FDA) GLP regulations.

### Single-Dose Toxicity

Single dose toxicity studies are summarized in End-of-Text Table 3.4. The acute toxicity of PROJECT X was evaluated in 2 GLP studies; 1 in rats (Study Project X-TX-0001) and 1 in monkeys (Study Project X-TX-0002). The animals were administered a single oral dose of PROJECT X, in a vehicle of 0.5% MC solution. In the single dose oral toxicity studies, the lethal doses were more than 60 mg/kg per day in rats and more than 45 mg/kg per day in monkeys. Key findings from these studies are discussed below.

### Rats

PROJECT X was orally administered once to SD rats (5/sex/group) at 30 and 60 mg/kg

(Study Project X-TX-0001). The dose of 60 mg/kg is the highest oral dose technically possible in rats, as 3 mg/mL is the highest feasible concentration of dosing suspension of PROJECT X in 0.5% MC. The maximum dose volume for a single oral dose toxicity study in rats is

20 mL/kg as specified in the Institutional Standard Operating Procedure (SOP) in Astellas based on the humane treatment of animals. Following administration, general signs were observed daily and body weight was measured periodically. Gross pathological examination was performed on the 14th day after dosing.

No deaths occurred up to a dose of 60 mg/kg. The only drug effect observed was a slight and transient loss of body weight or suppression of body weight gain noted in females dosed at 30 and 60 mg/kg.

### 4.3.1.2 Monkeys

PROJECT X was administered as a single oral dose to cynomolgus monkeys (1/sex/group) at a dose of 20 or 45 mg/kg (Study Project X-TX-0002). The dose of 45 mg/kg is the highest oral dose technically possible in monkeys, as 3 mg/mL is the highest feasible concentration of dosing suspension of PROJECT X in 0.5% MC and the maximum dose volume for a single oral dose toxicity study in monkeys is 15mL/kg as specified in the Institutional SOP in Astellas based on the humane treatment of animals. Systemic exposure was evaluated by determining the plasma PROJECT X concentrations. A 14-day observation period was established, and observation of general signs, measurement of body weight and food consumption, hematology, and blood chemistry measurements was performed during this period.

No deaths occurred in either group. Vomiting and an elevated fibrinogen level were seen at 20 mg/kg, as were decreases in lymphocyte count and albumin level at both 20 and 45 mg/kg. An increase in total bilirubin level was also noted at 45 mg/kg. A reduction in lymphocyte count in both dose groups was attributed to the pharmacological activity of PROJECT X.

The Cmax and AUC24 values for males increased almost in proportion to the dose level, whereas the values for females did not differ between dose groups (20 and 45 mg/kg). At 20 mg/kg, Cmax and AUC24 for the male were lower than for the female. At 45 mg/kg, Cmax and AUC24 for the male and female were comparable.

### 4.3.2 Repeat-Dose Toxicity

Nonpivotal dose range-finding repeated dose toxicity studies of PROJECT X conducted in rats (Studies Project X-TX-0101, Project X-TX-0102, Project X-TX-0103) and monkeys

(Study Project X-TX-0104) are summarized in End-of-Text Table 3.5. Tabulated results of PROJECT X pivotal 4-week oral repeated dose toxicity studies, with and without a 4-week recovery period, conducted in rats (Studies Project X-TX-0003 and Project X-TX-0009) and monkeys (Studies Project X-TX-0004 and Project X-TX-0010) can be found in End-of-Text Tables 3.6.1, 3.6.2,

3.6.3 and 3.6.4, respectively.

Repeated dose toxicity studies of PROJECT X were conducted up to 4 weeks in duration with and without a 4-week recovery in the rat and with a 4-week recovery in the monkey. High dose levels in the 4-week studies for the rats (10 mg/kg per day) and monkeys (60 mg/kg per day) were based on findings in the dose range-finding 2-week repeated dose toxicity study and a technically maximum feasible dose level/regimen, respectively. In the 2-week repeated dose study in rats (Study Project X-TX-0101), a dose level of 30 mg/kg per day was associated with toxicities described in End-of-Text Table 3.5. Of the greatest importance in the selection of the maximum tolerated dose (MTD) for the 4-week study were the dose related weight gain depression and weight loss (total weight gain, control: male, 72 g; female, 36 g vs. 30 mg/kg per day: male, 4 g; female, -4 g) as well as the effects on the hematopoiesis (decreased reticulocytes) at 30 mg/kg per day in the 2-week study. When projected over a 4- week study, these changes were considered unacceptably severe. Consequently, the MTD for the 4-week study in rats was set at 10 mg/kg per day. For the first 4-week repeated dose toxicity study in monkeys (Study Project X-TX-0004), a dose of 10 mg/kg per day was well tolerated and produced only increased lung weight in addition to changes related to the pharmacological action of the test article: decreased lymphocyte count, atrophy of the germinal center, atrophy of the periarterial lymphoid sheath, and/or widening of the marginal zone in the spleen. Therefore, in the additional 4-week repeated dose toxicity study in monkeys

(Study Project X-TX-0010) once daily (qd) and twice daily (bid) doses of 30 mg/kg (30 and 60 mg/kg per day, respectively) were employed as the maximum feasible dose levels determined by the highest possible concentration in 0.5% MC (3 mg/mL) and the highest feasible dosing volumes in a repeated dose study in monkeys (10 mL/kg qd [total dose:

30 mg/kg per day] and 10 mL/kg bid with a 4-hour interval [total dose: 60 mg/kg per day]).

