**A 4-Week Repeated Intravenous Dose Toxicity Study of PROJECT 9 in Cynomolgus Monkeys Followed by a 6-Week Recovery Period**

**11 SUMMARY**

PROJECT 9 was diluted with formulation buffer (PROJECT 9 placebo) and administered intravenously once weekly for 4 weeks at dose levels of 0 (control), 0.3, 1, 3, and 30 mg/kg to 4 male and 4 female cynomolgus monkeys (age at initiation of dosing: 3 to 4 years) per group in order to investigate its toxicity. Gross pathology at the end of the dosing period was conducted 7 days after the last dosing (4th dosing). Three male and three female animals per group were added to the 3 and 30 mg/kg groups to assess the reversibility of toxic changes during a subsequent 6-week recovery period. For the control group, formulation buffer was administered in the same manner as test article. Systemic exposure to PROJECT 9 and anti- PROJECT 9 antibody production were also assessed.

The following observations and examinations were performed: clinical signs, general behavior and neurobehavioral function, body weight, food consumption, ophthalmology, electrocardiography, body temperature, blood pressure, respiratory rate, urinalysis, hematology, blood chemistry, immunoglobulin analysis, peripheral blood immunophenotyping, gross pathology, organ weights, histopathology, toxicokinetics, and anti-PROJECT 9 antibody analysis.

No animal died or was euthanized due to moribundity.

At 0.3, 1, and 3 mg/kg, no toxicologically significant changes were noted.

At 30 mg/kg, the following changes were noted: increased reticulocyte ratio (male and female), eosinophil count (male and females), decreased platelet count (males), enlargement of the spleen and high spleen weights (males). Histopathology findings included congestion (males) and hyperplasia of the red pulp (male and female) in the spleen, hypertrophy of Kupffer cells in the liver (male and female), increase in hematopoiesis in the femoral bone marrow (males and female), atrophy in the thymus (males), hypertrophy of sinus histiocytes in the submandibular lymph node (male and female), edema and mixed inflammatory cell infiltration in the subserosa and lamina propria in the gallbladder (male), and vacuolation in the urothelium in the urinary bladder (males).

All changes observed during the dosing period recovered or tended toward recovery during the 6-week recovery period.

At the end of the recovery period, the following histopathology findings were observed at 30 mg/kg: fibrosis capsule in male, hemorrhage and brown pigment in the capsule in the spleen in male, and intimal thickening and perivascular fibrosis in the artery in the liver and intimal thickening and perivascular fibrosis in the artery of the subserosal in the gallbladder in male.

Anti-PROJECT 9 antibodies were detected at 0.3 mg/kg and greater from Day 15 until recovery Day 42. On Day 29, anti-PROJECT 9 antibodies were detected in 4 males and 2 female at 0.3 mg/kg, in 2 males and 3 females at 1 mg/kg, in 2 males and 2 females at 3 mg/kg, and in 3 males and 1 female at 30 mg/kg. In toxicokinetics, the mean Cmax and AUC168 values of PROJECT 9 were as shown in the following table:

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| --- | --- | --- | --- | --- | --- |
| Dose Level  (mg/kg) | Day | Cmax (µg/mL) | | AUC168 (µg·h/mL) | |
| Male | Female | Male | Female |
| 0.3 | 1\* | 7.65 | 7.99 | 626 | 704 |
| 22\*\* | 2.99 | 10.1 | 135 | 1010 |
| 1 | 1\* | 25.3 | 24.6 | 2230 | 2160 |
| 22\*\* | 27.4 | 15.0 | 2370 | 1340 |
| 3 | 1\* | 74.5 | 82.6 | 6790 | 7510 |
| 22\*\* | 108 | 129 | 10800 | 12900 |
| 30 | 1\* | 824 | 795 | 71500 | 72000 |
| 22\*\* | 1630 | 1540 | 177000 | 165000 |

\*: 1st dosing, \*\*: 4th dosing

Cmax and AUC168 values on Day 1 increased dose proportionally. The Cmax and AUC168 values on Day 22 were higher than those on Day 1 for all the animals in the 30 mg/kg group. However, the Cmax and AUC168 values on Day 22 were lower than those on Day 1 for anti- PROJECT 9 antibody positive animals in the 0.3 to 3 mg/kg dosing groups. The decrease in these values after repeated dosing was considered to be related to the production of anti- PROJECT 9 antibodies.

It was concluded that, under the conditions of the present study, the no-observed-adverse- effect level (NOAEL) was 3 mg/kg/week for males and females since changes of toxicological significance were noted at 30 mg/kg in hematology, gross pathology, organ weights, and histopathology. All changes observed during the dosing period were considered to have recovered or tended toward recovery during the 6-week recovery period.