### 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 9 -TX-0001) [Section [4.3.2.1](#_bookmark39)]. At doses up to 30 mg/kg, no effects on the CNS (clinical observations and body temperature), cardiovascular (ECG and blood pressure) or respiratory (respiration rate) systems were noted. In a preliminary single dose toxicity study (Study Project 9 -TX-5001), no changes in cytokine release (interleukin [IL]-2, IL-4, IL-5, IL-6, tumor necrosis factor (TNF), and interferon [IFN]-γ) were observed at doses up to 100 mg/kg.

### Pharmacodynamic Drug Interactions

As of the date of the preparation of this IB, no nonclinical pharmacodynamic drug interaction studies of PROJECT 9 have been conducted.

## Toxicology

PROJECT 9 binds to monkey Igβ but not to rat or mouse Igβ, suggesting monkeys are the relevant species for PROJECT 9 pharmacology and toxicology studies. A preliminary single intravenous dose toxicity study (Study Project 9-TX-5001), an immunological assessment study by 4-week repeated intravenous dosing in cynomolgus monkeys (Study Project 9-TX-5002) and a cytokine release assay (Study Project 9-TX-5003) were conducted. The safety of PROJECT 9 was then evaluated in 1 pivotal 4-week intravenous repeat-dose toxicity study in cynomolgus monkeys (Study Project 9-TX-0001). PROJECT 9 was tested for tissue cross-reactivity in humans in Study Project 9-TX-0003 and in monkeys in Study Project 9-TX-0004. The pivotal 4-week intravenous repeat-dose toxicity study in cynomolgus monkeys (Study Project 9-TX-0001) and the human tissue cross-reactivity study (Study Project 9-TX-0003) were conducted under appropriate national and international guidelines/guidances and in accordance with Good Laboratory Practice (GLP) standards. In 4 of the toxicity studies conducted with PROJECT 9 (Studies Project 9-TX-0001, Project 9-TX-0003, Project 9-TX-0004 and Project 9-TX-5003), the

concentration of the citrate buffer used to prepare the dosing formulations (35 mmol/L) was higher than the citrate concentration anticipated to be used in the clinical studies

(20 mmol/L). Results from stability studies have confirmed there is acceptable stability for PROJECT 9 with either concentration of citrate buffer (20 and 35 mmol/L). The difference in citrate concentration was considered to be of a magnitude that was unlikely to impact the results and conclusions of these 4 studies. A tabulated overview of these studies is provided in [End-of-Text Table 3.1].

### Single-dose Toxicity

In the preliminary intravenous single dose toxicity study of PROJECT 9 (dose level: 0 mg/kg, 3 mg/kg, 30 mg/kg, and 100 mg/kg) in cynomolgus monkeys (Study Project 9-TX-5001),

decreased erythrocyte count and hematocrit value and focal necrosis in the spleen were noted at the 100 mg/kg dose level. No changes in cytokine release (IL-2, IL-4, IL-5, IL-6, TNF, and IFN-γ) were observed in this study. The NOAEL was estimated to be 30 mg/kg in this study.

### Repeat-dose Toxicity

### 4.3.2.1 4-week Repeated Intravenous Dose Toxicity Study in Cynomolgus Monkeys

A tabulated summary of the results of the GLP-compliant repeat-dose toxicity study (Study Project 9-TX-0001) is presented in [End-of-Text Table 3.2.1]. The safety of PROJECT 9 was evaluated following weekly intravenous administration (0 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg and 30 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 male and 3 female animals were added to the 3 mg/kg and 30 mg/kg group to assess the reversibility of toxicity following a 6-week recovery period.

No animal died or was euthanized due to moribundity.

At the 30 mg/kg dose level, increased reticulocyte ratio and eosinophil count, decreased platelet count, enlargement of the spleen and high spleen weights were observed.

Histopathology findings included congestion and hyperplasia of the red pulp in the spleen, hypertrophy of Kupffer cells in the liver, increase in hematopoiesis in the femoral bone marrow, atrophy in the thymus, hypertrophy of sinus histiocytes in the submandibular lymph node, edema and mixed inflammatory cell infiltration in the subserosa and lamina propria in the gallbladder, and vacuolation in the urothelium in the urinary bladder (all at the end of dosing period), fibrosis, hemorrhage and brown pigment in the capsule in the spleen, intimal thickening and perivascular fibrosis in the artery of the liver and in the subserosal artery of the gallbladder (at the end of recovery period). 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, fibrosis, hemorrhage and brown pigment in the capsule in the spleen, and intimal thickening and perivascular fibrosis in the artery of the liver and in the subserosal artery of the gallbladder were observed at the 30 mg/kg dose level.

No test article-related toxic changes were noted at 0.3 mg/kg, 1 mg/kg, or 3 mg/kg.

