### Safety Pharmacology

Four core battery safety pharmacology studies of PROJECT 5 were performed [End-of-Text Table 1.2]. These safety pharmacology studies were performed in accordance with good laboratory practice (GLP).

PROJECT 5 is not expected to affect the rapidly activating component of the delayed rectifier potassium current as only a slight, statistically insignificant (12%) inhibition of hERG current was observed at the highest concentration tested of PROJECT 5 (10-5 mol/L) in human embryonic kidney 293 (HEK-293) cells (Study Project 5-PT-0001). Likewise, PROJECT 5 did not affect action potentials in isolated guinea pig papillary muscle tissue at concentrations up to 10-5 mol/L (Study Project 5-PT-0002).

In the rat, PROJECT 5 had no effect on the CNS (modified Irwin’s method) of rats at up to 100 mg/kg orally (Study Project 5-PT-0003).

Findings in the cynomolgus monkey were limited to loose/watery stools and a tendency towards reduced blood potassium concentrations at 60 mg/kg orally (Study Project 5-PT-0004). There were no other effects on the CNS, circulatory system (electrocardiogram [ECG], blood pressure or heart rate), or respiratory system at up to 60 mg/kg.

Four follow-up studies of PROJECT 5 and its metabolites (M1, M2 and M4) to investigate cardiovascular effects were performed [End-of-Text Table 1.2].

PROJECT 5 had no effect on hNav1.5, hCav1.2-β2-α2δ, hKvLQT1/hminK or hKv4.3 currents up to 10 μmol/L in HEK-293 or Chinese hamster ovary (CHO) cells (Study Project 5-PT-0005). M1 had no effect on hNav1.5, hCav1.2-β2-α2δ, hKvLQT1/hminK, hKv4.3 or hERG currents up to 0.1 μmol/L, and M2 and M4 had no effect on these currents up to 10 μmol/L in HEK293 cells or CHO cells (Study Project 5-PT-0006). M1 had no effect on hKir2.1 and hKir6.2/SUR2A current up to 0.1 μmol/L, and PROJECT 5, M2 and M4 had no effect on these currents up to 10 μmol/L in HEK293 cells (Study Project 5-PT-0007). PROJECT 5, M1, M2 and M4 had no effects on the NCX1 Na+/Ca2+ exchange transporter (human SLC8A1 gene) expressed in HEK293 cells at any concentration using a Fluo-8 calcium kit and a Fluorescence Imaging Plate Reader (FLIPRTETRA™) instrument (Study Project 5-PT-0008).

Based on the above, PROJECT 5 and its metabolites had no effects on any cardiac ion channels or the NCX1 Na+/Ca2+ exchange transporter up to the highest concentrations examined.

## Toxicology

A total of 40 toxicology studies were conducted in mice, rats, rabbits and monkeys as part of the PROJECT 5 development program [End-of-Text Table 3.1].

All pivotal toxicology studies were conducted in accordance with the standards of Japanese GLP (Notifications and Ordinances, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare). These standards are also in accordance with the FDA and EU GLP regulations.

### Single-dose Toxicity

Single-dose toxicity studies of PROJECT 5 were conducted at dose levels up to 2000 mg/kg in the rat and monkey (Studies Project 5-TX-0009 [rat] and Project 5-TX-0010 [monkey]). No mortality was observed in either study [End-of-Text Table 3.4].

No clinical abnormalities were noted in the rat. Slightly decreased body weight was observed in 2 males at 2000 mg/kg. At necropsy, dark red spots were noted in the glandular stomach in 1 male in each of the 1000 mg/kg and 2000 mg/kg groups. Histopathologic findings in the glandular stomach consisted of mucosal hemorrhage, epithelial basophilic changes with mitotic figure and calcification.

Findings in the monkey included vomiting at doses of 500 and 2000 mg/kg; increase in serum glutamic oxaloacetic transaminase (also referred to as aspartate aminotransferase [AST]) and decrease in serum potassium at 500 mg/kg; and increase in total bilirubin and decrease in serum sodium at 2000 mg/kg.

### Repeated-dose Toxicity

### Toxicokinetics

An overview of toxicity studies that included a toxicokinetic evaluation is provided in [End-of-Text Table 3.2]; toxicokinetic data are presented in [End-of-Text Table 3.3.] Repeated-dose toxicity studies of PROJECT 5 were conducted up to a 26-week duration in Crl:CD(SD) rats and up to a 52-week duration in cynomolgus monkeys

(Studies Project 5-TX-0030 [rat] and Project 5-TX-0029 [monkey]). PROJECT 5 Cmax and area under the concentration-time curve from 0 to 24 h (AUC24) increased with dose. In some studies, there was a tendency for Cmax and/or AUC24 to decrease with repeated dosing. Sex differences (i.e., higher exposure in females) were observed in the 4-, 13-, and 26-week rat and the 13-week cynomolgus monkey studies, but not in the 4-week monkey study, while higher exposure was observed in males at higher doses in the 52-week monkey study (Studies Project 5-TX-0011 [4-week rat], Project 5-TX-0040 [4-week rat], Project 5-TX-0024

[13-week rat], Project 5-TX-0030 [26-week rat], Project 5-TX-0012 [4-week monkey], Project 5-TX- 0041 [4-week monkey], Project 5-TX-0023 [13-week monkey] and Project 5-TX-0029 [52-week monkey]). In rat and monkey 4-week repeated-dose toxicokinetics Studies Project 5-TX-0040 and Project 5-TX-0041, pharmacokinetics of PROJECT 5 and 3 human metabolites (M1, M2 and M4) were analyzed.

In the rat 4-week toxicokinetic study, Cmax and AUC24 for PROJECT 5 were increased with increasing dose on days 1, 14 and 28 and did not change clearly after repeated dosing. No clear sex difference was noted among Cmax and AUC24 for PROJECT 5 except for Cmax at

30 mg/kg, which was higher in females than males. In the same study, Cmax and AUC24 increased dose proportionally in both males and females at all 3 evaluated timepoints for M1,

M2 and M4 metabolites. After repeated dosing, Cmax and AUC24 did not change clearly, with no consistent sex differences observed for M4 metabolite. Likewise, after repeated dosing, Cmax and AUC24 of M2 and M1 metabolites did not change clearly in females, while both parameters decreased in males. At all timepoints, and at both evaluated dose groups, Cmax and AUC24 were greater in females than males. At the final dosing in males, the systemic exposure of PROJECT 5 was the highest, and that of M2 was decreased to the same or lower level of M4. In contrast, systemic exposure to the parent compound and metabolites in females was the highest in PROJECT 5, decreasing in the order M2, M1 and M4 throughout the dosing period (Study Project 5-TX-0040).

In the monkey 4-week toxicokinetic study, systemic exposure to the parent compound and its metabolites in males was the highest for PROJECT 5 decreasing in the order M2, M4 and M1 at the initiation of dosing. No increase in Cmax and AUC24 parameters of PROJECT 5 were noted with increasing dose and large individual differences in PROJECT 5 exposure were observed. For the parent compound, Cmax and AUC24 in females were generally higher than those in males, though, after repeated dosing, there were no clear changes in PROJECT 5 exposure. For the M1, M2 and M4 metabolites, the exposure was lower than that of PROJECT 5, while the profile (dose relation, sex difference, and effect of repeated dosing) was similar to that of parent compound. Among metabolites, the highest exposure was to M2, followed by M4 and M1 (Study Project 5-TX-0041).

### Toxicity

Repeated-dose toxicity studies of PROJECT 5 are summarized in [End-of-Text Table 3.5] (nonpivotal studies) as well as in [End-of-Text Tables 3.6.1 to 3.6.6] (pivotal studies). Repeated-dose toxicity studies of PROJECT 5 were conducted for up to a 26-week duration in rats and up to a 52-week duration in monkeys. From nonclinical studies of PROJECT 5 conducted to date in rats and cynomolgus monkeys, findings considered to be related to PROJECT 5 have included effects on the GI system, hematopoietic system, immune system and muscle tissue (as evidenced by increases in creatine phosphokinase [CPK] and lactate dehydrogenase), which were reversible upon dosing cessation. The doses at which these changes were observed, and their corresponding AUC24 values, are shown in [Table 2](#_bookmark49) for rats and in [Table 3](#_bookmark50) for monkeys. In general, changes were observed at high dose levels.

