### Safety Pharmacology

Safety pharmacology assessments of the major organ systems were performed as part of the 4-week intravenous toxicity study in cynomolgus monkeys (Study Project 14-TX-0001)

[[Section 4.3.2.1](#_bookmark26)]. At doses up to 100 mg/kg, no discernible effects on the CNS (clinical observations), cardiovascular (electrocardiography and blood pressure) or respiratory (respiration rate) systems were noted.

## Toxicology

The safety of PROJECT 14 has been evaluated in a total of 6 toxicology studies, 3 in vivo studies and 3 in vitro studies. A tabulated overview of these studies is provided in [End-of-Text Table 3.1]. A tabulated overview of the toxicokinetic studies and toxicokinetic data from these studies is provided in [End-of-Text Tables 3.2 and 3.3], respectively. The toxicology studies, with the exception of the exploratory cytokine release assessment, were conducted under appropriate national and international guidelines/guidances and in accordance with Good Laboratory Practice standards.

The organs of toxicity identified by these studies included the renal/urinary system and immunological (immune) response. Details of these findings are outlined below.

### Single-dose Toxicity

* + - 1. **Single Subcutaneous Dose Toxicity Study in Cynomolgus Monkeys**

A tabulated summary of the results of the single-dose toxicity study (Study Project 14-TX-0002) is presented in [End-of-Text Table 3.4.1]. The potential of PROJECT 14 (0, 10 or 50 mg/kg) to induce local irritation at the injection site as well as its systemic toxicity was investigated following a single subcutaneous injection in cynomolgus monkeys (2 males and 2 females per dose level). The animals were observed for 2 weeks following the injection.

No animal died at either dose level. No test article-related changes were observed in clinical signs, macroscopic examination (injection site), body weight, food consumption, gross pathology or histopathology. Toxicokinetic assessments showed that the mean Cmax values, mean AUC168 values and mean AUC336 values increased in a dose-proportional manner. There were no apparent gender differences in the toxicokinetic parameters.

In conclusion, no test article-associated systemic toxicity findings and no discernible subcutaneous irritation at the injection site was noted in cynomolgus monkeys at doses up to 50 mg/kg.

### Repeat-dose Toxicity

* + - 1. **4-Week Repeated Intravenous Dose Toxicity Study in Cynomolgus Monkeys**

A tabulated summary of the results of the repeat-dose toxicity study (Study Project 14-TX-0001) is presented in [End-of-Text Table 3.5.1]. The safety of PROJECT 14 was evaluated following weekly intravenous administration (0, 3, 10 and 100 mg/kg) to cynomolgus monkeys (4 males and 4 females per group) for 4 weeks. The animals were sacrificed 7 days after the last dosing (4th dosing). In addition, 3 males and 3 females were added to the 100 mg/kg group to assess the reversibility of toxicity findings following a 4-week recovery period.

No test article-related death occurred in any group; however, 1 female from the 100 mg/kg dose group was found dead on day 20 of dosing (5 days after the 3rd dosing). The cause of death was determined to be choking due to aspiration of a foreign body (vomit) and was therefore considered not drug related.

At the 100 mg/kg dose level, occult blood was noted in the urine of 3 males. In 1 male, low albumin concentration, low percent albumin and low albumin/globulin ratio as well as high percentages of globulin and γ-globulin were noted. In this same animal which was necropsied as planned, perivascular inflammatory cell infiltration was observed in various organs (heart, submandibular glands, esophagus, stomach, duodenum, ileum, cecum, rectum, urinary bladder, sciatic nerve, femoral skeletal muscle, lungs and pancreas) and inflammatory cell infiltration was reported in the alveoli and bronchioles. At the end of the 4-week recovery period, no test article-related changes were noted for any measured parameter, and the occult blood in the urine had disappeared.

No test article-related changes were noted at the 3 or 10 mg/kg dose levels.

Results from the toxicokinetic analy[sis are discussed in [Section 4.2.1.2](#_bookmark16)]. Antidrug antibody (ADA) assessments performed at the end of the treatment period showed the presence of anti-PROJECT 14 antibodies in 8 of 8 animals at the 3 mg/kg dose level, 2 of 8 animals at the

10 mg/kg dose level and 1 of 14 animals at the 100 mg/kg dose level.

In conclusion, under the conditions of this study, the NOAEL was 10 mg/kg in males due to the occult blood in the urine and inflammatory cell infiltration; while in females, the NOAEL was determined to be 100 mg/kg.

