**A Multiple Dose Toxicity Study of PROJECT 16-1 and PROJECT 16-2 Administered by intravenous Infusion to Cynomolgus Monkeys with a 6-week Recovery Period**

1. **SUMMARY AND CONCLUSION**

The objectives of this study were to determine the potential toxicity and TK profiles of PROJECT 16-1 when administered once every 2 weeks for a total of 4 doses (on Days 1, 15, 29, and 43) by a 30-minute intravenous (IV) infusion to cynomolgus monkeys, and to evaluate recovery from any effects over a dose-free period of 6 weeks. To aid in determination of the antibody-drug conjugate (ADC) toxicity, the unconjugated antibody (PROJECT 16-2) was dosed at the equivalent high dose of the ADC and the drug payload (the small molecule attached to the antibody, monomethyl auristatin E [MMAE]) was administered at the molar equivalent of the high dose group.

Based on adverse test article-related events at the high dose (3 mg/kg/dose) of PROJECT 16-1 that resulted in the mortality of 1 animal and the unscheduled euthanasia of 2 animals, dosing was discontinued prior to the Day 15 dose interval and the animals in the 3 mg/kg group received a single dose on Day 1. The Terminal Necropsy day for 1 male and 3 females of the surviving 3 mg/kg/dose animals was moved to Days 15 and 13. The remaining three 3 mg/kg/dose animals (1 male and 2 females) were designated as recovery animals and were euthanized on Days 50/51 (approximately 7 weeks). In addition, based on adverse test article-related events at the mid dose (1 mg/kg/dose) of PROJECT 16-1 that resulted in the mortality of 1 animal on Day 10, the dose level was lowered to 0.75 mg/kg/dose starting from Day 15.

The study design was as follows:

The following parameters and end points were evaluated in this study: clinical signs, body weights, food consumption, ophthalmology, electrocardiology (ECG), blood pressure, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), flow cytometry and receptor occupancy parameters, toxicokinetics (TK), anti-drug antibody (ADA) formation, gross necropsy findings, organ weights, and histopathologic examinations.

PROJECT 16-1 administration at 1 and 3 mg/kg resulted in mortality/morbidity. PROJECT 16-1 at 0.25 and 0.5 mg/kg/dose, the lowered PROJECT 16-1 at 0.75 mg/kg/dose from Day 15, PROJECT 16-2 at 3 mg/kg/dose and MMAE at 0.05 mg/kg/dose were generally well tolerated.

Eight PROJECT 16-1 dosed animals (1 animal at 1 mg/kg and 7 animals at 3 mg/kg/dose) were euthanized or died prior to their scheduled euthanasia. Of these, 2 animals (1 each at 1 and 3 mg/kg/dose) died on study and 6 animals (3 mg/kg/dose) underwent unscheduled euthanasia due to deteriorating health (between Days 11-15). The cause of morbidity and early death in all animals was related to PROJECT 16-1 administration as described below.

In general, all early death animals had similar clinical signs on the days preceding the unscheduled euthanasia. These clinical signs included skin lesions (generalized), abrasions (face/neck), decreased activity/lethargic, and hunched posture starting at Day 9. These clinical signs were considered secondary to PROJECT 16-1-related immune suppression and opportunistic bacterial infections.

PROJECT 16-1-related changes in hematology parameters and clinical chemistry data in these animals were similar to those identified for the surviving animals and included markedly decreased leukocyte counts (white blood cell count [WBC], neutrophils, lymphocytes, eosinophils, monocytes), decreased red cell parameters (hemoglobin [Hb], hematocrit [Hct], and red blood cell [RBC] count) and reticulocyte count, and changes indicative of an acute phase response (decreased albumin, increased globulin and/or fibrinogen). The Group 4 animals that were euthanized early presented with lymphocyte and monocyte values that were equivalent with the other Group 4 animals at all time points leading up to the collection of unscheduled samples prior to necropsy. At the time of unscheduled necropsy, variable changes were present for the monocyte, T-lymphocyte, and NK cell populations. The B-lymphocyte values for all Group 4 animals had recovered to prestudy baseline levels at the time of unscheduled necropsy.