Comparative systemic [exposure (animal versus human) is presented in Table 5.](#_bookmark65)

### Table 2 Summary of Treatment-Related Changes of Potential Clinical Interest in Repeated-Dose Toxicity Studies of PROJECT 5 in Rats (4, 13 and 26 weeks)

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment-related Change** | **Lowest Dose (mg/kg) Change Was Observed**  **AUC24 in ng·h/mL at Last TK Evaluation: [Male/Female]** | | |
| **Rat** | | |
| **4-wk GLP** | **13-wk GLP** | **26-wk GLP** |
| **Clinical Signs and General Condition** | | | |
| Subcutaneous mass | - | 100F†‡ [19558] | 100F‡ [30984] |
| Ulcer (skin) | - | - | 100F† [30984] |
| Crust (skin) | - | - | 100F† [30984] |
| Reddish urine | - | - | 100M† [10902] |
| **Hematology** | | | |
| ↓ White blood cells§ | 30M [5530]  100F [26728] | 100 [23280/19558] | 100 [10902/30984] |
| ↓ Lymphocytes§ | 30 [5530/9539] | 100 [23280/19558] | 100 [10902/30984] |
| **Blood Chemistry** | | | |
| ↓ Albumin | - | - | 100F [30984] |
| ↓ Albumin/globulin ratio | - | - | 100F [30984] |
| ↓ Total protein | - | - | 100F [30984] |
| **Organ Weights** | | | |
| ↓ Spleen (relative or absolute)§ | 30 [5530/9539] | 10 [2165/2684] | 100 [40902/30984] |
| ↓ Thymus (relative or absolute)§ | 30 [5530/9539] | 100 [23280/19558] | - |
| **Histopathology – Lymph Nodes** | | | |
| Atrophy of mandibular§ | 30M† [5530] | 10 [2165/2684] | 3M† [850]  10F† [3057] |
| Atrophy of mesenteric§ | 30M [5530]  100F [26728] | 1 [200/299]§ | 3M† [850]  100F [30984]¶ |
| Suppurative granuloma (mandibular  lymph node) | - | 100†¶  [23280/19558] | - |
| **Histopathology – Spleen** | | | |
| ↓ Extramedullary hematopoiesis | 30 [5530/9539**]**¶ | 100 [23280/19558]¶ | - |
| Atrophy of lymphoid follicles§ | 100 [19504/26728] | - | - |
| Atrophy of the white pulp§ | - | 100  [23280/19558] | 3M [850]  10 [2886/3057] |
| Congestion | - | 100  [23280/19558] | 10 [2886/3057]¶ |
| Deposition of hemosiderin | - | 100 [23280/19558]§ | 100 [40902/30984]¶ |
| **Histopathology – Sternal Bone Marrow** | | | |
| Hypocellularity | 30 [5530/9539] | **-** | 100 [40902/30984] |
| Hypercellularity | **-** | 100F†¶ [19558] | **-** |
| **Histopathology – Femoral Bone Marrow** | | | |
| Hypocellularity | 30 [5530/9539] | **-** | 100 [40902/30984] |
| Hypercellularity | **-** | 100F†¶ [19558] | - |
| **Histopathology – Femur** | | | |
| Focal necrosis of trabecular bone | 100M† [19504] | - | - |
| **Histopathology – Peyer’s Patches** | | | |
| Atrophy | **-** | 10F [2684]  100M [23280] | - |
| **Histopathology – Lung** | | | |
| Suppurative, multifocal pneumonia | **-** | 100F†¶ [19558] | - |
| *Table continued on next page* | | | |

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment-related Change** | **Lowest Dose (mg/kg) Change Was Observed**  **AUC24 in ng·h/mL at Last TK Evaluation: [Male/Female]** | | |
| **Rat** | | |
| **4-wk GLP** | **13-wk GLP** | **26-wk GLP** |
| **Histopathology – Glandular Stomach** | | | |
| Erosion | 30F† [9539]  100M† [19504] | **-** | - |
| Focal basophilic change, superficial  epithelial cells | 30M [5530] | **-** | - |
| **Histopathology – Kidney** | | | |
| Epithelial hypertrophy in the  papillary collecting duct | **-** | **-** | 100  [40902/30984] |
| Epithelial cell hyperplasia in the  papillary | **-** | **-** | 100  [40902/30984] |
| **Histopathology – Cecum** | | | |
| Single cell necrosis of the glandular  epithelium | **-** | 10M [2165]  100F [19558] | - |
| **Histopathology – Rectum** | | | |
| Single cell necrosis of the glandular  epithelium | - | 100M† [23280] | - |
| Focal regeneration of the epithelium | - | 100M† [23280] | - |
| **Histopathology – Ovaries** | | | |
| Necrosis of lutein cells | 100F [26728] | 100F [19558] | - |
| **Histopathology – Thymus** | | | |
| Atrophy§ | 30 [5530/9539] | 100 [23280/19558] | 3M† [850]¶  10F† [3057]¶ |
| **Histopathology – Skin** | | | |
| Ulcer | - | 100F†‡ [19558] | - |
| **Histopathology – Subcutis** | | | |
| Suppurative granuloma | - | 100F†‡ [19558] | 100F‡ [30984] |

-: not observed; ↓: decreased; AUC24: area under the curve from time 0 to 24 h; F: females only; GLP: Good Laboratory Practice; M: males only; TK: toxicokinetics.

† Finding in 1 animal.

‡ Finding due to bacterial infection related to immunosuppressive activity of the drug.

§ Finding was attributed to the pharmacological effect of the drug.

¶ Finding also observed in vehicle control animals.

Source: Studies Project 5-TX-0011, Project 5-TX-0024, and Project 5-TX-0030

### Table 3 Summary of Treatment-Related Changes of Potential Clinical Interest in Repeated-Dose Toxicity Studies of PROJECT 5 in Cynomolgus Monkeys (4, 13 and 52 weeks)

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment-related Change** | **Lowest Dose (mg/kg) Change Was Observed**  **AUC24 in ng·h/mL at Last TK Evaluation: [Male/Female]** | | |
| **Monkey** | | |
| **4-wk GLP** | **13-wk GLP** | **52-wk GLP** |
| **Clinical Signs and General Condition** | | | |
| Soft stool, watery stool, diarrhea | 30 [3637/2653] | 60/30 [†] | 15 [2887/1521] |
| Salivation | 60 [7550/7489] | 60/30 [†] | - |
| Vomiting | 60 [7550/7489] | 60/30 [†] | - |
| Abnormal stool color (red/occult  blood) | - | 60/30 [†] | - |
| ↓ Activity, ↓ Response, Abnormal  position | - | 60/30 [†] | - |
| Hypothermia | - | 60/30 [†] | - |
| Pale oral mucosa | - | 60/30 [†] | - |
| ↓ Food consumption and body  weight | 30 [3637/2653] | 60/30 [†] | 15 [2887/1521] |
| **Hematology** | | | |
| ↓ RBC, hemoglobin, hematocrit | 60 [7550/7489] | 15 [2573/1990] | - |
| ↑ RBC, hemoglobin, hematocrit | 60 [7550/7489]‡ | 60/30‡§ [†] | - |
| ↓ MCV | - | 60/30§ [†] | - |
| ↑ MCHC | - | 60/30§ [†] | - |
| ↓ MCHC | - | - | - |
| ↓Reticulocyte ratio | - | 15 [2573/1990] | - |
| ↑ White blood cells | 60 [7550/7489] | 15F [1990] - 60/30 [†] | - |
| ↑ Neutrophils | 60 [7550/7489] | 15F [1990] - 60/30 [†] | 4F [664]  15 [2887/1521] |
| ↓ Lymphocytes | - | 60/30 [†] | 15 [2887/1521] |
| ↑ Monocytes | - | 60/30 [†] | - |
| ↑ Platelets | - | 60/30 [†] | - |
| ↑ Prothrombin time | - | 60/30 [†] | - |
| ↑ APTT | - | 60/30 [†] | - |
| **Blood Chemistry** | | | |
| ↑ or ↓ Albumin | 60 [7550/7489] | 60/30 [†] | 4 [706/664] |
| ↑ or ↓ Globulin | - | 60/30 [†] | 4 [706/664] |
| ↓ Albumin/globulin ratio | - | 60/30 [†] | 4 [706/664] |
| ↑ or ↓ Total protein | - | 60/30 [†] | - |
| ↑ Alkaline phosphatase | - | 60/30 [†] | - |
| ↑ Triglycerides | - | 60/30 [†] | - |
| ↑ Blood urea nitrogen | - | 60/30 [†] | - |
| ↑ Creatinine | 60 [7550/7489] | 60/30 [†] | - |
| ↓ Inorganic phosphorus | - | 8 [743.5/715.5] | - |
| ↑ Inorganic phosphorus | - | 60/30 [†] | - |
| ↑ Glucose | - | 60/30 [†] | - |
| ↑ Total bilirubin | - | 60/30 [†] | - |
| ↓ Sodium | 60 [7550/7489] | 60/30 [†] | - |
| ↓ Chloride | 60 [7550/7489] | 60/30 [†] | - |
| ↑ Chloride | 60 [7550/7489] | - | - |
| ↓ Calcium | - | 60/30 [†] | - |
| *Table continued on next page* | | | |

