**A 13-Week Repeated Oral Dose Toxicity Study of Project R in Cynomolgus Monkeys Followed by an 8-Week Reversibility Study**

**12 SUMMARY AND CONCLUSION**

Project R was administered orally once daily for 13 weeks at dose levels of 0, 1, 3, 10, and 100 mg/kg as ASP9603 to 4 male and 4 female cynomolgus monkeys per group in order to investigate its toxicity. Three males and three females were added to the 100 mg/kg group in order to assess the reversibility of toxicity during a subsequent 8-week recovery period. Systemic exposure to ASP9603 was also evaluated. Animals in the control group received 0.5 w/v% methylcellulose solution in the same manner as the test article groups.

The following examinations were performed in this study: clinical signs, body weight, food consumption, ophthalmology, electrocardiography, urinalysis, hematology, blood chemistry, bone marrow examination, gross pathology, organ weights, histopathology, testosterone analysis, and toxicokinetics.

In the 1 mg/kg group, no toxic changes were noted.

In the 3 mg/kg group, dilation of the bile canaliculus in the liver was observed in all males and some females.

In the 10 mg/kg group, increased neutrophil count was noted in 1 female at Week 13 of dosing. Increased total, direct, and indirect bilirubin levels were noted in males and females, and increased alkaline phosphatase activity was noted in 1 female at Weeks 7 and 13. Increased liver weight was noted in males. Dilation of the bile canaliculus in the liver was observed in all males and females. Hypertrophy and decrease in lipid in the zona fasciculata cells in the adrenal were observed in 1 male and 1 female.

In the 100 mg/kg group, abnormal urine color, yellowish-brown and bilirubin positive, was observed in 1 male between Day 41 and Day 46 of dosing. Decreased body weight was noted in 4 males and 3 females. Decreased food consumption was noted sporadically in 1 male and 2 females. Increased neutrophil count was noted in females at Weeks 7 and/or 13, and increased platelet count was noted in males and in females at Week 7 or 13. Increased total, direct, and indirect bilirubin levels, and increased alkaline phosphatase activity were noted in males and females at Weeks 7 and 13. Decreased total cholesterol in females at Week 7 and 13, and increased triglycerides in 1 male at Week 7 and in 2 females at Week 13 were noted. Increased liver and adrenal weights were noted in males and females. Dilation of the bile canaliculus in all males and females, pigmentation suspected to be due to bile in the canaliculus in some females, and hypertrophy of the centrilobular/midzonal hepatocytes in some males and females were observed in the liver. Hypertrophy and decrease in lipid in the zona fasciculata cells were observed in the adrenal in all males and females.

In 1 female, swelling at the head in clinical signs, and hydrothorax, ascites, pericardial fluid, and subcutaneous edema at the neck and head in gross pathology, and edema in the subcutaneous tissue in histopathology were observed. Decreased total protein and albumin at Weeks 7 and 13. The following changes were considered to be secondary: decreased lymphocyte count and chloride, and increased aspartate aminotransferase, blood urea nitrogen, inorganic phosphorus, and potassium at Weeks 7 and/or 13, and decreased bone marrow nucleated cell count, atrophy of the myocardium and muscle fiber (femoral muscle and tongue), atrophy of the subepicardial adipocytes, atrophy of the white pulp in the spleen, atrophy of the lymph follicles in the lymph nodes (submandibular and mesenteric), and hypocellularity in the sternal bone marrow noted in 1 female, and atrophy of the thymus noted in males and females.

The following changes were considered to be due to the pharmacological effect (anti-androgen receptor) of the test article and not to be toxicologically significant: Increased testosterone level and decreased absolute and relative seminal vesicle weights in males and decreased absolute and relative uterus weights in females at 1 mg/kg and greater Decreased absolute and relative prostate weights and atrophy/hypoplasia of the gland in the seminal vesicle at 3 mg/kg and greater Atrophy/hypoplasia of the gland in the prostate at 10 mg/kg and greater.

In toxicokinetics, Cmax and AUC24 increased almost dose-proportionally up to 10 mg/kg, but less than dose proportionally at 100 mg/kg. Cmax and AUC24 were increased 2 to 5-fold on Day 49, and then remained constant on Day 91. There was no clear sex difference in any parameter during the dosing period.

The changes noted during or at the end of 13-week treatment at 100 mg/kg had recovered by the end of the recovery period.

In conclusion, under the conditions of this study, the no-observed-adverse-effect level (NOAEL) was 1 mg/kg/day as ASP9603 for males and females. Reversibility of toxicity during the 8-week recovery period was noted.