Macroscopic and microscopic findings in all 8 animals indicate that the cause of morbidity and early death in all animals was related to a marked decrease in red blood cell mass, moderate to marked bone marrow hypocellularity (erythroid and myeloid), marked systemic lymphoid depletion characterized by a marked decrease in the number of lymphocytes in the thymus, spleen, mandibular lymph node, mesenteric lymphoid, and gut-associated lymphoid tissue (immunosuppression), and severe opportunistic bacterial infections. In the spleen, lymph nodes, and gut-associated lymphoid tissue, the diagnosis “decreased number and size, lymphoid follicle/germinal center” was used to further characterize the distribution of the lymphoid depletion. The microscopic findings identified in the lymphoid tissues (decreased number of lymphocytes) and bone marrow were attributed to PROJECT 16-1 administration. In the bone marrow, erythroid progenitors were decreased in number (hypocellularity) in all early death animals, while myeloid progenitors were often increased in number (hypercellularity) with a shift toward predominance of early progenitors. This shift was considered to be most likely in response to the increased tissue demand due to secondary infections (primarily bacterial). There were no other direct test article-related findings. All other microscopic finding in the early death animal was considered to be related to debilitation, inanition, bacterial infection, bacterial septicemia and/or circulatory failure/hypoxia (shock).

There were no test article-related changes in body weight, ophthalmology, electrocardiography, blood pressure, or physical examination parameters in any animals. While there were no PROJECT 16-1-related changes in food consumption in any Group 2 (0.5 mg/kg/dose), 3 (1.0/0.75 mg/kg/dose) or 7 (0.25 mg/kg/dose) animals, 2 Group 4 (3 mg/kg/dose) animals had low food consumption on Days 13 and 14, which was likely related to the overall poor health condition caused by marked changes in hematology parameters.

PROJECT 16-1-related clinical signs in surviving Group 3 (1/0.75 mg/kg/dose) and Group 4 (3 mg/kg/dose) animals included skin lesions, moderate swelling on face, abrasions, and/or limited use of limb (which was associated with skin lesions) in individual animals (one Group 3 and 2 Group 4 animals) starting Day 9 (similar to unscheduled animals described above). In general, these clinical signs correlated with changes in clinical pathology parameters and were considered secondary to PROJECT 16-1-related immune suppression and opportunistic bacterial infections.

There were no PROJECT 16-1-related clinical signs in Group 2 (0.5 mg/kg/dose) or Group 7 (0.25 mg/kg/dose) animals and no PROJECT 16-2 or MMAE-related clinical signs were present in Group 5 and 6 animals.

There were moderate to marked, adverse PROJECT 16-1-related changes in hematology parameters at 0.5, 1/0.75, and 3 mg/kg/dose that included decreases in indicators of erythrocyte mass (Hb, Hct, and RBC), reticulocytes, and WBC (neutrophils, lymphocytes, monocytes, eosinophils, basophils, and large unstained cells [LUC]) between Days 2-11. In general, these changes were reversed in a majority of animals by Days 16-18; however, toxicokinetic data indicated significant decrease in PROJECT 16-1 exposure post-Day 15, which was likely due to ADA formation.

At 0.25 mg/kg/dose there were minimal, non-adverse PROJECT 16-1-related decreases in erythrocyte mass and in some leukocytes.

MMAE-related changes in hematology parameters included minimal, non-adverse decreases in RBC mass and moderate decreases in neutrophils.

There were no PROJECT 16-2-related changes in hematology parameters in Group 5 animals.

There were no PROJECT 16-1, PROJECT 16-2 or MMAE-related changes in coagulation and urinalysis parameters. In addition, there were no PROJECT 16-1-related changes in clinical chemistry parameters at 0.25 or 0.5 mg/kg/dose, and no PROJECT 16-2-related changes in Group 5 animals.