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment-related Change** | **Lowest Dose (mg/kg) Change Was Observed**  **AUC24 in ng·h/mL at Last TK Evaluation: [Male/Female]** | | |
| **Monkey** | | |
| **4-wk GLP** | **13-wk GLP** | **52-wk GLP** |
| ↓ Potassium | - | 60/30 [†] | - |
| ↑ Lactate dehydrogenase | 60 [7550/7489] | 60/30 [†] | - |
| ↑ Creatine phosphokinase | 60 [7550/7489] | 60/30§ [†] | - |
| ↑ Troponin I and Troponin T | - | 60/30 [†] | - |
| **Urinalysis** | | | |
| ↓ pH | 60 [7550/7489] | **-** | **-** |
| ↓ Chloride | 60 [7550/7489] | 60/30 [†] | - |
| ↓ Sodium | - | 60/30 [†] | 4 [706/664] |
| Cast in urinary sediment | 60 [7550/7489] | **-** | **-** |
| **Organ Weights** | | | |
| ↑ Lung (relative or absolute) | - | - | 4 [706/664]§ |
| ↓ Thymus (relative or absolute)‡ | 30 [3637/2653] | - | - |
| **Histopathology – Lymph Nodes** | | | |
| Follicular atrophy (submandibular) | - | 60/30§ [†] | - |
| Single cell necrosis (submandibular) | - | 60/30§ [†] | - |
| **Histopathology – Spleen** | | | |
| Atrophy of lymphoid follicles‡ | - | 60/30 [†] | - |
| Atrophy of the germinal center‡ | - | - | 2 [291/ 316] |
| **Histopathology – Sternal Bone Marrow** | | | |
| Hypocellularity | **-** | 60/30 [†] | **-** |
| ↑ Granulocytes | 30 [3637/2653] | **-** | **-** |
| **Histopathology – Femoral Bone Marrow** | | | |
| Fibrosis, bone marrow | - | **-** | 4 [706/664]¶ |
| Abscess wall | - | **-** | 4 [706/664]¶ |
| **Histopathology – Femur** | | | |
| Hyperplasia, bone | - | **-** | 4 [706/664]¶ |
| **Histopathology – Liver and Kidney** | | | |
| Mononuclear cell infiltration | - | **-** | 4 [706/664]¶ |
| **Histopathology – Skin** | | | |
| Abscess all | - | **-** | 4 [706/664]¶ |
| **Histopathology – Lung** | | | |
| Cellular infiltration, mononuclear  cell | - | **-** | 8 [1784/763]¶ |
| Cell debris, lumen, bronchus | - | **-** | 4 [706/664]¶ |
| Abscess wall | - | **-** | 4 [706/664]¶ |
| **Histopathology – Glandular Stomach** | | | |
| Erosion | - | 60/30 [†] | - |
| Inflammatory cell infiltration in the  lamina propria | - | 60/30 [†] | - |
| Mucosal haemorrhage | - | 60/30 [†] | - |
| Epithelial regeneration | - | 60/30 [†] | - |
| **Histopathology – Cecum** | | | |
| Atrophy, epithelial cell | - | - | 15 [2887/1521] |
| Inflammatory cell infiltration in the  lamina propria | - | 8 [743.5/715.5] | 15 [2887/1521] |
| Erosion | - | 60/30§ [†] | - |
| Haemorrhage | - | 60/30§ [†] | - |
| *Table continued on next page* | | | |

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment-related Change** | **Lowest Dose (mg/kg) Change Was Observed**  **AUC24 in ng·h/mL at Last TK Evaluation: [Male/Female]** | | |
| **Monkey** | | |
| **4-wk GLP** | **13-wk GLP** | **52-Wwk GLP** |
| **Histopathology – Ileum** | | | |
| Inflammatory cell infiltration in the  lamina propria | - | 60/30§ [†] | - |
| Mucosal haemorrhage | - | 60/30§ [†] | - |
| **Histopathology – Duodenum** | | | |
| Mucosal haemorrhage | - | 60/30§ [†] | - |
| **Histopathology – Colon** | | | |
| Atrophy, epithelial cell | - | - | 15 [2887/1521] |
| Colitis | - | - | 15 [2887/1521] |
| Inflammatory cell infiltration in the  lamina propria | - | 8 [743.5/715.5] | - |
| **Histopathology – Rectum** | | | |
| Focal regeneration of the epithelium | - | 8§ [743.5/715.5] | - |
| Inflammatory cell infiltration in the  lamina propria | - | 8 [743.5/715.5] | 15 [2887/1521] |
| Erosion | - | 8§ [743.5/715.5] | - |
| Mucosal haemorrhage | - | 8§ [743.5/715.5] | - |
| **Histopathology – Vagina** | | | |
| Atrophy, mucosa | - | - | 4F [664]§ |
| **Histopathology – Thymus** | | | |
| Atrophy‡ | 30 [3637/2653] | 60/30 [†] | - |
| **Histopathology – Adrenal Gland** | | | |
| Hypertrophy of the fascicular zone | - | 60/30 [†] | - |

-: not observed; ↑: Increased; ↓: decreased; APTT: activated partial thromboplastin time; AUC24: area under the curve from time 0 to 24 h; F: females only; GLP: Good laboratory practices; MCHC: mean corpuscular hemoglobin volume; MCV: mean corpuscular volume; RBC: red blood cell count; TK: toxicokinetics.

† Monkeys were administered 60 mg/kg per day from days 1 to 32 (males) or from days 1 to 29 (female) and 30 mg/kg per day for the remainder of the 13-week treatment period. Moribund sacrifices occurred on day 21 (male), and days 17, 26 and 64 (females), respectively.

**AUC24 (ng·h/mL)**

**60 mg/kg 30 mg/kg**

Males Day 32: 4885.5 (n = 5) Day 91: 3115.3 (n = 5)

Females Day 29: 6461.9 (n = 4) Day 91: 4963.5 (n = 3)

‡ Finding was attributed to dehydration in animals experiencing clinical symptoms.

§ Finding in 1 animal.

¶ Finding due to bacterial infection related to immunosuppressive activity of the drug. Source: Studies Project 5-TX-0012, Project 5-TX-0023 and Project 5-TX-0029

In the 4-week dose range-finding (Study Project 5-TX-0019) and 13-week GLP toxicity (Study Project 5-TX-0023) studies in monkeys, animals became moribund and were sacrificed after showing clinical signs suggesting effects on GI organs, such as soft stool, watery stool, diarrhea and vomiting (≥ 60 mg/kg per day in the 4-week study and 60/30 mg/kg per day in the 13-week study). Based on the clinical signs and time-course of deterioration, it is considered that the moribund condition was induced by malnutrition and dehydration resulting from decreased food consumption and severe GI damage. In the 26-week rat study, 1 male receiving PROJECT 5 100 mg/kg per day was found dead with spleen, bone marrow, lymph node, papillary collecting duct and kidney alterations (Study Project 5-TX-0030).

Rats and monkeys that received repeated doses of PROJECT 5 had GI disorders during the dosing period. In rats, erosion and regenerative reactions in the stomach were observed at doses of ≥ 30 mg/kg per day (2- and 4-week studies). In the 13-week study, single cell necrosis of mucosal epithelium in the cecum at ≥ 10 mg/kg per day and single cell necrosis and focal regeneration of the mucosal epithelium in the rectum were noted in 1 male at 100 mg/kg per day. In monkeys, soft stool, diarrhea and vomiting were observed at

≥ 30 mg/kg per day (males) and 60 mg/kg per day (females) in the definitive 4-week study, 60/30 mg/kg per day in the 13-week study, and 15 mg/kg per day in the 52-week study. In the 13- and 52-week studies, histopathological changes of the GI tract such as inflammatory cell infiltration, erosion and hemorrhage were observed in the stomach, cecum, ileum, duodenum, colon or rectum. Reversibility of these changes was confirmed upon drug discontinuation in the 4- and 13-week studies.

In the oral repeated-dose toxicity studies in rats and monkeys, some changes were observed in hematological parameters and lymphoid organs such as decreases in white blood cell (WBC) and/or lymphocyte counts as well as atrophic changes in lymphoid organs such as the thymus, spleen and lymph nodes. These changes are considered related to the pharmacologic activity of PROJECT 5.

Rats and monkeys that received repeated doses of PROJECT 5 experienced changes in hematopoietic parameters during the dosing period that reversed by the end of the recovery period. Hypocellularity of the bone marrow was observed in the 4-week rat study

(100 mg/kg per day), 26-week rat study (100 mg/kg per day) and 13-week monkey study (60/30 mg/kg per day), and inhibition of splenic extramedullary hematopoiesis was observed in the 4- (30 mg/kg per day or higher) and 13-week (100 mg/kg per day) rat studies. Anemic changes such as decreases in red blood cell (RBC) count, hemoglobin, and hematocrit were noted in monkeys that received PROJECT 5 at 60 mg/kg per day and higher in the 4-week toxicity study and 15 mg/kg per day and higher in the 13-week study. PROJECT 5 inhibits human JAK3 enzyme activity at 0.7 nmol/L (IC50), but also inhibits human JAK2 enzyme activity at 5.0 nmol/L (IC50), indicating that the JAK3 selectivity is approximately

7-fold. JAK2 is involved in signal transduction of erythropoietin, the prime regulator of RBC production [Ghaffari et al, 2001]; therefore, loss of selectivity at high plasma PROJECT 5 concentrations may have resulted in inhibition of JAK2 enzyme activity that may have caused the anemic changes seen in rats and monkeys that received high doses of

PROJECT 5. Increases in neutrophil counts and/or increased granulocytes in the bone marrow were also observed in monkeys that received PROJECT 5 (30 mg/kg per day and higher in the 4-week study, 15 mg/kg per day and higher in the 13-week study, and 4 mg/kg per day and higher in the 52-week study). JAK3 is involved in the differentiation of neutrophils [Grossman et al, 1999]. Mice deficient in JAK3 show an increase in the number of neutrophils in peripheral blood; therefore, it is likely that inhibition of JAK3 enzyme activity caused the leukocytosis seen in monkeys that received high doses of PROJECT 5.