### Toxicokinetics

An overview of toxicity studies that included a TK evaluation is provided in End-of-Text Table 3.2; TK data are presented in End-of-Text Table 3.3.

In rats, indices of exposure (Cmax and AUC24) on day 1 increased almost dose dependently, whereas on days 14 and 28 the increase was more than dose-dependent in both male and female rats. In monkeys, indices of exposure increased almost dose proportionally however, the increase was just less than dose proportional. No clear sex differences were noted in any TK parameter throughout the dosing period in both rats and monkeys in the 4-week repeated dose studies, except for higher exposure values in female monkeys at doses greater than

10 mg/kg per day.

### Toxicity

Major findings from toxicology studies of PROJECT X conducted to date in rats and monkeys included an increase in lung weight, decreased lymphocyte counts and histopathological changes in the spleen, thymus and lymph nodes. Reduction in lymphocyte count and corresponding histopathological changes were consistent with the pharmacological action of PROJECT X. These findings recovered or tended to recover in the 4 weeks after dosing cessation in both rats and monkeys. At higher dose levels in monkeys, general condition deteriorated with decreased food consumption and body weight, and some animals were subsequently sacrificed in moribund condition. In Study Project X-TX-0010 toxic changes in the bone marrow, liver, kidney and gastrointestinal tracts and adrenals as well as QT and PR prolongations were noted.

### Rat - 4-Week Study

PROJECT X was orally administered once daily for 4-weeks to male and female SD rats (10/sex/dose group) at 0 (0.5% MC), 0.01, 0.1, 1, and 10 mg/kg per day

(Study Project X-TX-0003). Five animals of each sex were added to the control and highest dose groups in order to assess the reversibility of any toxicity in a subsequent 4-week recovery period. The following items were examined: clinical signs, body weight, food consumption, opthalmology, clinical pathology (hematology and blood chemistry), urinalysis, respiratory function (respiratory rate, tidal volume and minute volume, using the whole body plethysmograph method), and pathology (gross pathology, organ weight and histopathology). Plasma drug concentrations were also measured (3/sex for control, and 6/sex for

PROJECT X-treated groups) on days 1, 14 and 28.

At 0.01 mg/kg per day, no treatment-related toxic effects were evident in any animal.

At 0.1 mg/kg per day, decreased total protein concentration and beta-globulin ratio, and increased lung weight were noted in males.

At 1 mg/kg per day, decreased beta-globulin ratio and increased lung weight were noted in males and females. Decreased total protein concentration and increased total cholesterol concentration were noted in males. Decreased food consumption was noted in females.

At 10 mg/kg per day, decreased body weight gain and food consumption, increased total cholesterol concentration, decreased beta-globulin ratio, increased lung weight, alveolar accumulation of foam cells in the lungs, and dilatation of the fundic glands in the stomach were noted in males and females. Decreased total protein concentration and increased total bilirubin were noted in males.

Other findings included decreased leukocyte count (mainly due to decreased lymphocyte count) in males and females, decreased gamma-globulin ratio in males, increased lymphocytes in the thymic medulla in males and females, and several findings in the spleen (decreased organ weight irrespective of macroscopic size of the spleen, decreased lymphocytes in the periarterial lymphoid sheath, narrowing of the marginal zones, prominent lymphoid follicles, and atrophy of the lymphoid follicles) were noted at 0.1 mg/kg per day and higher. These changes were considered to be related to the pharmacological action of the test article; therefore, they were not considered to be toxicological findings.

At the end of the recovery period in the 10 mg/kg per day group, decreased leukocyte count, decreased beta-globulin and gamma-globulin ratios, and increased relative lung weight were noted in males, and decreased lymphocytes in the periarterial lymphoid sheath and atrophy of the lymphoid follicles in the spleen were noted in males and females. The changes in the recovery group were of lesser magnitude compared to those at the end of the dosing period, which suggests that PROJECT X treatment-related changes are reversible. Other changes noted during the dosing period disappeared during the 4-week recovery period.

The Cmax and AUC24 on day 1 increased almost dose-dependently, whereas the values on days 14 and 28 increased more than dose-dependently for both males and females. There were no sex differences in any TK parameters throughout the dosing period.

It was concluded that, under the conditions of this study, the NOAEL was 0.01 mg/kg per day for males and 0.1 mg/kg per day for females. However, considering results in the additional 4-week study in rats (Study Project X-TX-0009), the NOAEL for male rats has been reassessed to be 0.06 mg/kg per day (see Section 4.3.2.2.2).

### Rat – 4-Week Study (Additional)

The initial 4-week repeated dose toxicity study in rats (Study Project X-TX-0003) was conducted at 0.01, 0.1, 1, and 10 mg/kg per day and the NOAEL was 0.01 and 0.1 mg/kg per day in males and females, respectively, based on the increased lung weight. Based on the 10-fold difference in dose levels and exposures between the NOAEL and the dose level in males at which lung weight was increased, an additional 4-week repeated dose toxicity study in rats was conducted at 0.03 and 0.06 mg/kg per day to seek a more precise understanding of the relationship between dose/exposure and increased lung weight (Study Project X-TX-0009).

