**4-Week Toxicity Study of Project 15-1, PROJECT 15-2, and Project 15-3 in Cynomolgus Monkeys with a 6-Week Recovery Period (Non-GLP)**

1. **SUMMARY AND CONCLUSION**

**Objective:** To compare toxicity of three test articles in cynomolgus monkeys following intravenous administration, once weekly, for 4 consecutive weeks and to assess the reversibility of effects following a 6 week recovery period.

***Materials and Methods***

The initial dose administration was reported as Day 1, with subsequent days consecutively numbered. Days on study prior to the initial dose administration were consecutively numbered with the final day of acclimation referenced as Day -1. Eleven male and eleven female naïve, cynomolgus monkeys of Cambodian origin were assigned to one of eight dose groups as shown in In-text Table 1. Male animals weighed 2.07 to 2.69 kg and were 2 to 3 years old, and female animals weighed 1.94 to 2.84 kg and were 2 to 3 years old at the time of the physical examination.

**In-text Table 1: Study design**

The test articles were Project 15-1, PROJECT 15-2, and Project 15-3, and the control article was 20 mM histidine, 5% trehalose, pH 6.0. Test and control article were administered at the protocol-required strength as outlined in Intext Table 1. Doses were administered via 30 minute (±3 minutes) intravenous (IV) infusion once weekly for four consecutive weeks.

Nominal doses are referred to in this report, contingent on the assumption of accurate preparation and stability of the dosing formulations.

Study assessments included twice daily clinical observations; daily food consumption; body weight (twice in acclimation, once weekly throughout the study, and once on the day of necropsy); electrocardiography (ECG) (once during acclimation and on Day 23); ophthalmology (once during acclimation and on Days 9 and 23); urinalysis and coagulation (once during acclimation and on Days 26 and 70); hematology and serum chemistry (once during acclimation and on Days 4, 11, 25, and 70). Blood was also drawn at regular intervals for toxicokinetic (TK) analysis.

At necropsy gross observations were recorded, organ weights were collected, and specific tissues were collected. Histopathology was conducted on sections of collected tissues stained with hematoxylin and eosin (H&E).

***Results and Conclusions***

Intravenous administration of Project 15-1 (Group 2), PROJECT 15-2 (Groups 3 to 5), and Project 15-3 (Groups 6 to 8) to cynomolgus monkeys once every week for four consecutive was well tolerated. No mortalities occurred and no test article-related changes were observed in urinalysis, coagulation, ECG, ophthalmology, and organ weight assessments.

Test article-related postural changes (hunched) were noted in Group 5, and reductions in food consumption associated with minimal decreases in body weight were observed in Groups 5 and 8.

Test article-related changes in hematology included decreased red cell mass (red blood cells, hemoglobin, and/or hematocrit) in one Group 5 male on Days 25 and 70, accompanied by increased red cell distribution width (RDW) on Day 70.

Monocytes were mildly increased in Group 2 animals on Days 4, 11, and 25, one Group 5 male on Day 70, and one Group 8 male on Days 11 and 25. Test article-related changes in serum chemistry data included minimally decreased albumin and the albumin to globulin ratio (A/G) in Group 5 animals on Days 25 and 70. The A/G was also minimally decreased in one Group 8 female on Day 25. Globulin was minimally increased in one Group 4 male on Day 25, one Group 5 male on Days 25 and 70, and one Group 8 female on Day 25. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatine kinase (CK) were minimally to moderately increased in multiple animals across all test article-treated groups. The increased ALT and AST may be related to hepatic changes noted on histopathology but also may be due to muscle damage (consistent with the increased CK). These increases tended to be highest in Group 5.

Peak serum concentrations were generally attained instantaneously upon completion of intravenous (IV) injection for each of the three test articles. The area under the serum concentration curve (AUCT) and maximum concentration (Cmax) for both antibody-drug conjugates (ADC) and total antibody (Tab) for PROJECT 15-2 and Project 15-3 antibody drug conjugates showed dose dependent increases, approximately proportional to dose.

Project 15-1 exposure determined using TAb concentrations was, generally, higher in comparison to the corresponding ADC profiles. However exposure of PROJECT 15-2 and Project 15-3 determined using TAb concentrations was similar to the corresponding ADC profiles, indicating negligible unconjugation of the ADC. ADC and TAb exposures of PROJECT 15-2 were lower in comparison to those of Project 15-3 at similar dose levels.

Serum concentration of cys-mcMMAF (payload released from Project 15-1) increased gradually after dosing and reached a maximum by 6 to 12 hours postdose for Project 15-1. Mean concentrations were >200,000-fold lower than ADC and TAb (Project 15-1, parent drug) concentrations. In contrast, serum concentrations of pAF-AGL-185-30 (payload released from PROJECT 15-2) increased gradually after dosing, reached a maximum by 6 to 168 hours postdose, and were >900,000-fold lower than ADC and TAb (PROJECT 15-2, parent drug) concentrations.

Gross and microscopic pathology findings of potential toxicologic significance were observed in the kidneys, spleen, and liver. Findings at terminal necropsy were present in Groups 5 through 8. Following a 6 week recovery period, animals in Group 8 had no treatment-related findings, suggestive of complete recovery. In contrast, one of the two Group 5 animals had persistent kidney and liver changes, considered to be related to test article treatment, indicating partial reversibility.