In the 4-week dose range-finding study in monkeys, mild multifocal muscle necrosis was observed in 1 female that received PROJECT 5 at 60 mg/kg per day. In the definitive 4-week toxicity study in monkeys, no muscle change was detected by histopathology, but increased levels of CPK and lactate dehydrogenase that disappeared during the recovery period were observed in 2 males at 60 mg/kg per day. In the 13-week toxicity study in monkeys, at the highest dose level of 60/30 mg/kg per day, increased muscular fraction of isoenzymes of CPK and lactose dehydrogenase were noted in 1 male that was sacrificed in extremis, and a transient increase of muscular fraction of isoenzymes of CPK was observed in another male at week 7. However, no histopathological changes of the muscle were observed in those animals, leaving the relationship between elevation of enzymes and muscular damage unknown. In the same study, slightly increased troponin T and troponin I were noted in

2 males each without accompanying histopathological changes in the heart.

Rats treated for 13 weeks (Study Project 5-TX-0024) or 26 weeks (Study Project 5-TX-0030) and monkeys treated for 52 weeks (Study Project 5-TX-0029) exhibited changes considered to be due to an opportunistic infection resulting from immunosuppression caused by PROJECT 5.

### Genotoxicity

Genotoxicity studies were conducted in vitro and in vivo [End-of-Text Tables 3.7.1, 3.7.2,

3.8.1 and 3.8.2]. PROJECT 5 was negative in the reverse mutation test in bacteria but was positive in an in vitro chromosomal aberration test in CHL cells (Studies Project 5-TX-0006 and Project 5-TX-0007). In the latter study, an increased incidence of chromosomal structural aberrations was noted at 120 to 180 μg/mL in the 6-h test without metabolic activation and at 140 to 160 μg/mL in the 6-h test with metabolic activation. The incidence of polyploidy cells was increased at 2.5 to 90 μg/mL in the 6-h test without metabolic activation and at 80 to

160 μg/mL in the 6-h test with metabolic activation.

PROJECT 5 was negative in 2 in vivo genotoxicity studies, the unscheduled DNA synthesis test in the rat (Studies Project 5-TX-0008 and Project 5-TX-0020) and the micronucleus test in the mouse (Study Project 5-TX-0003).

### Carcinogenicity

Preliminary 2-week and 13-week studies of PROJECT 5 were conducted in B6C3F1 mice and Wistar rats to obtain information for dose selection in subsequent 24-month carcinogenicity studies. These studies are summarized in [End-of-Text Tables 3.9.1, 3.9.2 and 3.9.3].

Four-week repeated-dose toxicokinetics studies in B6C3F1 mice and Wistar rats were conducted to obtain information of human metabolites in these strains of the mouse and rat, and results are summarized in [End-of-Text Table 3.3]. Twenty-four-month carcinogenicity studies in B6C3F1 mice and Wistar rats were conducted and results are summarized in

[End-of-Text Tables 3.9.4 and 3.9.5].

In the preliminary 2-week repeated-dose studies in the B6C3F1 mouse and Wistar rat [End-of-Text Table 3.9.1] (Studies Project 5-TX-0034 and Project 5-TX-0035), mortality was

observed in the mouse at 500 and 1500 mg/kg per day and in the rat at 1500 mg/kg per day.

It was concluded in both studies that the nontoxic dose level was lower than 200 mg/kg per day for both sexes.

In the preliminary 13-week repeated-dose toxicity study in B6C3F1 mice, 1 female dosed at 300 mg/kg per day was found dead on day 12 and showed severe changes in the forestomach (perforation and severe ulcer/erosion). Body weight gain in male mice at 300 mg/kg per day was 27.9% less than the control group, indicating that this dose level was beyond the maximal tolerated dose. At doses of 30 and 100 mg/kg per day, only minimal histopathological changes were observed in a few animals in addition to changes that were considered to be related to the pharmacological action of PROJECT 5. The body weight gain in males at 100 mg/kg per day was 18.1% less than the control group but it was not considered to be dose limiting given that the body weight curve was comparable to the control group.

In the preliminary 13-week repeated-dose toxicity study in Wistar rats, 1 male dosed at

300 mg/kg per day was sacrificed in extremis on day 52 after showing decreased body weight and food consumption, decreased spontaneous movement, bradypnea, pale skin, and hypothermia. In surviving animals at the same dose level, body weight gain in males and females was 8.3% and 10.3% less than the control animals, respectively. Therefore, a dose level of 300 mg/kg per day was considered to be beyond the maximum tolerated dose in both male and female rats. At 100 mg/kg per day, no prominent changes in body weight were noted; however, histopathological changes were observed in various organs including the GI tract, liver and kidney. At 30 mg/kg per day, histopathological changes were observed in the GI tract and submandibular and mesenteric lymph nodes.

In the definitive 24-month carcinogenicity study in B6C3F1 mice (0 [vehicle], 0 [vehicle], 10, 40 and 100 mg/kg per day) [End-of-Text Table 3.9.4] (Study Project 5-TX-0039), PROJECT 5 showed no tumorigenic potential.

As nonneoplastic changes, histopathological examination revealed an increase in incidence of squamous cell hyperplasia in both sexes at 100 mg/kg per day and an increase in incidence of erosion/ulcer in the forestomach in males at 100 mg/kg per day. In the liver, histopathological examination revealed an increased incidence of vacuolation in the hepatocytes in females at 40 and 100 mg/kg per day and an increased incidence of clear altered cell focus in the liver was observed in males at 100 mg/kg per day.

Toxicokinetics data revealed dose-related systemic exposure to PROJECT 5 and its metabolites (M2 and M4). Cmax and AUC24 of PROJECT 5 and each metabolite increased with the increase in dose level in both sexes on each dosing day. Cmax and AUC24 of PROJECT 5 on any sampling day showed no marked sex differences at any dose. Cmax and AUC24 of M2 in males were lower than those in females at all dose levels on each sampling day. Cmax and AUC24 of M4 on day 1 showed no marked sex differences at any dose, while Cmax and AUC24 of females in weeks 13 and 26 were slightly higher than those of males. Cmax and AUC24 of PROJECT 5, M2 and M4 in week 13 of administration in both sexes at all doses except for AUC24 of M2 in females at 10 mg/kg per day were slightly lower than those on day 1 of

administration; however, those in week 13 of administration were comparable to those in week 26 of administration.

In the definitive 24-month carcinogenicity study in Wistar rats (0 [vehicle], 0 [vehicle], 5, 20 and 50 mg/kg per day) [End-of-Text Table 3.9.5] (Study Project 5-TX-0038), the increased incidence of proliferative changes (benign and malignant thymomas, and hyperplasia) in the thymus was noted in females at 20 and 50 mg/kg per day [[Table 4](#_bookmark53)].

### Table 4 Summary of Neoplastic Changes and Tumor-related Proliferative Changes in Wistar Rats

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sex**  **Dose (mg/kg/day) Number of animals** | **Male** | | | | | **Female** | | | | |
| **0 †** | **0‡** | **5** | **20** | **50** | **0†** | **0‡** | **5** | **20** | **50** |
| **54** | **55** | **55** | **55** | **54** | **55** | **55** | **55** | **55** | **55** |
| Thymus | | | | | | | | | | |
| Hyperplasia (Total) | 2 | 1 | 2 | 6 | 0 | 4 | 0 | 4 | 6 | 6 |
| minimal | 0 | 0 | 0 | 3 | 0 | 1 | 0 | 2 | 2 | 1 |
| mild | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 2 | 4 |
| moderate | 1 | 1 | 2 | 2 | 0 | 2 | 0 | 2 | 2 | 1 |
| Thymoma, Benign | 6 | 5 | 7 | 7 | 6 | 5§¶ | 8†† | 13 | 18 ‡‡§§ | 26‡‡§§¶¶ |
| Thymoma, Malignant | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 |
| Thymoma, Benign + Thymoma, Malignant | 6 | 6 | 7 | 7 | 6 | 5§¶ | 8†† | 14 | 19‡‡§§ | 28‡‡§§¶¶ |
| Hyperplasia + Thymoma, Benign  + Thymoma, Malignant | 8 | 7 | 9 | 12 | 6 | 9§¶ | 8†† | 16 | 22‡‡§§¶¶ | 33‡‡§§¶¶ |

Numbers in the table indicate the number of animals with respective findings.