PROJECT X was orally administered once daily for 4 weeks to male and female rats SD (10/sex per group) at 0 (0.5% MC), 0.03, and 0.06 mg/kg per day (Study Project X-TX-0009). The following observations and examinations were performed in this study: clinical signs, body weight, food consumption, ophthalmology, urinalysis, hematology, blood chemistry, gross pathology, organ weights, and histopathology. Plasma drug concentrations were also measured on Days 1, 14, and 28.

No toxic changes were noted in any group.

Low leukocyte count, mainly due to low lymphocyte count, and an increase in lymphocytes in the thymic medulla were noted in males and females in the 0.03 and 0.06 mg/kg groups, and low gamma-globulin ratio was noted in males in the 0.06 mg/kg group. Splenic changes (low organ weight, a decrease in lymphocytes in the periarterial lymphoid sheath, and/or prominent lymphoid follicles) were noted in males and females in the 0.06 mg/kg group.

These changes were considered to be related to the pharmacological effect of the test article; therefore, they were not considered to be toxicological findings.

In toxicokinetics, Cmax and AUC24 on days 1, 14, and 28 increased almost dose proportionally in males and females. Following repeated dosing, Cmax and AUC24 on day 14 were greater than those on day 1, and Cmax and AUC24 on day 28 were similar to those on day 14. There were no sex differences in any TK parameters throughout the dosing period.

It was concluded that, under the conditions of this study, the NOAEL was 0.06 mg/kg per day for males and females because no toxic changes, including in lung weight, were noted in any group. Based on results from the former 4-week study in rats (Study Project X-TX-0003, Section 4.3.2.1.1.), the NOAEL in a 4-week repeated dose toxicity study was determined to be 0.06 and 0.1 mg/kg per day for male and female rats, respectively.

### Monkeys – 4-Week Study

PROJECT X was orally administered once daily for 4 weeks to 3 male and 3 female cynomolgus monkeys per group at 0 (0.5% MC), 0.01, 0.1, 1, and 10 mg/kg per day (Study Project X-TX-0004). Three animals of each sex were added to the 10 mg/kg per day group in order to assess the reversibility of any toxicity in a subsequent 4-week recovery period. The following items were examined: clinical signs, body weight, food consumption, ophthalmology (including fluorescein fundus angiography), electrocardiography, clinical pathology (hematology, blood chemistry, and immunophenotyping), urinalysis, and pathology (gross pathology, organ weight, and histopathology). Plasma drug concentrations were also measured on days 1, 14 and 28.

At 0.01 and 0.1 mg/kg per day, no toxic changes were noted in any examination. At 1 mg/kg per day, increased lung weight was noted in 1 male.

At 10 mg/kg per day, total protein, albumin, and globulin levels decreased in 1 male and 2 females. Increased lung weight was noted in all males and 1 female.

Attributed to the pharmacological action of the test article, the following changes were noted in a dose-dependent manner: decreases in lymphocyte count, atrophy of the germinal center, atrophy of the periarterial lymphoid sheath, and/or widening of marginal zone in the spleen in males and females dosed with 0.1 mg/kg per day or more. Immunophenotyping analysis gave the following results: decreases in CD3+CD4+ (helper T) cell and CD3+CD8+ (suppressor/ killer T) cell counts (due to CD28+CD95- [naïve] cell and CD28+CD95+ [central memory] cell counts), and CD3-CD20+ (B) cell count for males and females dosed with

0.1 mg/kg per day or more; decreases in CD28-CD95+ (effector memory) cell counts in both CD3+CD4+ and CD3+ CD8+ lymphocytes for males and females dosed with 1 mg/kg per day or more.

After the 4-week recovery period, the changes noted in the 10 mg/kg per day group during the dosing period recovered or tended to recover.

The Cmax and AUC24 increased almost proportionally, except for 10 mg/kg per day males which was less than dose proportional. No clear sex difference was noted in any TK parameters. On days 14 and 28, no clear changes were noted in any TK parameters, compared to those on day 1.

The NOAEL was judged to be 0.1 mg/kg per day for males and 1 mg/kg per day for females.

### 4.3.2.2.4 Monkey – 4-Week Study (Additional)

In the first 4-week repeated dose toxicity study in monkeys (Study Project X-TX-0004), a dose of 10 mg/kg per day was well-tolerated and produced only increased lung weight in addition to changes related to the pharmacological action of the test article (Section 4.3.2.3.). Therefore, the additional 4-week repeated dose toxicity study in monkeys was conducted to cover a full toxicity profile of PROJECT X in monkeys at higher dose levels using a maximum feasible dose (Study Project X-TX-0010).

PROJECT X was orally administered once daily for 4 weeks to 4 male and 4 female cynomolgus monkeys per group at 0 (0.5% MC), 30 and 60 mg/kg per day (30 mg/kg qd and 30 mg/kg bid with a 4-hour interval). Four animals of each sex were added to the 30 and 60 mg/kg per day group in order to assess the reversibility of any toxicity in a subsequent 4-week recovery period. The following items were examined: clinical signs, body weight, food consumption,

ophthalmology (including fluorescein fundus angiography), electrocardiography, clinical pathology (hematology, blood chemistry, and immunophenotyping), urinalysis, and pathology (gross pathology, organ weight, and histopathology). Plasma drug concentrations were also measured on Days 1, 14 and 28.