### 13-Week Repeated Intravenous Dose Toxicity Study in Cynomolgus Monkeys

A tabulated summary of the results of the repeat-dose toxicity study (Study Project 14-TX-0004) is presented in [End-of-Text Table 3.5.2]. The toxicity of PROJECT 14 was evaluated following weekly intravenous administration (0, 25, 50 and 100 mg/kg) to cynomolgus monkeys

(4 males and 4 females per group) for 13 weeks. The animals were sacrificed 7 days after the last dosing (13th dosing). In addition, 3 males and 3 females were added to the 100 mg/kg dose group to assess the reversibility of toxicity findings following a 13-week recovery period.

No mortality was observed in any dose group.

At the 100 mg/kg dose level, high fibrinogen levels in 1 male and 1 female, high total bilirubin levels in 1 male and high thyroid weight in 1 male were noted. At the end of the recovery period, no test article-related changes were noted for any measured parameter, and all changes noted during the dosing period recovered.

No test article-related changes were noted at the 25 or 50 mg/kg dose levels.

Results from the toxicokinetic analysis are discussed in [Sec[tion 4.2.1.2](#_bookmark16)]. ADA assessments performed at the end of the treatment period showed the presence of anti-PROJECT 14 antibody in 2 of 8 animals at the 25 mg/kg dose level.

In conclusion, under the conditions of this study, the NOAEL was 50 mg/kg in males and females due to the increased fibrinogen and total bilirubin levels as well as elevated thyroid weight noted at the dose of 100 mg/kg.

### Genotoxicity

The genotoxicity studies routinely conducted for pharmaceuticals are not applicable to biotechnology-derived pharmaceuticals. There is no indication that PROJECT 14 would require a special approach to evaluate genotoxicity.

### Carcinogenicity

No carcinogenicity studies with PROJECT 14 have been conducted as of the preparation of this IB.

### Reproductive and Developmental Toxicity

No reproductive and developmental toxicity studies with PROJECT 14 have been conducted as of the preparation of this IB.

### Local Tolerance

Local irritation was evaluated as part of the single subcutaneous dose toxicity study

(Study Project 14-TX-[0002) [Section 4.3.1.1](#_bookmark24)] and the 4-week repeated intravenous dose toxicity study (Study Project 14-TX-0001) [Section [4.3.2.1](#_bookmark26)]. In both studies, no notable macroscopic or microscopic changes were observed at the injection sites.

### Other Toxicity Studies

* + - 1. **In Vitro Tissue Cross-reactivity Study in Human Tissues**

A tabulated summary of the results of the tissue cross-reactivity study (Study Project 14-TX-0003) is presented in [End-of-Text Table 3.6.1]. PROJECT 14 was applied to cryosections of 36 different normal human tissues (3 donors per tissue, where available) at 2 concentrations (0.5 and 5 µg/mL). Control antibody, with a different antigenic specificity from PROJECT 14 (designated HuIgG1), was applied to the same panel of tissues. In order to confirm antibody binding under the conditions of the study, recombinant human TSLPR Fc Chimera UV-resin spot slides (designated rhTSLPR) served as the positive control material; and human hypercalcemia of malignancy peptide (amino acid residues 1 - 34) UV-resin spot slides (designated PTHrP 1 - 34) served as the negative control material.

PROJECT 14 produced moderate to intense staining of the positive control material at both staining concentrations. PROJECT 14 did not specifically react with the negative control material at either staining concentration. The control article, HuIgG1, did not specifically react with either the positive or negative control materials.

PROJECT 14 stained mast cells in the urinary bladder, breast, esophagus, small intestine, heart, lymph node, parathyroid, prostate, skin, skeletal muscle, thymus, ureter and cervix. As TSLPR is reportedly expressed by mast cells, the mast cell staining observed with PROJECT 14 in this study was anticipated.

In addition, PROJECT 14 stained extracellular granules in the eye (sclera), vascular wall of the pancreas, perivascular region of the placenta and vascular wall of the endometrium as well as extracellular proteinaceous material in the kidney tubules. The specificity of these stainings was not determined, although mAb binding to extracellular sites is generally considered of little to no toxicologic significance.

These data showed that PROJECT 14 had little or no discernible tissue cross-reactivity and that the tissue binding that did occur was related to known expression of TSLPR.