† Control-I

‡ Control-II

§ P < 0.005 (statistically significant difference for positive trend among combined control-I and control-II groups and dose groups, Peto’s test)

¶ P < 0.005 (statistically significant difference for positive trend among control-I group and dose groups, Peto’s test)

†† P < 0.005 (statistically significant difference for positive trend among control-II group and dose groups, Peto’s test)

‡‡ P < 0.01 (statistically significant difference for positive between combined control-I and control-II groups and dose groups, Peto’s test)

§§ P < 0.01 (statistically significant difference between control-I group and dose groups, Peto’s test)

¶¶ P < 0.01 (statistically significant difference between control-II group and dose groups, Peto’s test) Source: Study Project 5-TX-0038 Table 11-1

As nonneoplastic changes, test article-related effects were observed in the liver, kidneys, digestive tract and Harderian glands. In the liver, histopathological examination revealed hepatocytic degeneration characterized by microvesicular cytoplasm and cytoplasmic inclusion, increases in incidence and severity of oval cell hyperplasia and an increased incidence of eosinophilic altered cell focus in males at 50 mg/kg per day. In the digestive system, histopathological examination revealed an increased incidence of erosion/ulcer in the glandular stomach and duodenum in died and moribund-sacrificed males at 50 mg/kg per

day. In the kidneys, histopathological examination revealed increased incidence and severity of pelvic inflammation and urothelial hyperplasia in males at 50 mg/kg per day. In the Harderian glands, histopathological examination revealed multinucleated acinar cells in males at all doses and in females at 50 mg/kg per day.

Toxicokinetics data revealed dose-related systemic exposure to PROJECT 5 and its metabolites (M1, M2 and M4). Cmax and AUC24 of PROJECT 5 and each metabolite increased with the increase in dose level in both sexes on each dosing day except for Cmax of M1 in males on day 1 of administration. Cmax and/or AUC24 of PROJECT 5, M1 and M2 in males were lower than those of females at each dose on each dosing day. Cmax and AUC24 of M4 on day 1 showed no marked sex differences at any dose. In weeks 13 and 26, there was also no sex difference in Cmax at 50 mg/kg per day; however, Cmax and AUC24 in females at other doses were lower than those of males. No clear effects were observed in Cmax or AUC24 of PROJECT 5 and M1 in either sex at any dose by repeated dosing. Cmax and/or AUC24 of M2 in week 13 of administration in both sexes at 5 and 20 mg/kg per day were slightly lower than those on day 1 of administration; however, they were comparable to those in week 26 of administration. At 50 mg/kg per day, no clear effects were observed in Cmax or AUC24 of M2 in either sex by repeated dosing. Cmax and/or AUC24 of M4 in week 13 of administration in females at each dose were slightly lower than those on day 1 of administration; however, they were comparable to those in week 26 of administration. In males, no clear effects were observed in Cmax or AUC24 of M4 at any dose by repeated dosing.

### Reproductive and Developmental Toxicity

A fertility and early embryonic developmental toxicity study was conducted in the rat. Embryo-fetal reproductive and developmental toxicity studies were conducted in the rat and rabbit. A pre- and postnatal developmental toxicity study was conducted in the rat.

A tabulated summary of data from nonpivotal reproductive and developmental toxicity studies can be found in [End-of-Text Table 3.10]. Pivotal reproductive and developmental toxicity studies are presented in [End-of-Text Tables 3.11, 3.12.1, 3.12.2, 3.12.3 and 3.13].

### Fertility and Early Embryonic Development

The effects of PROJECT 5 (0 [vehicle], 10, 30 and 100 mg/kg per day) were studied in male and female rats prior to cohabitation, throughout mating, and up through the implantation stage of gestation [End-of-Text Table 3.11] (Study Project 5-TX-0026).

No toxicological effects from the administration of PROJECT 5 were noted in regard to general condition, body weight, food consumption, estrous cycle or gross pathological findings in any dose group.

In the 100 mg/kg per day group, viability of embryos was decreased after implantation and the number of live embryos was decreased compared with the control group. No test

article-related effects were noted in the indices of fertility in males and females such as days until copulation, copulation index, fertility index or number of corpora lutea in any dose group. Moreover, no test article related effects were noted in the indices of early embryonic

development until implantation such as number of implantations or index of preimplantation losses in any dose group.

The NOAEL was considered to be 100 mg/kg per day for general toxicity in males and females and fertility of males and females and 30 mg/kg per day for early embryonic development.

### Embryo-fetal Development

Embryo-fetal toxicity studies of PROJECT 5 were conducted at doses up to 1000 mg/kg per day in the rat and up to 30 mg/kg per day in the rabbit [End-of-Text Tables 3.10, 3.12.1, 3.12.2 and 3.12.3].

In the dose-finding embryo-fetal toxicity study in the rat, 1 dam at 1000 mg/kg per day died and the rest of the treatment group experienced signs including decreased food consumption, decreased body weight, gestational body weight gain, vaginal hemorrhage, decreased spontaneous motility, reddish fur around the eyes and paleness of the auricle, as well as small spleen and dark red foci in the glandular stomach (Study Project 5-TX-0001). Three of the

4 surviving dams at 1000 mg/kg per day experienced the loss of the entire litter. Postimplantation loss rate was increased and fetal body weights were decreased at doses

≥ 300 mg/kg per day. External abnormalities affecting the extremities occurred at an increased incidence at 300 mg/kg per day (12.5% versus 0% [control]).

In the oral GLP embryo-fetal toxicity study in rats (Study Project 5-TX-0002), the maternal NOAEL was 300 mg/kg per day, but increased incidence of skeletal abnormalities and variations and visceral variations were noted in fetuses at all doses tested (30, 100 and 300 mg/kg per day), and increased incidence of visceral and external abnormalities was observed at 300 mg/kg per day. Fetal body weights were decreased at doses of 100 and

300 mg/kg per day. The NOAEL for embryo-fetal development was not determined for this study.

An additional low-dose study was conducted to determine an embryo-fetal development NOAEL in rats (Study Project 5-TX-0025). PROJECT 5 was administered at doses of 1, 3 and 10 mg/kg per day. In dams, no abnormal signs were seen in any group. No test

article-related effects on body weight, food consumption, necropsy findings or number of implantations was noted in any group.

In fetuses, no test article-related effects on the number of live fetuses, number of

embryo-fetal deaths, postimplantation loss, fetal weights, placental weights, sex ratio, fetal external or skeletal findings or skeletal ossification were noted in any group. Thus PROJECT 5 showed no potential for inducing fetal growth retardation, fetal lethality or teratogenicity in this study.

Toxicokinetic data indicated systemic exposure to the test article was achieved during the dosing period. The Cmax and AUC24 for doses of 1 to 10 mg/kg per day increased

dose-proportionally after administration on GD7 and GD17. The Cmax and AUC24 values on GD17 at 1 and 3 mg/kg per day were almost comparable to those at 10 mg/kg per day, but were greater than corresponding values on GD7.

After administration of PROJECT 5 at 1, 3 and 10 mg/kg per day, the NOAEL was considered to be 10 mg/kg per day for embryo-fetal development.

In the oral dose-finding embryo-fetal toxicity study in rabbits (Study Project 5-TX-0004), increased mortality (5 deaths) and morbidity in dams were observed at 30 mg/kg per day and the effects of this dose level on fetuses could not be determined; findings in dams included decreased food consumption and suppression of body weight gain, decrease in spontaneous activity, tachypnea and external genital bleeding. At 10 mg/kg per day, decreased food consumption was noted in dams, and a high postimplantation loss rate and tendency toward low number of live fetuses were noted in litter data. No toxicity was observed in dams or fetuses at 3 mg/kg per day.

In the definitive oral embryo-fetal toxicity study in the rabbit (Study Project 5-TX-0005), 1 dam died and 2 others had a complete loss of their litters at 10 mg/kg per day. Decreased food consumption and external genital bleeding in dams were noted at 10 mg/kg per day. The number of embryo-fetal deaths, postimplantation loss and frequency of fused sternebra and thready fusion of sternebra were significantly higher and the number of live fetuses was significantly lower for offspring of dams that received PROJECT 5 at 10 mg/kg per day. The increased frequency of sternebral findings was not considered to be caused by teratogenicity of the test article because the frequency of fused sternebra was within the background range; thready fusion of sternebra is commonly observed as a spontaneous change and fusion should have occurred if development had been allowed to continue. In the rabbit study, the NOAEL was considered to be 3 mg/kg per day for general toxicity and reproductive function of dams and 3 mg/kg per day for offspring.

### Pre- and Postnatal Development

The effects of PROJECT 5 (0 [vehicle], 3, 10 and 100 mg/kg per day) on pre- and postnatal development and maternal functions were studied in female rats during period from implantation to the day before weaning (from GD7 to day 20 of lactation) [End-of-Text Table 3.13] (Study Project 5-TX-0042).