One male and 3 females in the 30 mg/kg per day group and 4 males and 2 females in

60 mg/kg per day group were sacrificed due to deteriorated condition. Findings described below were observed in both the animals sacrificed in extremis and the animals sacrificed in terminal; however, the findings tended to increase in the incidence rate and the degree in animals sacrificed in extremis. With regard to clinical signs, vomiting, a decrease in spontaneous activity, stagger, and/or vertical nystagmus were observed. Additionally, abnormal position (sitting position, crouching, and lateral position), suppressed response to stimulation, disappearance of touch response, and/or hypothermia were observed when animals were considered moribund. Decreased food consumption and decreased body weight as well as decreased urine volume and increased urinary protein and glucose concentration were noted. Decreased erythrocyte count, hematocrit value, hemoglobin concentration, and/or reticulocyte ratio, prolongation of activated partial thromboplastin time and/or prothrombin time were noted. Increased aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, total cholesterol, and/or blood urea nitrogen, decreased total protein due to decreased albumin, and/or decreased inorganic phosphorus, sodium, and/or chloride were noted. In histopathology, findings included: hypocellularity in the bone marrow, atrophy of the fundic gland or pyloric mucosa, and/or extension of the gastric pits in the stomach, atrophy of the mucosa in the ileum and/or colon, or erosion in the colon, degeneration/necrosis in the centrilobular hepatocytes, hyaline droplets and vacuolation in the renal tubules, and depletion of lipids and/or hypertrophy in the zona fasciculata cells in the adrenals. Prolongation of QRS interval, QTcF in a small number of animals sacrificed in extremis, and slight PR prolongation in some animals were also observed.

At both levels, the following changes were considered due to exaggerated pharmacological action of the test article: decreased peripheral lymphocyte count, and decreased total

CD3+ (T) cell, CD3+CD4+ (helper T) cell, CD3+CD8+ (suppressor/killer T) cell, and

CD3-CD20+ (B) cell, and CD28+CD95- (naïve) cell, CD28+CD95+ (central memory) cell, and CD28-CD95+ (effector memory) cell counts in both CD3+CD4+ and CD3+CD8+ lymphocytes, atrophy of the germinal center, atrophy of the marginal zone, and/or widening of the marginal zone in the spleen, and marked atrophy/involution of the thymus.

In toxicokinetics, the mean Cmax and AUC24 at doses of 30 and 60 mg/kg per day increased in a dose-dependent manner on days 1 and 14 for both male and female. No marked difference in the Cmax and AUC24 between 30 and 60 mg/kg per day was observed on day 28 in males.

The mean Cmax and AUC24 at 30 and 60 mg/kg/ per day were greater than those at 10 mg/kg per day in the previous monkey study (Project X-TX-0004). The mean concentrations of PROJECT X in the plasma of females were higher than those of males on days 1 and 14.

After the 4-week recovery period, all changes noted during the dosing period had recovered or tended toward recovery.

It was concluded that, under the conditions of this study, higher dose levels, 30 and 60 mg/kg per day as PROJECT X, caused moribundity with deteriorated general conditions, decreases in food consumption and body weight as well as changes in hematology and biochemistry with histopathological changes in the adrenals, liver, kidneys, and bone marrow.

Histopathological abnormalities in the gastrointestinal tract were also identified.

### 4.3.3 Genotoxicity

PROJECT X has been evaluated in 2 GLP in vitro genotoxicity studies: a bacterial reverse mutation assay (Study Project X-TX-0005) and a chromosome aberration test

(Study Project X-TX-0006). Tabulated results of these studies can be found in End-of-Text tables

3.8.1 and 3.8.2, respectively.

PROJECT X did not induce gene mutation when tested in 5 strains of bacteria (*Salmonella typhimurium* [TA98, TA100, TA1535, and TA1537] and *Escherichia coli* [WP2*uvrA*]). PROJECT X did not induce chromosomal aberrations in Chinese hamster lung (CHL/IU) cells, regardless of treatment length or the presence or absence of metabolic activation.

In conclusion, PROJECT X had no genotoxic potential in a reverse mutation test or chromosome aberration test.

### Carcinogenicity

No carcinogenicity studies of PROJECT X have been conducted to date.

### Reproductive and Developmental Toxicity

Nonpivotal dose range-finding reproductive and developmental toxicity studies of PROJECT X conducted in rats (Study Project X-TX-0105) and rabbits (Studies Project X-TX-0106 and

Project X-TX-0107) are summarized in End-of-Text Table 3.7. Tabulated results of pivotal reproductive and developmental toxicity studies conducted to evaluate the effects of PROJECT X on embryo-fetal development conducted in rats (Study Project X-TX-0007) and rabbits (Study Project X-TX-0008) can be found in End-of-Text Tables 3.8.1 and 3.8.2, respectively.

Key findings are presented below.

### Embryo-fetal Development in Rats

In the dose-finding embryo-fetal toxicity study in the rat, effects on dams included decreases in food consumption and body weight (Study Project X-TX-0105) at all dose levels. The embryo- fetal viability deteriorated severely and all embryo-fetuses in all dams died for all dose groups (3, 10 and 30 mg/kg per day).

In the definitive GLP embryo-fetal development study in rats (Study Project X-TX-0007), PROJECT X was administered orally to groups of SD rats, each consisting of 20 copulated females at dose levels of 0 (0.5% MC), 0.03, 0.1, 0.3, and 1 mg/kg per day once daily from gestation days (GD) 7 to GD17. Dams underwent Cesarean section on GD20. A toxicokinetic-satellite group consisting of 8 copulated females (4 copulated females for the

control group) was set up for each dose group to determine the plasma drug concentration of PROJECT X.