PROJECT 16-1-related changes in clinical chemistry parameters at 1/0.75 mg/kg/dose and 3 mg/kg animals included minimal decreases in albumin and increases in globulin resulting in decreased albumin:globulin (A:G) ratio. Similar changes were present in MMAE-treated Group 6 males. PROJECT 16-1-related, dose-independent, transient, and recoverable decreases were present for all lymphocyte subsets in Groups 2-4 and Group 7 at the 48 hr post-EOI time point. Similar dose-dependent decreases were also present for monocytes at the 48 hr post-EOI timepoint for Groups 2-4 and Group 7. The Group 5 animals that were dosed with PROJECT 16-2 also presented with reductions in the number of lymphocyte subsets and monocyte populations, which generally trended at the same post dose timepoints; however, these reductions were generally not of the same magnitude as those present for the animals dosed with PROJECT 16-1, and the levels of these reductions were often inconsistent between male and female animals. Although the levels of reduction present for the PROJECT 16-2 dosed group were not of the same magnitude as those present for the PROJECT 16-1 dose groups, the lymphocyte subset and monocyte populations for each dose group generally returned to prestudy values by the next predose time point (ie, Day 15, Day 29, or Day 43), and remained variable across all subsequent timepoints. The differences in the magnitude of depletion between the cell subsets were likely due to varying levels of PROJECT 16-1 target expression on each cell population. The prestudy receptor occupancy assessment demonstrated that the B-lymphocyte and Monocyte populations had the greatest expression of the PROJECT 16-1 target molecule (>90%), followed by the NK cells (approximately 43%-69%), and that T lymphocytes had virtually no expression of the PROJECT 16-1 target molecule (<1%). These data correlated with the levels of PROJECT 16-1 binding present at the Day 1, 1 hr post-EOI, at which time the B-lymphocytes and Monocytes presented with the highest level of PROJECT 16-1 binding (mean values of approximately 71%-87% of B-lymphocytes and 63% to 94% of Monocytes), followed by NK cells (mean values of approximately 26%-38% of NK cells), and finally the T lymphocytes, which presented with no binding of PROJECT 16-1 at any post dose time point. Collectively, the receptor occupancy and test article binding data demonstrated that the greatest levels of prestudy target expression were directly correlated to the levels of depletion for each cell population.

ADC and TAb PK parameters for all groups were reported and calculated for the first dose only due to limited exposure beyond Day 7, which was attributed to the incidence of immunogenicity in animals. Following IV injections of PROJECT 16-1 (small molecule toxin conjugated antibody) and PROJECT 16-2 (unconjugated antibody), the peak serum concentrations were attained within 5 minutes upon IV infusion. Area under the serum concentration-time curves (AUC) and Cmax for PROJECT 16-1 appeared to increase approximately proportionally to dose between 0.25 and 3 mg/kg.

The AUC determined using the TAb concentrations was generally larger in comparison to ADC areas due to unconjugation of the ADC over time. Serum concentration of unconjugated MMAE increased gradually after dosing and reached a maximum by 12-24 hours post dose. Serum MMAE concentrations were approximately >10,000-fold lower than ADC and TAb (PROJECT 16-1, parent drug) concentrations, respectively. Exposure of the “naked” antibody (PROJECT 16-2) (Cmax and AUCτ(0-7))was similar to the ADC and TAb at 3 mg/kg dose of PROJECT 16-1. IV Injection of the small molecule drug, MMAE, at a molar equivalent dose to the payload of a 3 mg/kg PROJECT 16-1 infusion dose showed that the mean serum MMAE half-life was 0.708 ± 0.0847 and 0.861 ± 0.179 days after the first and last dose, respectively. The T1/2 λz determined for the MMAE drug payload following IV infusion of 3 mg/kg PROJECT 16-1 (T1/2 λz = 2.48 ± 0.375 days) was partially attributed to its formation and was associated with systemic levels of the drug conjugated Ab (PROJECT 16-1). Incidences of seroconversion in male and female cynomolgus monkeys were 0%, 70%, 50%, 30%, 60%and 60% for Dose Groups 1, 2, 3, 4, 5, and 7, respectively.