At the end of the last dosing period (day 29), ADA were detected in 6 of 8 animals at the

0.3 mg/kg dose level, in 5 of 8 animals at the 1 mg/kg dose level, and in 4 of 14 animals at the 3 mg/kg and 30 mg/kg dose levels. ADA were also observed during the recovery period. The Cmax and AUC168 values of PROJECT 9 increased dose proportionally on day 1, but the exposure levels after the last dosing were affected by the production of ADA. 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 ADA. No apparent sex differences were noted at any dose level.

In conclusion, under the conditions of this study, the NOAEL was 3 mg/kg for male and female animals due to the toxic changes in hematology, gross pathology, organ weights, and histopathology seen at the 30 mg/kg dose.

### Genotoxicity

Genotoxicity studies are not routinely conducted for biotechnology-derived pharmaceuticals. There are no pharmacological or toxicological effects that would warrant genotoxicity evaluations.

### Carcinogenicity

No carcinogenicity studies have been conducted.

### Reproductive and Developmental Toxicity

No reproductive and developmental toxicity studies have been conducted.

### Local Tolerance

No notable macroscopic or microscopic changes were observed at the injection sites in the 4- week repeat dose toxicity study in cynomolgus monkeys (Study Project 9-TX-0001).

### Other Toxicity Studies

* + - 1. **Exploratory Immunological Assessment by 4-Week Repeated Intravenous Dosing in Cynomolgus Monkeys**

In an exploratory study, the immunological effects of PROJECT 9 were evaluated following weekly intravenous administration (0 mg/kg, 3 mg/kg, and 30 mg/kg) to cynomolgus monkeys (3 males per group) for 4 weeks. The animals were sacrificed 7 days after the last dosing (4th dosing) (Study Project 9-TX-5002).

In this study, decreases in lymphocyte count and CD3-CD20+ cell ratio and count in peripheral blood, atrophic changes of the thymus, spleen, submandibular and mesenteric lymph nodes, Peyer’s patch, and/or inguinal lymph node were observed at 3 mg/kg and

30 mg/kg. In the immunohistochemical assessment, decreases in CD20+ cells in the spleen, submandibular and mesenteric lymph nodes, Peyer’s patch, thymus, and/or inguinal lymph node at 3 mg/kg and 30 mg/kg, and decreases in CD3+, CD4+, and CD8+ cells in the thymus at 3 mg/kg and 30 mg/kg and in the spleen at 30 mg/kg were observed. Among the above- stated findings, decreases in CD20+ cells in the blood and tissues were considered to be a pharmacological effect of PROJECT 9.

### In vitro Tissue Cross-reactivity Study in Human Tissues

PROJECT 9 was applied to cryosections of 36 different normal human tissues (at least 3 donors per tissue, where available) at 2 concentrations (2 µg/mL and 10 µg/mL)

(Study Project 9-TX-0003). A control antibody with a different antigenic specificity from PROJECT 9 (designated HuIgG1) was applied to the same panel of tissues. In order to confirm

antibody binding under the conditions of the study, recombinant human CD79b UV-resin spot slides (designated rhCD79b) 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 9 produced weak to strong staining of the positive control material at both staining concentrations. PROJECT 9 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 9 stained the membrane and cytoplasm in mononuclear cells in most human tissues examined (including lymphocytes in blood smears, Kupffer cells in liver, and Hofbauer cells in placenta), hematopoietic cells in the bone marrow, and basal epithelium in ducts of the salivary gland. As the expression of Igβ has been previously reported on the surface of

B cells as well as on monocytes, hematopoietic progenitor cells, and T cells, as has the expression of FcγRIIB on monocytes/macrophages and/or dendritic cells , the staining observed in mononuclear cells and hematopoietic cells with PROJECT 9 in this study was anticipated. However, the staining in ductal epithelium of the salivary gland, which is not a known site of Igβ or FcγRIIB expression, was an unanticipated cross-reactivity of the test article.

All other reactivity with PROJECT 9 in the human tissue panel was cytoplasmic in nature, and monoclonal antibody binding to cytoplasmic sites in tissue cross-reactivity studies generally is considered of little to no toxicological significance due to the inability of antibody drugs to access the cytoplasmic compartment in vivo.

In a study using cynomolgus monkey tissues [Project 9-TX-0004], the staining in the ductal epithelium of the salivary gland was seen only in the cytoplasm.

These data showed that PROJECT 9 had little discernible tissue cross reactivity and that the tissue binding that did occur was related to known expression of Igβ or FcγRIIB, except for the salivary gland staining.

### In vitro Tissue Cross-reactivity Study in Cynomolgus Monkey Tissues

PROJECT 9 was applied to cryosections of 36 different normal cynomolgus monkey tissues (at least 2 animals per tissue, where available) at 2 concentrations (0.5 µg/mL and 5 µg/mL) (Study Project 9-TX-0004). A control antibody with a different antigenic specificity from PROJECT 9 (designated HuIgG1) was applied to the same panel of tissues. In order to confirm antibody binding under the conditions of the study, recombinant human CD79b UV-resin spot slides (designated rhCD79b) 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 9 produced weak to strong staining of the positive control material at both staining concentrations. PROJECT 9 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 9 stained mononuclear cells in lymphoid tissues and non lymphoid tissues (including the adrenal, bladder, Fallopian tube, ovary, pancreas, peripheral nerve, placenta, prostate, salivary gland, striated skeletal muscle, testis, and uterus), lymphocytes in blood smears, hematopoietic cells (including megakaryocytes) in the bone marrow, and trophoblasts in the placenta and epithelium in the prostate.