The effects on dams included decreased food consumption at 0.1 mg/kg per day or more and suppressed body weight gain at 1 mg/kg per day. Effects on embryo-fetal development included high values for the incidences of visceral variation (thymic remnant in neck) and visceral abnormalities (ventricular septal defects, abnormal lobation of liver), low values for the number of ossified sacro-caudal vertebrae as well as a marked increase in the index of embryo-fetal deaths and external abnormalities in the live fetuses such as generalized edema. There were no effects on embryo-fetal development at 0.03 mg/kg per day. PROJECT X induced teratogenic effects at 0.1 mg/kg per day, fetal growth inhibition effects at 0.3 mg/kg per day and embryo-fetal lethal effect at 1 mg/kg per day in rats.

Plasma Cmax and AUC24 increased almost dose proportionally on GD7 and GD17. The NOAEL was estimated to be 0.03 mg/kg per day for both dams and embryo-fetal development.

### Embryo-fetal Development in Rabbits

In the oral dose-finding study in rabbits (Study Project X-TX-0106), decreases in body weight, food consumption and fecal output as well as abortion (in 2/4 dams) were observed at

30 mg/kg per day, and fetal lethality was observed at 3 mg/kg per day or more.

In the pivotal GLP embryo-fetal development study in rabbits (Study Project X-TX-0008), PROJECT X was administered orally to groups of New Zealand white rabbits, each consisting of 17 to 20 copulated females at dose levels of 0 (0.5% MC), 0.3, 1, and 3 mg/kg per day once daily from GD6 to GD18. Dams underwent Cesarean section on GD28. Plasma drug concentrations on the first and final days of dosing (GD6 and GD18) were also measured.

No treatment related findings were recorded on dams and embryo-fetal development at any dose tested.

Plasma Cmax values were comparable on both GD6 and GD18. Indices of exposure (Cmax and AUC24) increased almost dose proportionally on both GD6 and GD18.

Based on TK parameters the exposure level in rabbits at 3 mg/kg per day (NOAEL) is more than 5 times higher than that in rats administered 0.1 mg/kg per day at which there were teratogenic effects [Rat Cmax: 35.711 ng/mL and rabbit Cmax: 218.122 ng/mL; Rat AUC24: 620 ng·h/mL and rabbit AUC24: 3380 ng·h/mL].

### Local Tolerance

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

### Other Toxicity Studies

No other toxicity studies of PROJECT X have been conducted to date.

## Integrated Nonclinical Overview and Conclusion: Potential Clinical Relevance

### Pharmacology

PROJECT X is a new molecular entity discovered by Astellas Pharma Inc. that has an agonistic effect on S1P receptors, specifically S1P1 and S1P5. S1P has been implicated in the regulation of many cellular functions including proliferation, apoptosis, survival, adhesion, differentiation, and migration [[Hla, 2004](#_bookmark0)]. S1P receptors are divided into 5 subtypes (S1P1 to S1P5). Pharmacological and genetic approaches have revealed that stimulation of S1P1 causes sequestration of lymphocytes in lymphoid tissues [[Forrest et al, 2004](#_bookmark0); [Fujishiro et al,](#_bookmark0) [2006](#_bookmark0)], while stimulation of S1P3 causes bradycardia [Peters & Alewijnse, 2007].

PROJECT X showed potent binding affinities for human S1P1 and S1P5, and preferably stimulates these 2 subtypes over S1P2, S1P3 and S1P4. PROJECT X decreased the number of lymphocytes in peripheral blood in rats, which is thought to be mediated through the stimulation of S1P1. The pharmacologically active dose (ED50) in rats is 0.023 mg/kg with a predicted clinically effective Cmax of 5.46 ng/mL and AUC24 of 95.68 ng·h/mL, based on the lymphopenia effects observed following multiple daily doses of PROJECT X. PROJECT X prevented the development of EAE, an animal model of MS. As infiltration of T cells into the central nervous system is thought to be involved in the development of EAE [Hickey et al, 1991], PROJECT X might ameliorate EAE by inhibiting encephalitogenic T-cell responses through the sequestration of T cells in the secondary lymphoid tissues.

The less-potent effect of PROJECT X on the cardiovascular system as compared with its lymphopenia-inducing effect may be ascribed to the high selectivity of PROJECT X for S1P1 over S1P3. Further discussion regarding cardiovascular findings of PROJECT X is made in Section 4.4.2.3. By contrast, AS1920697 (FTY720 phosphate) showed similar agonistic potency on S1P1 and S1P3. In HSV-1 infected mice, PROJECT X does not alter the host’s defense system against HSV-1 infection (up to 0.3 mg/kg per day) unless higher doses of PROJECT X are administered, exceeding the half maximal effective dose (ED50) for lymphopenia in uninfected mice.

### Toxicology

Data from nonclinical toxicokinetic and metabolic studies conducted with PROJECT X indicate that adequate doses were tested in appropriate in vitro and in vivo models to sufficiently evaluate the potential toxicity profile of PROJECT X in human studies. All findings were evaluated for relevance to human risk assessment and impact on clinical trial design.