### In Vitro Cytokine Release Assessment in Human Peripheral Blood

A tabulated summary of the results of the cytokine-release assessment study

(Study Project 14-TX-5001) is presented in [End-of-Text Table 3.6.2]. Whole blood samples from 10 healthy volunteers were incubated with PROJECT 14 to a final concentration of 0, 10, 30 or 100 µg/mL for 24 h at 37°C and under an atmosphere of 5% CO2. Alemtuzumab or a monoclonal anti-human CD28 antibody (ANC28.1/5D10) at a concentration of 1 µg/mL served as the positive control while bevacizumab at a concentration of 10 µg/mL or PBS served as the negative control. After incubation, the blood was centrifuged and the cytokine (IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, interferon- γ and tumor necrosis factor-α) concentrations in the plasma were recorded. An increased cytokine concentration 2 times greater than background (PBS control) and greater than that observed in blood treated with bevacizumab in 3 volunteers or more at each concentration was considered to be positive.

PROJECT 14 at concentrations up to 100 µg/mL did not result in positive cytokine release from whole human blood. Positive and negative control mAbs yielded the expected results, confirming the assay validity.

### Immunofluorescence Examination for Immune Complex Localization in Selected Tissues From the 4-Week Repeated Intravenous Dose Toxicity Study of PROJECT 14 in Cynomolgus Monkeys

A tabulated summary of the results from the immunofluorescence examination

(Study Project 14-TX-0005) is presented in [End-of-Text Table 3.6.3]. To evaluate if immune complex deposition occurred in the kidneys and/or in sites of perivascular inflammation in animals from the previous 4-week repeated intravenous dose toxicity study in cynomolgus monkeys (Study Project 14-TX-0001), the kidneys from vehicle-treated controls and

PROJECT 14-treated (100 mg/kg dose group) animals were stained for human

immunoglobulin G (hIgG), cIgG, cynomolgus monkey IgM (cIgM) and cynomolgus monkey C3 by immunofluorescence. Selected organs (heart, lungs, pancreas, submandibular glands, sciatic nerve, esophagus, duodenum, cecum, stomach, ileum, rectum, urinary bladder and skeletal muscle) from 1 vehicle control animal and 1 PROJECT 14-treated animal with perivascular inflammation were also immunofluorescently stained and evaluated for immune complex deposition.

None of the tissues from the vehicle-treated animals stained positive for hIgG in either the vascular or extravascular space, while all of the vehicle-treated animals and all of the PROJECT 14-treated monkeys stained positive for cIgG and cIgM in the vascular space. None of the vehicle-treated and none of the PROJECT 14-treated animals stained positive for cIgG or cIgM in the extravascular space. Finally, none of the vehicle-treated and none of the PROJECT 14-treated animals stained positive for C3 in either the vascular or the extravascular space.

These data showed that no discernible immune complex deposition occurred in the kidney vasculature or perivascular space nor was there immune complex deposition in the glomeruli of PROJECT 14-treated monkeys. In addition, there was no discernible evidence of immune complex deposition in either the vascular or perivascular space of the other evaluated tissues harvested from vehicle controls or PROJECT 14-treated animals including the monkey with the histopathological finding of perivascular inflammation.

## Integrated Nonclinical Overview and Conclusion: Potential Clinical Relevance

### Rationale for Animal Selection

The nonclinical safety profile of PROJECT 14 has been evaluated according to ICH’s Harmonised Tripartite Guideline, Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals (S6[R1]) (Jun 2011); and all findings were evaluated for relevance to human risk. PROJECT 14 was demonstrated to bind to the TSLPR of human and cynomolgus monkey, but did not bind to the TSLPR of rodents; accordingly, the cynomolgus monkey was chosen as the toxicology model for this drug and no toxicity studies were conducted in rodents.

### Exposure Ratios

Exposure ratios including safety margins were calculated based on a simulated human exposure at a predicted efficacious dose [[Table 1](#_bookmark36)]. The human systemic exposure was simulated using a linear human pharmacokinetics model assuming monthly dosing and an efficacious plasma concentration of 1 µg/mL.