One dam in the 100 mg/kg per day group was confirmed to start parturition before administration in the morning on GD22 but parturition was not completed by 17:00. The dam died during parturition on GD22 (17:10). There were no abnormalities in the body weight gain or food consumption until GD21, and there were no abnormalities in general signs during the time of parturition on GD22. Eight live-born pups were delivered, and there were no abnormalities in these pups. At necropsy, 5 fetuses were left in the uterus and the head of 1 fetus was protruded from the cervical region of the uterus to the vagina. The cause of death was considered dystocia with parturient prolongation. Other than this mortality, there were no changes or abnormalities caused by the test article in the general signs, body weight, food consumption, or gross pathological findings in any dose group. There were no effects of the test article on the delivery index, length of the gestation period, number of implantation sites, still-birth index, number of live-born pups, or live-birth index, and there were no abnormalities in the nursing condition of dams in any dose group.

In the 100 mg/kg per day group, a decrease in the viability index on day 4 after birth and suppressed body weight gain were noted. In this group, although there were no statistically significant differences, there were skeletal abnormalities in the vertebrae and ribs (absent cervical vertebra and cervical arch, fused rib and thoracic arch). There were no similar changes in the 10 mg/kg per day or lower dose groups. In addition, there were wavy rib and delayed ossification that were observed in the embryo-fetal development study (Study Project 5- TX-0002); however, no pups with these changes were observed on day 21 after birth in this study.

No adverse effects of the test article were noted in the external observation, sex ratio, viability on day 21 after birth, physical development, examination of sensory and reflex function, behavior tests (open field test, rotor rod test, water-filled multiple T-maze test), mating and fertility, body weight of F1 dams, intrauterine findings at the middle of gestation in F1 dams and gross pathological finding at any stage after birth.

Toxicokinetics data indicated Cmax and AUC24 for both PROJECT 5 and M2 were increased with the increase in dose level on each day. For PROJECT 5, increases in Cmax by repeated dosing were observed at 3 mg/kg per day. Decreases in Cmax by repeated dosing were observed at 10 and 100 mg/kg per day. Slight decreases in AUC24 by repeated dosing were observed in all dose groups. For M2, no differences in Cmax by repeated dosing were observed at 3 mg/kg per day. Decreases in Cmax by repeated dosing were observed at 10 and 100 mg/kg per day. Decreases in AUC24 by repeated dosing were observed in all dose groups.

The NOAEL was considered to be 10 mg/kg per day for general toxicity and reproductive performance in dams and for growth of F1 animals, and 100 mg/kg per day for reproductive performance in F1 animals, physical development and behavior of F1 animals.

### Local Tolerance

No local tolerance studies were conducted with PROJECT 5.

### Other Toxicity Studies

### Immunophenotyping Study in Monkeys

An oral immunophenotyping study of PROJECT 5 was conducted in the cynomolgus monkey (Study Project 5-TX-0013). PROJECT 5 was administered for 6 weeks at doses of 0 (vehicle), 1, 3 and 10 mg/kg per day with a 3-week recovery period. The blood obtained from monkeys dosed orally with PROJECT 5 was analyzed by flow cytometry to examine the lymphocyte subsets. Aliquots of the blood samples were stimulated ex vivo by IL-2, and then phosphorylated STAT5+ cell ratio in T cells (CD3+ lymphocytes) was determined. No animals died, and no test article-related or clinically significant changes were noted in clinical signs, body weight, food consumption or hematology [End-of-Text-Table 3.16].

An immunophenotyping study of PROJECT 5 in the cynomolgus monkey revealed changes related to the mechanism of action of the drug (i.e., JAK inhibition). Findings included a decreased ratio of phosphorylated STAT5+ CD3+ to total CD3+ lymphocytes, decreased

percentages of CD3-CD16+ and CD16+ (NK) lymphocytes in the total lymphocyte pool compared with predose values at 3 and 10 mg/kg per day, and decreased numbers of CD3- CD16+ and CD16+ (NK) lymphocytes at 10 mg/kg per day versus control. On day 41, percentages of CD28+CD95- (naïve) of the CD3+CD4+ (helper T cells) lymphocytes were reduced compared with the predose period at doses of 3 and 10 mg/kg per day. The number of CD28+CD95- (naïve) lymphocytes was decreased at a dose of 10 mg/kg per day relative to control. Immunophenotyping changes noted during the dosing period recovered during the

3-week recovery period.

These effects might be attributable to JAK3 inhibition on T or NK cells, resulting in a subsequent decrease in these cells.

### Combination Toxicity

Three 4-week and four 13-week combination toxicity studies in rats were conducted in order to assess the effects of combination treatment of PROJECT 5 and tacrolimus

(Studies Project 5-TX-0022 [4 weeks] and Project 5-TX-0028 [13 weeks]), PROJECT 5 and MMF (Studies Project 5-TX-0021 [4 weeks] and Project 5-TX-0027 [13 weeks]) and PROJECT 5 and MTX (Studies Project 5-TX-0031 [4 weeks] and Project 5-TX-0032 and Project 5-TX-0033 [13 weeks]).

Results of these studies are summarized in [End-of-Text Tables 3.16 and 3.16.1 to 3.16.6].

In the 4-week repeated oral dose combination toxicity study with PROJECT 5 and tacrolimus in rats (Study Project 5-TX-0022, dose levels of PROJECT 5/tacrolimus: 0/0, 0/5, 30/0, 3/5, 10/5 and 30/5 mg/kg per day), changes observed in either the tacrolimus or PROJECT 5 alone group (such as renal toxicity, pancreatic toxicity and immune system changes) did not worsen with the combination treatment. The additive effects with the combination treatment were enhancement of decreased lymphocyte count and aggregation of thymic changes related to tacrolimus and those related to PROJECT 5. No novel toxicities were seen with combination treatment. In toxicokinetics for the combination treatment, Cmax and/or AUC24 of PROJECT 5 in males were increased after repeated dosing, but there were high individual variations in Cmax and AUC24 of tacrolimus; therefore, the effects of tacrolimus in combination treatment were unclear.

In the 13-week repeated oral dose combination toxicity study with PROJECT 5 and tacrolimus in rats (Study Project 5-TX-0028, dose levels of PROJECT 5/tacrolimus: 0/0, 0/3.2, 30/0, 3/3.2, 10/3.2 and 30/3.2 mg/kg per day), changes observed in either the tacrolimus or PROJECT 5 alone group (such as immune system changes caused by pharmacological action, ocular toxicity, renal toxicity, pancreatic islet toxicity and brain and nerve toxicity) did not worsen with the combination treatment. Additive effects with combination treatment were suppression of the immune system caused by pharmacological action (low lymphocyte count, atrophy, increased cortex/medulla ratio and lymphocytolysis in the thymus). No novel toxicity was seen with combination treatment. In toxicokinetics, no clear differences were noted in the toxicokinetic parameters between the PROJECT 5 alone group and the combination treatment group.

In the 4-week combination study with PROJECT 5 and MMF in rats, (Study Project 5-TX-0021, dose levels of PROJECT 5/MMF: 0/0, 0/20, 30/0, 3/20, 10/20 and 30/20 mg/kg per day), noteworthy findings included individual or interactive responses in hematological parameters, lower thymus and spleen weights, thymic and splenic atrophy, bone marrow hypocellularity and decreased mononuclear cell infiltrates in the liver. Compared to the changes observed in animals given PROJECT 5 alone at 30 mg/kg per day or MMF alone at 20 mg/kg per day, treatment with PROJECT 5 at dose levels of 3, 10 or 30 mg/kg per day in combination with MMF at 20 mg/kg per day for 4 weeks did not cause exaggerated toxicological changes, although several pharmacological changes were apparent. In rats

given a 30 mg/kg per day dose of PROJECT 5 alone, the AUC24 values for PROJECT 5 in plasma on day 28 were 5266 and 7973 ng∙h/mL in males and females, respectively. In the males and females also given MMF (20 mg/kg per day), the corresponding values for PROJECT 5 were 7087 and 9982 ng·h/mL.

In the 13-week combination study with PROJECT 5 and MMF in rats (Study Project 5-TX-0027, dose levels of PROJECT 5/MMF: 0/0, 0/20, 30/0, 3/20, 10/20 and 30/20 mg/kg per day), suppression of the immune system caused by pharmacological action was noted in the PROJECT 5 alone, MMF alone, and combination treatment groups, and almost all findings did not worsen with combination treatment. Synergistic effects, thymic atrophy (due to pharmacological action) and anemia, were noted in females in the high-dose combination treatment group. No novel toxicity was seen with combination treatment. In toxicokinetics, no clear differences were noted in the toxicokinetic parameters between the PROJECT 5 alone group and the combination treatment group.