No PROJECT 16-1, PROJECT 16-2, or MMAE-related organ weight changes were noted in the Terminal Euthanasia dose groups at Day 50 or Recovery Euthanasia dose groups at Day 50/51 or Day 93. At Terminal Euthanasia Day 50, PROJECT 16-1-related microscopic findings were present in the spleen, lymph nodes (mandibular and mesenteric), and gut associated lymphoid tissue [GALT]) and bone marrow at ≥ 1/0.75 mg/kg/dose and kidney at 0.5 mg/kg/dose. PROJECT 16-1-related microscopic findings in the spleen, lymph nodes, and/or GALT were similar to those identified in the early death animals; lymphoid depletion characterized by a mild or marked decrease in the number and size of lymphoid follicle germinal centers. Bone marrow findings were characterized by mild myeloid hypocellularity (with a shift towards greater numbers of early myeloid progenitors as compared to late) and minimal erythroid hypercellularity (decreased in the myeloid to erythroid ratio). The bone marrow findings were considered to represent a regenerative/recovery response. In the kidney, moderate glomerulonephritis (increased glomerular mesangium) was identified in one 0.5 mg/kg/dose group male. Based on the nature of the microscopic findings, the glomerular changes were considered to be most likely associated with an immune complex deposition. MMAE-related microscopic findings were identified in the bone marrow only (0.05 mg/kg/dose). The bone marrow changes were similar in character, yet increased in incidence and severity as compared to those described in the 1/0.75 mg/kg/dose PROJECT 16-1 dose group. There were no PROJECT 16-2-related microscopic findings.

At Recovery Euthanasia Day 50/51 (3 mg/kg/dose PROJECT 16-1 dose group), PROJECT 16-1 -related microscopic findings were present in the spleen, lymph nodes (mandibular and/or mesenteric), and/or GALT in 2 of the 3 animals. Similar to the Terminal Euthanasia, lymphoid depletion characterized by a decrease in number/size of germinal centers was noted. In 1 animal, a mild increase in protein deposition and lymphoid necrosis (active inflammation and necrosis) was evident in germinal centers. There was no evidence of PROJECT 16-1- related microscopic findings in the other target tissues (thymus, bone marrow, or kidney) after the 49 or 50 day dose-free interval.

At Recovery Euthanasia Day 93, no PROJECT 16-1, PROJECT 16-2, or MMAE-related microscopic findings were noted in the target tissues evaluated (spleen, thymus, lymph nodes [mandibular and mesenteric], GALT, bone marrow [sternum], and kidney).

In conclusion, eight PROJECT 16-1 dosed animals (1 animal at 1 mg/kg/dose and 7 animals at 3 mg/kg/dose) were euthanized or died prior to their scheduled euthanasia. Of these, 2 animals (1 each at 1 and 3 mg/kg/dose) died on study and 6 animals (3 mg/kg/dose) underwent unscheduled euthanasia due to deteriorating health. Toxicokinetic parameters for all groups were reported and calculated for the first dose only due to limited exposure beyond Day 7, which was attributed to the incidence of immunogenicity in animals. Area under the serum concentration-time curves (AUC) and Cmax for PROJECT 16-1 appeared to increase approximately proportionally to dose between 0.25 and 3 mg/kg. Macroscopic and microscopic findings in animals that died or were euthanized early indicated that the cause of morbidity and early death in all animals was related to a PROJECT 16-1-related immunosuppression characterized by marked systemic lymphoid depletion, bone marrow suppression characterized by moderate to marked bone marrow hypocellularity, an associated decrease in red blood cell mass, and secondary infection. PROJECT 16-1 at 0.25 and 0.5 mg/kg/dose, the lowered PROJECT 16-1 at 0.75 mg/kg/dose from Day 15, PROJECT 16-2 at 3 mg/kg/dose, and MMAE at 0.05 mg/kg/dose were generally well tolerated. However, PROJECT 16-1 and PROJECT 16-2 exposures were limited due to anti-drug antibody formation.