### Table 1 Exposure Ratios Based on Animal Cmax/AUC and Predicted Human Cmax/AUC of PROJECT 14

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study Type (Study No.)** | **Sex/No. of Animals** | **Dose† (mg/kg)** | **Cmax (µg/mL)** | | | | **AUC168 (µg.h/mL)** | | | |
| **First Dose** | **Last Dose** | **Exposure Ratios‡** | | **First Dose** | **Last Dose** | **Exposure Ratios‡** | |
| **First** | **Last** | **First** | **Last** |
| Monkey/ single, sc  (Project 14-TX-0002) | M/2 | 10 | 152 | NA | 5.9 | NA | 20300 | NA | 22 | NA |
| F/2 | 10 | 112 | NA | 4.3 | NA | 15800 | NA | 17 | NA |
| M/2 | 50 | 741 | NA | 29 | NA | 97500 | NA | 105 | NA |
| F/2 | 50 | 767 | NA | 30 | NA | 95800 | NA | 103 | NA |
| Monkey/ 4-week, iv  (Project 14-TX-0001) | M/4 | 3 | 89.0 | 66.7 | 3.4 | 2.6 | 7740 | 843 | 8.3 | 0.9 |
| F/4 | 3 | 76.3 | 35.6 | 3.0 | 1.4 | 7230 | 289 | 7.8 | 0.3 |
| M/4 | 10 | 392 | 457 | 15 | 18 | 26700 | 32200 | 29 | 35 |
| F/4 | 10 | 269 | 340 | 10 | 13 | 22400 | 21800 | 24 | 24 |
| M/7 | 100 | 3510 | 5710 | 136 | 221 | 239000 | 527000 | 258 | 568 |
| F/7§ | 100 | 2880 | 5550 | 112 | 215 | 249000 | 581000 | 269 | 627 |
| Monkey/ 13-week, iv  (Project 14-TX-0004) | M/4 | 25 | 686 | 1900 | 27 | 74 | 48800 | 198000 | 53 | 214 |
| F/4 | 25 | 670 | 1030 | 26 | 40 | 44900 | 88000 | 48 | 95 |
| M/4 | 50 | 1570 | 4010 | 61 | 155 | 109000 | 437000 | 118 | 471 |
| F/4 | 50 | 1450 | 3180 | 56 | 123 | 97300 | 326000 | 105 | 352 |
| M/7 | 100 | 4200 | 8300 | 163 | 322 | 370000 | 815000 | 399 | 879 |
| F/7 | 100 | 3460 | 5840 | 134 | 226 | 314000 | 640000 | 339 | 690 |

NA: not applicable; NOAEL: no-observed-adverse-effect level

† The underlined dose represents the NOAEL.

‡ The exposure ratios were calculated as (AUC168 x 4 [or Cmax]) / (estimated human systemic exposure level at the efficacious dose). The estimated human systemic exposure level of Cmax or AUCinf was 25.8 µg/mL or 3709 µg·h/mL, respectively, at the estimated efficacious dose of 70 mg, which would be needed to maintain the target trough concentration of 1 µg/mL for 28 days [Report Project 14-ME-9001].

§ 6 animals on day 22

### Mortality

There were no drug-related deaths in the nonclinical studies. In the 4-week repeat-dose toxicity study, 1 female monkey at the 100 mg/kg dose level was found dead 5 days after the third dose, and the cause of death was determined as foreign body (vomit) occlusion of the airway. This female showed no abnormalities in clinical signs, body weight or food consumption. On the morning of day 20 (5 days after the third dosing), vomitus was found. At necropsy, the lungs or trachea/bronchus had dark red focus or retention of white foamy fluid, high lung weight and a foreign body in the bronchus lumen. Histologically, the lungs showed inflammatory cell infiltration in the alveoli, congestion and edema. These pathological findings are frequently observed in the lungs following aspiration of foreign objects (vomit) [Araos et al, 2015; Marik, 2001; Raidoo et al, 1990]. No mortality was observed in the 13-week repeat-dose toxicity study. It was indicated at the study site that vomitus occasionally occurs in untreated monkeys. This point coupled with the fact that it occurred well after the peak serum concentration and that there were no indications that the animal was in distress as late as 1 day before it was found dead leads to the conclusion that the death was accidental and unrelated to the test article.

### Target Organs of Toxicity

The primary target organ toxicities identified in the toxicity studies were effects on renal/urinary system (occult blood in the urine) and an immunological (immune) response (perivascular inflammatory cell infiltration in multiple organs as well as inflammatory cell infiltration in the alveoli and bronchioles associated with changes in blood chemistry including high globulin and γ-globulin ratio). Although these findings were observed in the 4-week repeat-dose toxicity study, they were not confirmed in the 13-week repeat-dose toxicity study in cynomolgus monkeys.