A summary of the findings of potential clinical interest observed in the pivotal rat and monkey repeated-dose studies is presented in ([Table 7](#_bookmark65)).

### Table 7 Summary of Treatment-Related Changes of Potential Clinical Interest in Pivotal Repeated-Dose Toxicity Studies of PROJECT X in Rats and Monkeys

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment-Related Change** | **Dose Level of Observed Response (mg/kg) [HED] (mg/kg)**† | | | | |
| **Rat** | | **Monkey** | | |
| **Male** | **Female** | **Male** | **Female** | |
| **Clinical Signs and General Condition** | | | | | |
| Moribundity | -- | -- | 30 [9.7] | 30 [9.7] | |
| Vomiting, decreased activity,  staggering | -- | -- | 30 [9.7] | 30 [9.7] | |
| ↓ Food consumption and body weight | 10 [1.6] | 1 [0.16] | 30 [9.7] | 30 [9.7] | |
| **Hematology** | | | | | |
| ↓ Lymphocytes‡ | 0.03 [0.005] | 0.03 [0.005] | 0.1 [0.032] | 1 [0.32] | |
| ↓ Leukocyte count‡ | 0.03 [0.005] | 0.03 [0.005] | 0.1 [0.032] | 0.1 [0.032] | |
| ↓ Reticulocytes | -- | -- | 30 [9.7] | 30 [9.7] | |
| ↓ Monocytes, neutrophils | -- | -- | 30 [9.7] | 30 [9.7] | |
| **Blood Chemistry** | | | | | |
| ↑ AST, ALT | -- | -- | 30 [9.7] | 30 [9.7] | |
| ↑ BUN | -- | -- | 30 [9.7] | 30 [9.7] | |
| ↓ beta globulin ratio | 0.1 [0.016] | 1 [0.16] | -- | -- | |
| ↓ gamma globulin ratio | 0.06 [0.010] | -- | -- | -- | |
| ↓ globulin | -- | -- | 10 [3.2] | 10 [3.2] | |
| ↑ Total cholesterol | 1 [0.16] | 10 [1.6] | 30 [9.7] | 30 [9.7] | |
| ↓ Total protein | 0.06 [0.010] | -- | 10 [3.2] | 10 [3.2] | |
| ↑ Total bilirubin | 10 [1.6] | -- | 30 [9.7] | 30 [9.7] | |
| ↓ Albumin | -- | -- | 10 [3.2] | 10 [3.2] | |
| ↓ Sodium | -- | -- | 30 [9.7] | 30 [9.7] | |
| ↓ Chloride | -- | -- | 30 [9.7] | 30 [9.7] | |
| **ECG** | | | | | |
| QT/PR prolongation | NT | NT | 30 [9.7] | 30 [9.7] | |
| **Organ Weights** | | | | | |
| ↓ Spleen (relative or absolute)‡ | 0.06 [0.010] | 0.06 [0.010] | -- | | **--** |
| ↑ Lung (relative or absolute) | 0.06 [0.010] | 0.1 [0.016] | 1 [0.32]§ | | 10 [3.2]§ |
| **Histopathology - Liver** | | | | | |
| Degeneration/necrosis of hepatocytes | -- | -- | 30 [9.7] | | 30 [9.7] |
| **Histopathology – Kidney** | | | | | |
| Hyalin cast | -- | -- | 30 [9.7] | | 30 [9.7] |
| Vacuolation of renal tubule | -- | -- | -- | | 60 [19.4] |
| **Histopathology - Lung** | | | | | |
| Foam cell accumulation | 10 [1.6] | 10 [1.6] | -- | | -- |
| **Histopathology – Bone Marrow** | | | | | |
| Hypocellularity | -- | -- | 30 [9.7] | | 30 [9.7] |
| **Histopathology - Spleen** | | | | | |
| ↓ Lymphocytes in the periarterial  lymphoid sheath‡ | 0.06 [0.010] | 0.06 [0.010] | 1 [0.32]§ | | 0.1 [0.032]§ |
| Atrophy of lymphoid follicles‡ | 0.1 [0.016] | 0.1 [0.016] | -- | | -- |
| Prominent lymphoid follicles‡ | 0.1 [0.016] | 0.06 [0.010] | -- | | -- |
| Narrowing of the marginal zones‡ | 0.1 [0.016] | 0.1 [0.016] | 60 [19.4] | | 60 [19.4] |
| Widening of the marginal zones‡ | -- | -- | 1 [0.32] | | 0.1 [0.032] |
| Atrophy at the germinal center‡ | -- | -- | 0.1 [0.032] | | 0.1 [0.032] |
| *Table continued on next page* | | | | | |

*Table 7 (continued)*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment-Related Change** | **Dose Level of Observed Response (mg/kg) [HED] (mg/kg)**† | | | | |
| **Rat** | | **Monkey** | | |
| **Male** | **Female** | **Male** | **Female** | |
| **Histopathology – GI Tracts** | | | | | |
| Glandular dilatation of stomach | 10 [1.6] | 10 [1.6] | -- | | -- |
| Mucosal atrophy of stomach and ileum | -- | -- | 60 [19.4] | | 30 [9.7] |
| Erosion and mucosal atrophy of colon | -- | -- | 60 [19.4] | | 60 [19.4] |
| **Histopathology – Thymus** | | | | | |
| ↑ Lymphocytes in thymic medulla‡ | 0.03 [0.005] | 0.03 [0.005] | -- | | -- |
| **Histopathology – Adrenals** | | | | | |
| Hypertrophy of cortical cells | -- | -- | 30 [9.7] | | 30 [9.7] |
| Lipid depletion of cortical cells | -- | -- | 30 [9.7] | | 30 [9.7] |

