**A 4-Week (5-Dose) Study of PROJECT 11 by Intravenous Infusion in Cynomolgus Monkeys with a 4-Week Recovery Period**

**11 SUMMARY**

The objectives of this study were to determine the potential toxicity of PROJECT 11, a human anti‑GITR IgG4 S228P tetravalent antibody, when given by intravenous infusion once weekly for 4 doses (Days 1, 8, 15, and 22) to cynomolgus monkeys, and to evaluate the potential reversibility of any findings after a 4-week recovery period. In addition, the toxicokinetic characteristics of PROJECT 11 were monitored.

The study design was as follows:

Text Table 1  
Experimental Design

| **Group No.** | **Test Material** | **Dose Level (mg/kg/week)a** | **Dose Volume (mL/kg)b** | **Dose Concentration (mg/mL)** | **No. of Animalsc** | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Main Study** | | **Recovery Study** | |
| **Males** | **Females** | **Males** | **Females** |
| 1 | Control Articled | 0 | 7.8 | 0 | 3 | 3 | 2 | 2 |
| 2 | PROJECT 11 | 50 | 7.8 | 6.4 | 3 | 3 | - | - |
| 3 | PROJECT 11 | 100 | 7.8 | 12.8 | 3 | 3 | - | - |
| 4 | PROJECT 11 | 200 | 7.8 | 25.7 | 3 | 3 | 2 | 2 |
| - = not applicable.  a Dosing (1 hour infusion) was stagger-started with a 1 day interval between dosing Set A and Set B. Males (Set A) were dosed initially, followed by the females (Set B). The main study and recovery animals underwent necropsy on Day 30 and 57, respectively. Due to an error in dose volume administered (low dose males on Day 1) and errors in formulation concentration on Day 1 and Day 15 for males only, actual dose levels administered for males in Groups 2, 3, and 4 (respectively) were 102, 165, and 147 mg/kg (Day 1) and 105, 178, and 145 mg/kg (Day 15). Due to unexpected infusion reactions in a total of 4 males (from Groups 2, 3 and 4) on Day 22, infusions were stopped early for all males in Groups 1-4 with only partial dose levels administered. On Day 22, females were pretreated with diphenhydramine (5 mg/kg, IM) 15 minutes prior to infusions and received their full dose of PROJECT 11, except for one Group 3 female who had an infusion reaction and received only 20% of the target dose. Due to infusion reactions observed on Day 22, the 5th scheduled dose on Day 29 was not administered to males and females.  b Dose volume was administered via a 60-min infusion, except Day 22 infusions which were stopped early for all males and one female. Dose volume administered on Day 1 to low dose males was 8.6 mL/kg.  c Scheduled necropsy was on Day 30 for the main groups and on Day 57 for the recovery groups. Unscheduled necropsy occurred on Day 22 for 2 males (one in Group 2 and one in Group 4) due to severity of their infusion reactions.  d Control article (vehicle) was 20 mM histidine, 250 mM sucrose, 0.025% Polysorbate 80, pH 5.5. | | | | | | | | |

The following parameters and end points were evaluated in this study: clinical signs, body weights, qualitative food evaluation, ophthalmology, neurological examination, blood pressure, heart rate, respiratory rate, electrocardiology, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), toxicokinetic parameters, anti-drug antibody (ADA) analysis, immunophenotype, cytokine sample analysis, total hemolytic complement (CH50) analysis, gross necropsy findings, organ weights, and histopathologic examinations.

Dose formulations for all Set B (female) dosing occasions met acceptance criteria. Set A (males) were mis-dosed on Day 1 and on Day 15 per the table below.

Text Table 2

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Low Dose (Group 2) | | Mid Dose (Group 3) | | High Dose (Group 4) | |
| Dosing Occasion | Intended (mg/kg) | Actuala (mg/kg) | Intended (mg/kg) | Actual (mg/kg) | Intended (mg/kg) | Actual (mg/kg) |
| Day 1 | 50 | 102b | 100 | 165 | 200 | 147 |
| Day 15 | 50 | 105 | 100 | 178 | 200 | 145 |

a Based on Lonza Statement of Testing (ver 1.0) 29 Jun 2018; b Includes allowance for infusion inadvertently including the dead space volume.

Day 8 and Day 22 dose formulations for Set A met acceptance criteria.

After the first dose, PROJECT 11 demonstrated a serum half-life of 1 to 2 days. The increase in systemic exposure on Day 1 was approximately dose proportional and systemic exposure to PROJECT 11 was similar between males and females. On Day 22, males received dose levels of PROJECT 11 that were 50% or more below the intended dose level; therefore, comparisons of Day 22 versus Day 1 serum levels were not possible and further complicated by the presence of ADA. All females on Day 22 received their full dose (except #3603), which allowed comparisons of Day 22 versus Day 1 serum PROJECT 11 levels. In female animals with either no or a low ADA response, PROJECT 11 AUC0-tau and Cmax levels on Day 22 were either at or near Day 1 values**.** Overall, the notable decrease in exposure observed among ADA positive animals after repeated dosing correlated with the highest ADA response.