In the 4-week non-GLP repeated oral dose combination toxicity study with PROJECT 5 and MTX in rats (Study Project 5-TX-0031, dose levels of PROJECT 5/MTX: 0/0, 0/0.2, 30/0, 3/0.2, 10/0.2 and 30/0.2 mg/kg per day), additive effects with combination treatment were suppression of the immune system (low leukocyte count [mainly low lymphocyte count] and low spleen and thymus weights) and myelosuppression (hypocellularity in the femoral or sternal bone marrow). Other changes noted in the PROJECT 5 alone or MTX alone group (such as anemia, low eosinophil count and papillary necrosis in the kidney) did not worsen with combination treatment. No synergistic toxicity was seen with combination treatment, but expansion of the paracortex of the mesenteric lymph node (very slight to slight) was newly observed in the combination treatment groups. Cmax and AUC24 of MTX were slightly lower in the high-dose combination treatment group than in other groups, but there were no other obvious differences in Cmax and AUC24 values in toxicokinetic parameters of PROJECT 5 and MTX.

In the 13-week non-GLP repeated oral dose combination study with PROJECT 5 and MTX in rats (Study Project 5-TX-0032, dose levels of PROJECT 5/MTX: 0/0, 0/0.2, 30/0, 3/0.2, 10/0.2 and 30/0.2 mg/kg per day), many animals treated with MTX died or were sacrificed due to moribundity. Therefore, the effect of the combination of PROJECT 5 and MTX could not be evaluated.

In the 13-week GLP repeated oral dose combination toxicity study with PROJECT 5 and MTX in rats (Study Project 5-TX-0033, dose levels of PROJECT 5/MTX: 0/0, 0/0.1, 30/0, 3/0.1, 10/0.1 and 30/0.1 mg/kg per day), myelosuppression (hypocellularity in the femoral and/or sternal bone marrow) was noted in the PROJECT 5 alone, MTX alone, and combination treatment groups in males or females at all dose levels, and suppression of the immune system caused by pharmacological action (low leukocyte count [mainly low lymphocyte count], low spleen weight, follicular atrophy in the spleen in males and females, follicular atrophy in the mesenteric lymph nodes and atrophy of the thymus in males) related to treatment with PROJECT 5 was noted at the middle and/or high dose levels. However, these changes did not worsen with combination treatment. Cmax of MTX decreased in combination treatment with increasing doses of PROJECT 5 after repeated dosing.

### Phototoxicity

In order to investigate the potential phototoxicity of PROJECT 5, a phototoxicity study was performed with cultured mammalian Balb/c 3T3cells (Study Project 5-TX-0043), [End-of-Text Table 3.16]. The main test was performed at 3.35, 5.36, 8.58, 13.7, 22.0, 35.2, 56.3 and

90 μg/mL as PROJECT 5 in the presence and absence of UV-A irradiation. Test article precipitation in the treatment mixture was not observed at up to 90 μg/mL at the start of treatment, but was observed at 90 μg/mL at the end of treatment. Accordingly, the results for cell viability at 90 μg/mL were not evaluated. The result was judged from the mean photo effect (MPE) because the IC50 for cell viability could not be determined in either the presence or absence of irradiation. The MPE (actual value: 0.011) was less than 0.15. Therefore, PROJECT 5 was categorized as having no phototoxicity.

It was concluded that, under the conditions of this study, PROJECT 5 showed no potential to induce phototoxicity to cultured mammalian cells (Balb/c 3T3 cells).

### Metabolite Studies with AS2604202 (PROJECT 5 Metabolite M4)

AS2604202 (PROJECT 5 metabolite M4 ) was administered orally once daily for 1 week and up to 4 weeks at dose levels of 0 (vehicle control), 100, 300 and 1000 mg/kg per day to Crl:CD(SD) rats (Studies Project 5-TX-0044 and Project 5-TX-0045) in order to investigate its toxicity [End-of-Text Table 3.17].

In the 1-week toxicity study, no animal died and no toxicity was observed at up to 1000 mg/kg per day.

In toxicokinetics, Cmax and AUC24 increased more than dose proportionally on days 1 and 7. Cmax and AUC24 on day 7 were increased at 100 and 300 mg/kg per day and decreased at 1000 mg/kg per day compared with those on day 1. There were no sex differences except in AUC24 at 1000 mg/kg per day on day 7. AUC24 at 1000 mg/kg per day on day 7 was higher in males than in females.

It was concluded that, under the conditions of this study, the NOAEL was 1000 mg/kg per day for males and females.

In the 4-week toxicity study, no animal died and no toxicity was observed at up to 300 mg/kg per day. At 1000 mg/kg per day, dilatation of the cecum with increased content was observed in all males and 5 females. In males, low body weight gain and food consumption between days 1 to 3 of dosing, and slightly low urinary pH and liver weight were observed. In females, slightly high aspartate transaminase and alanine transaminase were observed.

The toxicological significance of these changes was considered to be low.

During the recovery period, all changes observed during the dosing period had recovered.

Cmax and AUC24 increased with dose, and in particular, the increase at 1000 mg/kg per day (days 1, 14 and 28 of dosing) was greater than dose proportional in both sexes. Cmax and AUC24 increased greater than dose proportionally in females at 300 mg/kg per day (days 1 and 14 of dosing). Cmax and AUC24 were almost constant during the dosing period, except for Cmax in both sexes at 100 mg/kg per day and in males at 300 mg/kg per day, which was increased by repeated dosing.

The NOAEL for males and females was 300 mg/kg per day.

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

Findings from nonclinical studies of PROJECT 5 conducted as of current IB release date suggest that the primary areas of toxicity are the GI system, hematopoietic system, muscle tissue and teratogenicity.

PROJECT 5 is an investigational oral immunosuppressant that acts by inhibiting JAK family members which play a crucial role in the signal pathway of various cytokine and growth hormone receptors [Banerjee, 2017]. In vitro kinase assay results suggest that PROJECT 5 inhibits all of the members, JAK1, JAK2, JAK3 and TYK2.

In the 4-week dose range-finding and 13-week toxicity study in monkeys, animals became moribund and were sacrificed at high dose levels of PROJECT 5. It is likely that the moribund condition was induced by malnutrition and dehydration resulting from decreased food consumption and severe GI pathology.

Rats, mice and monkeys that received repeated doses of PROJECT 5 experienced GI disorders. In rats, erosion and regenerative reactions in the stomach were observed at 30 mg/kg per day and higher in the 4-week study and single cell necrosis in the cecum and rectum was observed at 10 mg/kg per day and higher in the 13-week study. In mice, inflammatory cell infiltration and hyperplasia of the squamous cells in the forestomach were observed in males at 30 mg/kg per day and higher. Monkeys showed some clinical signs including vomiting and stool change, such as soft stool and diarrhea (≥ 30 mg/kg per day in 4-week studies, 60/30 mg/kg per day in the 13-week study and 15 mg/kg per day in the 52-week study).

Histopathological changes of the GI tract, such as erosion and inflammatory cell infiltration, were only observed at 8 mg/kg per day and higher in the 13-week study, and at 15 mg/kg per day in the 52-week study. Reversibility of these changes was confirmed in the 4- and

13-week studies in rats and monkeys.

Rats and monkeys that received PROJECT 5 experienced changes in hematopoietic parameters during the dosing period that had reversed by the end of the recovery period. Hypocellularity of the bone marrow and inhibition of splenic extramedullary hematopoiesis were observed in rats that received PROJECT 5 at 30 mg/kg per day or higher. Decreases in red blood cell count, hemoglobin and hematocrit and hypocellularity of the bone marrow were noted in monkeys at high doses. PROJECT 5 inhibits human JAK3 enzyme activity at 0.71 nmol/L (IC50), but also inhibits human JAK2 enzyme activity at 5.01 nmol/L (IC50), indicating that the JAK3 selectivity is approximately 7-fold. JAK2 is involved in signal transduction of erythropoietin, the prime regulator of red blood cell production [Ghaffari et al, 2001]; therefore, loss of selectivity at high plasma concentrations of PROJECT 5 may result in inhibition of JAK2 enzyme activity that may be responsible for anemic changes seen in rats and monkeys that received high doses of PROJECT 5. Increases in neutrophil counts and/or increased granulocytes in the bone marrow were also observed in monkeys that received PROJECT 5 (30 mg/kg per day and higher in the 4-week study, 15 mg/kg per day and higher in the 13-week study, and 4 mg/kg per day and higher in the 52-week study). JAK3 is involved in the differentiation of neutrophils [Grossman et al, 1999]. Mice deficient in JAK3 show an increase in the number of neutrophils in peripheral blood; therefore, it is likely that inhibition of JAK3 enzyme activity caused the leukocytosis seen in monkeys that received high doses of PROJECT 5.

In the 4-week dose range-finding study in monkeys, mild multifocal muscle necrosis was observed in 1 female that received PROJECT 5 at 60 mg/kg per day. In the definitive 4-week toxicity study in monkeys, no muscle change was detected by histopathology. Increased levels of CPK and lactate dehydrogenase were observed at 60 mg/kg per day, but had disappeared during the recovery period. In the 13-week toxicity study in monkeys, increased muscular fraction of isoenzymes of lactose dehydrogenase and CPK were noted in 1 male that was sacrificed in extremis, and a transient increase of muscular fraction of isoenzymes of CPK was observed in another male at week 7. However, no histopathological changes of the muscle were observed in those animals, leaving the relationship between elevation of enzymes and muscular damage unknown.