### Effects on Renal/Urinary System

In the 4-week repeat-dose toxicity study, occult blood (grade 1 - 3) was observed in urine samples from 3 male cynomolgus monkeys at the 100 mg/kg dose level. This finding was reversible by the end of a 4-week recovery period. The mechanism has not been identified; however, these findings were not associated with blood chemistry changes indicative of renal injury or with histopathological changes in either the kidneys or urinary bladder. In addition, the occult blood in the urine occurred at systemic exposures that are estimated to be at least 258-fold higher than the human systemic exposure at the clinically efficacious dose. This finding was not reproducible in the 13-week repeat-dose toxicity study even though the same dose of PROJECT 14 was administered. Based on the lack of reproducibility of the finding at the same dose level with longer treatment times, coupled to the fact that this finding is readily monitorable in clinics, it was concluded that risk for hematuria in humans at dose levels of PROJECT 14 to be administered in the clinic was minimal.

### Immunological (Immune) Response

In the 4-week repeat-dose toxicity study, 1 male cynomolgus monkey at the highest dose tested (100 mg/kg, at least 258-fold the systemic exposure at the projected clinically efficacious human dose) showed very slight to moderate perivascular inflammatory cell infiltration in the heart, submandibular glands, esophagus, stomach, duodenum, ileum, cecum, rectum, urinary bladder, sciatic nerve, femoral skeletal muscle, lungs and pancreas and moderate inflammatory cell infiltration in the alveoli and bronchioles. These histological findings were associated with blood chemistry changes such as low plasma albumin concentration, low percent albumin and low albumin/globulin ratio in addition to high percentages of globulin and γ-globulin. These data suggest a generalized, low-grade inflammatory response may have occurred in this animal [Frazier et al, 2015]. The mechanism of these changes has not been identified; however, it was speculated that it may

be associated with the immunogenic response to this drug in monkeys. The risk of a severe immunogenic response clinically appears unlikely since the finding was not reproducible with increased duration of treatment (absent in the 13-week repeat-dose toxicity study) and it is expected that the human mAb may be less immunogenic in humans. Furthermore, incubating PROJECT 14 with human blood did not induce proinflammatory cytokine release suggesting a low risk for a cytokine storm-type reaction clinically. Finally, these histological findings and changes in blood chemistry were noted only at the highest dose tested

(100 mg/kg; at least 258-fold the systemic exposure at the projected clinically efficacious human dose), which provides a safety margin for these findings of at least 24-fold. Based on these findings, it is concluded that the risk of a generalized immunology (immune) response to PROJECT 14 is low.

### Conclusions

Based on the currently available safety data, it was concluded that there are no observations or findings that preclude further clinical development of this mAb. Anti-PROJECT 14 antibody formation was observed in the animal studies [[Table 2](#_bookmark40)] suggesting that monitoring for possible immunogenic side effects as well as anti-PROJECT 14 antibody formation in the clinical studies should be considered.

### Table 2 Potential Safety Concern of PROJECT 14

|  |  |  |
| --- | --- | --- |
| **Key Safety Targets** | **Key Observations** | **Relevance to Human Usage** |
| Mortality | **100 mg/kg:** Mortality was observed in the morning 5 days after the third dose in 1 female cynomolgus monkey; however, the cause of death was considered to be aspiration of vomit and not test article related, based on the following findings:   * No abnormalities in clinical signs, body weight or food consumption up to the day before * Vomitus found on the day of death * Dark red focus and retention of white foamy fluid in the trachea/bronchus with increased lung weight and foreign body in the bronchus lumen * Inflammatory cell infiltration in the alveoli, congestion and edema in the lungs | None |
| Renal/Urinary System | **100 mg/kg:** Occult blood in the urine in 3 male cynomolgus monkeys | Possible |
| Immunological (Immune) Response | **100 mg/kg:** Findings all reported in 1 male cynomolgus monkey   * High globulin level and γ-globulin ratio * Perivascular inflammatory cell infiltration in the heart, submandibular glands, esophagus, stomach, duodenum, ileum, cecum, rectum, urinary bladder, sciatic nerve, femoral skeletal muscle, lungs and pancreas * Inflammatory cell infiltration in the alveoli and bronchioles | Possible |

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