Infusion reactions occurred in 5 animals (4 males, 1 female) during the fourth dose on Day 22 within approximately 15 minutes post start of infusion but were not observed on dosing Days 1, 8, or 15. The infusion reactions were not dose related (i.e., seen in at least one animal in each PROJECT 11 dose group) and were characterized during the infusion by loss of consciousness, pale skin, shallow or labored breathing, decreased heart rate, and/or decreased activity. The infusion reactions led to mortality in a low-dose male (Animal #2001) within 5 minutes of dose termination (delivered dose was 10 mg/kg) and to euthanasia of a moribund high-dose male (Animal #4004) within 40 minutes of dose termination (delivered dose was 23 mg/kg); no significant gross or microscopic changes were observed in either animal. Since severe infusion reactions occurred during intravenous infusion, dosing was halted for all males (Set A) on Day 22. The remaining males displayed no signs of infusion reactions after receiving only 12-50% of their intended dose. Females (Set B) dosed the following day (Day 22) were pretreated with diphenhydramine, and all received their intended dose of PROJECT 11, with the exception of one mid-dose female, who exhibited a loss of consciousness 12 minutes post start of infusion but recovered quickly after the infusion was discontinued (delivered dose was 20 mg/kg). In addition to adverse infusion reactions that occurred during dosing on Day 22, non-adverse clinical signs related to the infusion on Day 22 consisted of hypersalivation and/or excessive bleeding from the catheter site (mid-dose male and high-dose male), as well as slight tremors in the forelimbs (low-dose female). Except for the 2 animals that died/euthanized on Day 22, all other animals displaying infusion reactions and/or clinical signs fully recovered within an hour of after dosing.

In the 5 surviving animals with transient infusion reactions and/or clinical signs, other than the changes occurring immediately after dosing on Day 22, there were no other PROJECT 11-related effects (direct or secondary to an immune-mediated response) on body weights, qualitative food evaluation, ophthalmology, electrocardiology, blood pressure and heart rate, respiration, neurological examination, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), immunophenotype, urinalysis, and organ weights. No significant gross or histopathological changes were observed in the animals with infusion reactions/clinical signs that survived to their scheduled euthanasia on Day 30 (main group) or Day 57 (recovery group).

In the remainder of animals that reached scheduled necropsy, no PROJECT 11-related findings were observed for clinical signs, body weights, qualitative food evaluation, ophthalmology, electrocardiology, neurological examination, heart rate, respiratory rate, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), cytokines, immunophenotype, gross necropsy findings, organ weights, and histopathologic examinations.

Mean arterial pressure (MAP), systolic pressure and diastolic pressure were statistically significantly reduced postdose on Day 1, for males (but not females) administered at the high dose level, when compared to control animals. However, baseline MAP and diastolic blood pressure were reduced for high-dose males, relative to controls, with all blood pressure parameters increased for controls on Day 1 relative to their baseline values. Given this variability in blood pressure and absence of other changes (e.g., heart rate increase), the decrease in blood pressure is not considered toxicologically meaningful and of uncertain relationship to PROJECT 11.

Based on the high levels of ADA detected by Day 22 in most animals administered PROJECT 11, the weight-of-evidence suggests that the observed infusions reactions/clinical signs were ADA-mediated. An ADA-mediated mechanism is also supported by transient activation of complement (i.e., depletion of complement activity) and coagulation/fibrinolytic pathways (i.e., decreased platelets and fibrinogen, increased coagulation parameter times, and/or excessive bleeding from the catheter site) and/or stimulation of pro-inflammatory cytokines (i.e., increased IL-6, IL-10, and/or TNF) that were seen in animals with infusion reactions and/or clinical signs from serum/blood collected approximately one hour after dosing on Day 22.

Intravenous infusion of PROJECT 11 once weekly for 4 weeks (total of 4 doses) to cynomolgus monkeys at 50, 100, or 200 mg/kg/week resulted in adverse infusion reactions in 5 animals on Day 22 that were unrelated to dose, with mortality/moribundity occurring immediately after dosing in one male at 50 mg/kg/week and one male at 200 mg/kg/week. Infusion reactions and/or clinical signs after the fourth weekly dose were typical of an ADA-mediated response, occurred in select animals from all PROJECT 11 dose groups, and were associated with high levels of ADA, transient activation of complement and coagulation/fibrinolytic pathways, and transient elevations in pro-inflammatory cytokines.

No changes attributed directly to PROJECT 11 were observed.  The no-observed-adverse-effect level (NOAEL) for non-ADA mediated toxicity is the highest dose tested (200 mg/kg/week).

Despite the variability in PROJECT 11 serum levels on Day 22 the majority of females maintained PROJECT 11 serum levels similar to Day 1 over the 4‑week dosing period. Exposure on Day 1 for females at 200 mg/kg/week was, AUC0-168 = 9630 + 3680,

AUC0-inf= 10000 + 3750 µg·day/mL and Cmax = 5380 + 2480 µg/mL.  Males on Day 1 were mis-dosed due to the formulation error, and instead of 200 mg/kg, a dose of 147 mg/kg was administered which generated AUC0-168 = 8460 + 2730, AUC0-inf = 8900 + 3030 µg·day/mL and Cmax = 5650 + 1240 µg/mL.  This mis-dose in males on Day 1 and a similar mis-dose again in males on Day 15 did not affect the quality or integrity of the study results nor the study conclusion.