**A 4-Week Repeated Oral Dose Toxicity Study of Project G in Beagle Dogs Followed by a 4-Week Recovery Period**

**12 SUMMARY AND CONCLUSION**

Project G was suspended in 0.5 w/v% methylcellulose solution and orally administered once daily for 4 weeks at dose levels of 0 (vehicle control), 10, 100 and 1000 mg/kg (as PROJECT G) to 4 male and 4 female beagle dogs per group in order to investigate its toxicity. Three males and three females were added to the 1000 mg/kg group to assess the reversibility of toxicity during the dosing period in a subsequent 4-week recovery period. Systemic exposure to PROJECT G and its metabolites (AS1056091 and AS2771664) was also assessed. The following observations and examinations were performed in this study: clinical signs, body weight, food consumption, ophthalmology, electrocardiography, urinalysis, hematology, blood chemistry, measurement of plasma CCK-8 and serum BCAA concentrations, gross pathology, organ weights, histopathology, electron microscopy, and toxicokinetics.

No animal died or was sacrificed due to moribundity in any group during the dosing or recovery period.

In the 10 mg/kg group, no test article-related changes were noted.

In the 100 and 1000 mg/kg groups, abnormal stool color (yellowish-white or white) was observed in males and/or females mainly before dosing or approximately 1 or 4 hours after dosing during the dosing period; however, it was considered possible that the test article had been excreted without being absorbed, and the change was considered to be toxicologically insignificant.

In the 1000 mg/kg group, decreased body weight was noted in males and females during the dosing period. Low specific gravity or tendency toward low specific gravity in urinalysis in males and females and low serum calcium in males and females were noted in the 1000 mg/kg group. Prolongation of prothrombin time and activated partial thromboplastin time in males, high alanine transaminase and lipase in males, and high alkaline phosphatase in males and females were noted in the 1000 mg/kg group. In the liver, clear cell change of the hepatocytes was observed in males and females in the 1000 mg/kg group, and an increase of glycogen granules in the clear cell changed hepatocytes was observed in electron microscopy. Low urinary urea nitrogen excretion in males and females, low serum urea nitrogen in males in the 100 mg/kg group and in males and females in the 1000 mg/kg group, and low total protein, albumin, and A/G in males and females in the 1000 mg/kg group were noted. Low concentrations of BCAA were noted in males and females in all test article groups, mainly 1 to 8 hours after dosing, and low AUC24 values in BCAA were noted in males and females in the 100 and 1000 mg/kg groups. These changes were considered to be related to the pharmacological effect (trypsin inhibitor) of the test article; therefore, they were not considered to be toxicologically significant.

Dark discoloration of the pancreas in males and acinar cell atrophy in the pancreas in males and females in the 1000 mg/kg group, and high serum amylase in males and females in the 1000 mg/kg group were noted. Low absolute and relative thymus weights in males, a tendency toward low absolute and relative thymus weights in females, atrophy of the thymus in males and females, and fatty bone marrow in males were observed in the 1000 mg/kg group. These changes were considered to be secondary changes related to decreases in body weight or stress due to deterioration in general condition resulting from test article-administration.

During the dosing and recovery periods, no toxicological changes were noted in food consumption, ophthalmology, electrocardiography, or plasma CCK-8 in any group.

The changes noted during the dosing period were not noted after the 4-week recovery period. In toxicokinetics, the mean Cmax and mean AUC24 values of PROJECT G, AS1056091 (M1), and AS2771664 (M2) increased less than dose-proportionally, and those values showed a tendency to be almost constant regardless of the administration period. No TK parameters (Cmax or AUC24 values) showed clear sex difference.

It was concluded that, under the conditions of this study, the no-observed-adverse-effect level of Project G was 100 mg/kg/day for males and females as PROJECT G because decreased body weight in both sexes, low specific gravity or tendency toward low specific gravity in both sexes, prolongation of prothrombin time and activated partial thromboplastin time in males, high alanine transaminase in males and high alkaline phosphatase in both sexes, and low serum calcium in both sexes were noted in the 1000 mg/kg group. The test articlerelated changes observed during the dosing period recovered during the 4-week recovery period.