In the embryo-fetal development studies in rats, an increased frequency of external abnormalities and visceral abnormalities and variations was observed at 300 mg/kg per day, and increased frequency of skeletal abnormalities and variations was observed at 30 mg/kg per day and higher. In the rat studies, the NOAEL was considered to be 300 mg/kg per day for dams and 10 mg/kg per day for embryo-fetal development. In the embryo-fetal development studies in rabbits, no teratogenic potential of PROJECT 5 was suggested.

In the pre- and postnatal development study in rats, 1 dam in the 100 mg/kg per day died during parturition on GD 22 because of dystocia with parturition prolongation. Decreased viability index on day 4 after birth and suppressed body weight gain in pups were noted at 100 mg/kg per day. At this dose, although there were no statistically significant differences, there were skeletal abnormalities in the vertebrae and ribs. The NOAEL was considered to be 10 mg/kg per day for general toxicity and reproductive performance in dams and for

growth of F1 animals, and 100 mg/kg per day for reproductive performance in F1 animals, physical development and behavior of F1 animals.

In the fertility and early embryonic development study, no test article-related effects were noted in indices of fertility in males and females, but the viability of embryos was decreased after implantation and the number of live embryos was decreased in the 100 mg/kg per day group.

PROJECT 5 was not considered to have mutagenic potential in a bacterial reverse mutation test; however, PROJECT 5 induced chromosomal aberrations in an in vitro chromosomal aberration test. PROJECT 5 had no detectable clastogenic potential in a mouse micronucleus test or in an unscheduled DNA synthesis test in rats. Based on these results, PROJECT 5 is not considered to show mutagenic or clastogenic potential in vivo.

In the 24-month carcinogenicity study in Wistar rats, the incidence of proliferative changes in the thymus was increased at 20 mg/kg per day or higher in females, indicating tumorigenic potential of PROJECT 5 in Wistar rats. Results from the 24-month carcinogenicity study in B6C3F1 mice indicated no tumorigenic potential. The mechanism of increased incidences of proliferative changes in the rat study is considered to be nongenotoxic, since PROJECT 5 is not considered to be genotoxic in vivo.

It is known that immunosuppressive drugs can increase the risk of neoplasia in animals and humans [Bugelski et al, 2010; Vial & Descotes, 2003]. Review of the pharmacology sections from the Summary Basis of Approval of the New Drug Applications for tofacitinib (a JAK inhibitor) and pimecrolimus (a calcineurin inhibitor) reports an increased incidence of benign thymoma in the thymus in the 2-year carcinogenicity studies in rats with these immunosuppressive drugs (XELJANZ prescribing information, May 2018; ELIDEL prescribing information, March 2014). In the 2-year carcinogenicity study of tofacitinib in Sprague Dawley rats, in addition to benign thymoma, other tumors such as benign Leydig cell tumor and malignant hibernoma were also induced. Leydig cell tumor and malignant hibernoma were not induced in the 24-month carcinogenicity studies of PROJECT 5 in mice and rats. The increased incidence of proliferative changes in the thymus was noted only in female rats with PROJECT 5 at 20 mg/kg per day or higher. This finding was not observed in mice even at the highest dose level. In addition, there was no increased incidence of malignant thymoma in mice or rats.

Based on the above, PROJECT 5 showed the proliferative changes in thymus, indicative of nongenotoxic tumorigenic potential in the 24-month carcinogenicity study in female Wistar rats. Immunosuppressive drugs are known to be associated with an increased risk of neoplasia and similar results were seen in carcinogenicity studies with tofacitinib or other immunosuppressive drugs. Therefore, the obtained results are not considered to raise a newly identified safety concern for tumorigenicity in human patients.

In the 4-week or 13-week combination toxicity studies in rats, combination treatments of PROJECT 5 and tacrolimus, PROJECT 5 and MMF, and PROJECT 5 and MTX did not cause synergistic exaggerated toxicities or novel toxicities except for very slight to slight expansion

of the paracortex of the mesenteric lymph node in PROJECT 5/MTX combination, and no obvious toxicokinetics interactions were observed.

PROJECT 5 showed no potential to induce phototoxicity to cultured mammalian cells (Balb/c 3T3 cells).

In a 4-week rat study with M4, PROJECT 5 metabolite, minor changes were seen at 1000 mg/kg per day and these changes were reversible after 4 weeks.

In summary, findings from nonclinical studies of PROJECT 5 conducted to date in rats and monkeys have included effects on the GI system, hematopoietic system and muscle tissue, all of which were reversible upon dosing cessation. The dose levels at which these toxicities were observed and comparison of exposures at those dose levels with those in human subjects are shown in [Table 5.](#_bookmark65)

### Table 5 Safety Margins Based on Human AUC and Animal AUC of PROJECT 5

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species/ Dosage** | **Dose** | **Sex (M/F)** | **AUC†**  **(ng·h/mL)** | **Safety Margin Based on 150 mg Human Dose** |
| Rat/  13-week po (Day 91 data) | 3 mg/kg (NOAEL) | M | 519.0 | 0.27 |
| 10 mg/kg (NOAEL) | F | 2684.4 | 1.38 |
| 10 mg/kg (LOAEL) | M | 2164.9 | 1.12 |
| 100 mg/kg (LOAEL) | F | 19558.0 | 10.08 |
| Rat/  26-week po (Day 182 data) | 3 mg/kg (NOAEL) | M | 849.5 | 0.44 |
| 10 mg/kg (NOAEL) | F | 3057.2 | 1.58 |
| 10 mg/kg (LOAEL) | M | 2886.1 | 1.49 |
| 100 mg/kg (LOAEL) | F | 30983.7 | 15.96 |
| Monkey/ 13-week po  (Day 91 data) | 15 mg/kg (NOAEL) | M | 2573.4 | 1.33 |
| 4 mg/kg (NOAEL) | F | 358.9 | 0.18 |
| 60/30 mg/kg‡ (LOAEL) | M | 3115.3 | 1.60 |
| 8 mg/kg (LOAEL) | F | 715.5 | 0.37 |
| Monkey/ 52-week po  (Day 364 data) | 8 mg/kg (NOAEL) | M | 1784.3 | 0.92 |
| 2 mg/kg (NOAEL) | F | 316.4 | 0.16 |
| 15 mg/kg (LOAEL) | M | 2886.9 | 1.49 |
| 4 mg/kg (LOAEL) | F | 663.7 | 0.34 |
| Human (Study  Project 5-CL-PK26) | 150 mg | M/F | 1941 | NA |

Note: Calculation of safety margins does not take plasma protein binding into consideration.

AUC: area under the concentration-time curve; F: females; LOAEL: lowest-observed-adverse-effect level; M: males; NOAEL: no-observed-adverse-effect level; NA: not available.

† Animal and human data were reported as the AUC24 and AUCtau, respectively.

‡ Monkeys were administered 60 mg/kg per day from days 1 to 31 (male; dose was changed beginning day 32) or from days 1 to 28 (female; dose was changed beginning day 29) and 30 mg/kg per day for the remainder of the 13-week treatment period. Moribund sacrifices occurred on day 21 (male), and days 17, 26 and

64 (females), respectively.

Source: Studies Project 5-TX-0024, Project 5-TX-0030, Project 5-TX-0023, Project 5-TX-0029 and Project 5-CL-PK26

In the embryo-fetal development study in rats, PROJECT 5 showed teratogenic effects. Therefore, care should be taken to not expose pregnant females or females of childbearing potential using inadequate contraception to PROJECT 5.

In conclusion, the results of nonclinical safety pharmacology and toxicology studies support further development of PROJECT 5.

## List of References

Banerjee S, Biehl A, Gadina M, Hasni S, Schwartz DM. JAK-STAT signaling as a target for inflammatory and autoimmune diseases: current and future prospects. Drugs. 2017;77:521-46.

Bugelski PJ, Volk A, Walker MR, Krayer JH, Martin P, Descotes J. Critical Review of Preclinical Approaches to Evaluate the Potential of Immunosuppressive Drugs to Influence Human Neoplasia. International Journal of Toxicology. 2010; 29(5): 435-66.

ELIDEL (pimecrolimus) prescribing information. Bridgewater, NJ 08807, US. Valeant Pharmaceuticals North America LLC. March 2014.

Ghaffari S, Kitidis C, Fleming MD, Neubauer H, Pfeffer K, Lodish HF. Erythropoiesis in the absence of janus-kinase 2: BCR-ABL induces red cell formation in JAK2-/- hematopoietic progenitors. Blood. 2001;98:2948-57.

Grossman WJ, Verbsky JW, Yang L, Berg LJ, Fields LE, Chaplin DD, et al. Dysregulated myelopoiesis in mice lacking Jak3. Blood. 1999;94:932-9.

Vial T, Descotes J. Immunosuppressive drugs and cancer. Toxicology. 2003; 185(3): 229-40.

XELJANZ® (tofacitinib) prescribing information, NY, 10017, US. Pfizer Labs, Division of Pfizer Inc. May 2018