-- :not observed; ↑: increase; ↓:decrease; HED: human equivalent dose; NT: not tested

† Lowest dose level at which a change in one or more of the listed parameters occurred as noted in report summary

‡ Finding was attributed to the pharmacological effect of the drug

§ Not observed at 30 and 60 mg/kg per day

HED was based on human weight of 60 kg and scaling on the basis of mg/m2

Sources: Study Project X-TX-0003, Study Project X-TX-0009, Study Project X-TX-0004 & Study Project X-TX-0010 (Draft)

### Effects on Lung

It has been reported that dyspnea was induced by fingolimod (FTY720) in clinical trials. Pulmonary function tests (including forced expiratory volume in 1 sec (FEV1), forced vital capacity and CO diffusion capacity) revealed that FEV1 values were slightly low [Martini et al., 2007; Horga & Montalban, 2008]. In the 4-week rat study of PROJECT X, lung weight was increased in males at 0.1 mg/kg per day or more and in females at 1 mg/kg per day or more. Foam cell accumulation in the lung was histopathologically observed in a few rats treated at 10 mg/kg per day. In the 4-week monkey study, lung weight was increased in males at 1 mg/kg per day or more and in females at 10 mg/kg per day; however, there were no histopathological findings in the lung. In both the 4-week rat and monkey studies, increased lung weight was not accompanied by any pulmonary histopathological changes; however, increased lung weight as well as perivascular and alveolar edema in the lung were noted in nonpivotal exploratory studies. It was, therefore, thought that increased lung weight in the 4-week studies was due to very slight edema which could not be detected by histopathological examination. The meaning of foam cell accumulation was unclear, but it may have no relation to the increased lung weight because there is no consistency between the individual severity of these two findings. After the 4-week recovery period, lung weight fully recovered in monkeys and female rats and tended to recover in male rats. In addition, no abnormality in the respiratory function tested using the whole body plethysmograph method was detected in the 4-week rat study. In the safety pharmacology study in monkeys with telemetry system, no abnormalities were detected in respiratory rate or blood gas parameters. Taken together, the toxicological significance and mechanisms of increased lung weight have been unknown to date, but no severe histopathological and respiratory functional abnormalities were observed even at high dose levels. The lung finding was observed at near the expected clinically effective dose. Therefore, clinical signs such as dyspnea and

respiratory function parameters, including forced expiratory volume in 1 sec (FEV1), should be carefully monitored in clinical trials.

### Effects on Embryo-Fetal Development

PROJECT X produced teratogenic effects in the embryo-fetal development study in rats. In the study a marked increase in the index of embryo-fetal deaths and teratogenic effect such as an increased rate of ventricular septal defects were noted in rats at 0.1 mg/kg per day (human equivalent dose [HED]: 0.016 mg/kg). Since an important role of S1P in embryo-fetal development was reported [Mizugishi et al., 2005], these findings were hypothesized to relate to a disruption of S1P signaling by PROJECT X. However, in rabbits, no embryo-fetal death and teratogenic effects were observed up to 3 mg/kg per day (HED: 0.97 mg/kg) at which the exposure levels were more than 5 times that in rats at 0.1 mg/kg per day, which showed teratogenic effects. In studies conducted with healthy female volunteers, Astellas plans to only include surgically sterile or postmenopausal women. In PROJECT X studies conducted with MS patients, prior to their enrollment and during their participation in clinical studies, Astellas plans to thoroughly counsel women of child-bearing potential regarding these findings and to require that appropriate contraception be used to prevent pregnancy.

### Effects on Cardiovascular Parameters

Bradycardia has been often reported in clinical trials with FTY720, and the cause of bradycardia is reported to be related to S1P3 which is one of the receptors activated by FTY720 [Horga & Montalban, 2008; Sensken et al., 2008]. PROJECT X, a highly

S1P1-selective agonist, has a low affinity for S1P3, and is thus expected to have a greater safety margin. A transient reduction of the heart rate by PROJECT X was identified in conscious rats only when PROJECT X was iv-infused at 3 mg/kg, a much higher dose than that induced lymphopenia with oral ED50 values of 0.10 mg/kg (single dose) and 0.023 mg/kg (multiple doses). The potential cardiovascular effects of PROJECT X were further evaluated in vitro and in vivo safety pharmacology studies and 4-week repeated dose toxicity studies in monkeys. However, no effect of PROJECT X on the heart rate was noted in these studies.

PROJECT X had no effect on the hERG current of HEK293 cells up to 0.153 mcmol/L (68 ng/mL), but inhibited the hERG current by 13.8% at the exposure concentration of 3.39 mcmol/L (1499 ng/mL), which is extremely higher than that at the ED50 for lymphopenia (Predicted clinically effective Cmax: 5.46 ng/mL, Cmax unbound is expected to be far lower than this value). In safety pharmacology in monkeys, no effect was found on blood pressure, heart rate, electrocardiogram parameters, and electrolytes at up to 10 mg/kg (mean Cmax at 10 mg/kg: 509 ng/mL). In 4-week repeated dose toxicity studies in monkeys, QT prolongation occurred only in animals at 30 and 60 mg/kg per day and was associated with deteriorated general condition, which led animals to subsequent sacrifice in moribundity (mean Cmax at 30 mg/kg per day on day 28: 1822 ng/mL for males and 1655 ng/mL for females). PR prolongation was minimal and within a narrow range in control animals. No cardiovascular abnormality was evident at up to 10 mg/kg per day in the 4-week toxicity study in monkeys (mean Cmax on day 28: 561 ng/mL for males and 982 ng/mL for females on day 14). Therefore, PROJECT X did not induce bradycardia in monkeys and may cause QT and PR prolongations only at doses above tolerated levels in humans. It is less likely to induce bradycardia or other cardiovascular abnormality in humans at effective dose levels.

Nevertheless, electrocardiograms should be closely monitored in clinical studies to ensure human safety.

### Effects on the Liver

No hepatotoxicity was observed in rats or monkeys after dosing with PROJECT X for 4 weeks at up to 10 mg/kg per day (78 to 149 times the expected clinically effective AUC24). At higher doses in the 4-week monkey study, increases in plasma AST, ALT, total bilirubin and total cholesterol were noted with hepatocytic degeneration/necrosis. These changes were observed only at high dose levels above the MTD (10 mg/kg) at 30 and 60 mg/kg per day.

Reversibility of these changes was noted after dosing cessation. Although the liver toxicity may occur only at doses above tolerated levels in humans, liver parameters should be monitored carefully.

### Effects on the Kidney

No renal toxicity was observed in rats or monkeys after dosing with PROJECT X for 4 weeks at up to 10 mg/kg per day (78 to 149 times the expected effective AUC24). At higher doses in the 4-week monkey study, increase in BUN was noted with hyaline casts in the kidney.

These changes were observed only at high dose levels above the MTD (10 mg/kg) at 30 and 60 mg/kg per day and even at 60 mg/kg per day only females showed renal tubular vacuolation. Reversibility of these changes was noted after dosing cessation. Although the renal toxicity may occur only at doses above tolerated levels in humans, renal parameters should be monitored carefully.

### Effects on Hematopoiesis

No hematopoietic toxicity was observed in rats or monkeys after dosing with PROJECT X for

4 weeks at up to 10 mg/kg per day (78 to 149 times the expected effective AUC24). At higher doses in the 4-week monkey study, decreased reticulocytes were noted with hypocellularity in the bone marrow. Hypocellularity in the bone marrow also induced decreased neutrophils, monocytes, and other leukocytes with the exception of lymphocytes. These changes were observed only in moribund animals at high dose levels at 30 and 60 mg/kg per day; therefore, they were thought to be related to a continuous deterioration of general condition.

With regard to the pharmacological effects, lymphocytes, involving T and B cells, decreased in a dose-dependent manner at approximately the effective dose or more; however, the degree of the reduction was particularly notable at high dose levels of 30 and 60 mg/kg per day.

Moreover, neutrophils, monocytes, and NK cells decreased only at high dose levels, more than 30 mg/kg per day. In general, decrease in these cells may include a risk of infection after chronic administration at higher doses, and should be monitored carefully.

### Effects on the Macula Lutea

In a clinical trial with FTY720, a few subjects showed macular edema, but the cause was unclear [Horga & Montalban, 2008]. In the 4-week studies with PROJECT X in rats and monkeys, no abnormality was detected in ophthalmology or histopathology examinations, including detailed observation of the macula in monkeys by means of histopathology and fluorescein fundus angiography. Based on the data, it is unlikely that PROJECT X has the potential to induce macular edema; however, clinical signs (including subjective symptoms such as decreased visual acuity) and ophthalmology should be monitored in clinical trials.

### Conclusions

Potential safety concerns relevant to human usage as well as strategies to mitigate potential risks are outlined in [Table 8](#_bookmark74)

### Table 8 Strategies to Mitigate Potential Risks for PROJECT X in Clinical Studies

|  |  |  |
| --- | --- | --- |
| **Potential Safety Concern (from nonclinical studies with PROJECT X**  **and clinical studies with fingolimod)** | **Observed in nonclinical studies with PROJECT X** | **Relevance to Human Usage and Planned Risk Management** |
| Increased lung weight in rats and  monkeys | Yes | Dyspnea and respiratory function will be  measured |
| Embryo-fetal death, teratogenicity in rats | Yes | Women of child-bearing potential will be excluded from phase 1 studies. Pregnancy testing and contraception will be required  for phase 2 and 3 studies |
| Immunosuppression | Yes | Infection and malignancy will be  monitored |
| Cardiovascular toxicity | Yes | Vital signs and cardiac monitoring (ECG, Holter) will be monitored for phase 1  studies |
| Ocular toxicity | No | A finding in fingolimod clinical trials, ophthalmology exams will be performed  during clinical trials |
| Hepatotoxicity | Yes | Liver function tests will be monitored |
| Nephrotoxicity | Yes | Renal function will be monitored |

ECG: electrocardiogram

In conclusion, none of the findings in the nonclinical safety pharmacology and toxicology studies described in this Investigator’s Brochure would preclude further development of PROJECT X in phase 1 clinical trials. These data do suggest that careful monitoring of respiratory function should occur during clinical trials with PROJECT X.

#### List of References

*Note: Literature references are available upon request.*

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