As described in Sectio[n 4.3.7.2](#_bookmark46), the staining observed in mononuclear cells and hematopoietic cells with PROJECT 9 in this study corresponded to the reported sites of Igβ or FcγRIIB expression. In contrast, the trophoblasts in the placenta and epithelium in the prostate are not known sites of Igβ or FcγRIIb expression and represents unanticipated cross-reactivity.

All other reactivity with PROJECT 9 in the cynomolgus monkey tissue panel was cytoplasmic in nature, and monoclonal antibody binding to cytoplasmic sites in tissue cross-reactivity studies generally is considered of little to no toxicological significance due to the inability of antibody drugs to access the cytoplasmic compartment in vivo.

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

Whole blood samples from 10 healthy subjects were incubated with PROJECT 9 to a final concentration of 0 µg/mL, 1 µg/mL, 10 µg/mL, 100 µg/mL or 1000 µg/mL for 24 h at 37°C and under an atmosphere of 5% CO2 (Study Project 9-TX-5003). Alemtuzumab or a monoclonal antihuman 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 phosphate-buffered saline (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, IFN- γ and TNF-α) concentrations in the plasma were recorded. An increased cytokine concentration 2 times or greater than the background (cytokine level in PBS), plus cytokine levels greater than the negative control (bevacizumab), in 3 or more donors at each concentration was considered to be positive.

PROJECT 9 at concentrations up to 1000 µg/mL had no discernable potential for cytokine release from whole human blood. Positive and negative control monoclonal antibodies yielded the expected results, confirming the assay validity.

## 4.4 Integrated Nonclinical Overview and Conclusion: Potential Clinical Relevance

### 4.4.1 Pharmacology

PROJECT 9 bound with higher affinity to FcγRIIB and induced greater FcγRIIB ITIM phosphorylation in the presence of BCR stimulation compared to an anti-human Igβ antibody with the Fc domain of native IgG1. PROJECT 9 inhibited human primary B cell proliferation induced by BCR stimulation and also inhibited in vitro IgG2 antibody production using B cells isolated from SLE patients. These results indicate that PROJECT 9 has an effective inhibitory activity on B cell function in SLE patients via Fc engineering.

PROJECT 9 bound to human and monkey Igβ, but not to rat or mouse Igβ, suggesting monkeys are the relevant species for PROJECT 9 pharmacology and toxicology studies. PROJECT 9

showed concentration-dependent Igβ receptor occupancy in B cells from monkeys, healthy subjects and SLE patients. In cynomolgus monkey TTx immunization models, PROJECT 9 prevented anti-TTx antibody production in a dose-related manner from 0.01 to 1 mg/kg and 1 mg/kg of PROJECT 9 was the dose exerting the maximum inhibitory effect on antibody production in monkeys. At a dose of 1 mg/kg and above, a full Igβ receptor occupancy in B cells was observed. PROJECT 9 (10 mg/kg) significantly decreased anti-TTx IgG titer by

71.3% whereas rituximab (10 mg/kg) decreased it by 20.3%. Rituximab completely depleted peripheral B cells while PROJECT 9 showed about an 80% reduction from day 14 to day 28.

Additionally, PROJECT 9 significantly inhibited anti-TTx IgG production induced by a secondary immunization with TTx antigen. These results indicate in vivo efficacy of PROJECT 9 on B cell activation including naive and memory B cells in cynomolgus monkey and suggest that PROJECT 9 with a full Igβ receptor occupancy is expected to inhibit B cells in SLE patients.

The recovery of peripheral blood B cells after single intravenous administration of PROJECT 9 (1 to 10 mg/kg) was examined in cynomolgus monkeys. A decrease in peripheral blood B cell counts was noted from 1 h after dosing. Recovery from the decrease in B cell counts was initiated from day 7 and was completed on day 42 at the 1 mg/kg dose and on day 49 at

10 mg/kg. These results indicate that the effect of PROJECT 9 on B cell counts in peripheral blood is reversible. Serum PROJECT 9 concentration also decreased time-dependently, suggesting that B cell decrease and subsequent recovery are related to the concentration of PROJECT 9 in peripheral blood.

PROJECT 9 did not show in vitro cytotoxic activity on human primary B cells, whereas rituximab decreased B cells, indicating PROJECT 9 does not have the potential for B cell cytotoxic activity, in contrast to rituximab.

In conclusion, the data from non-clinical pharmacology studies indicate that PROJECT 9 may have therapeutic potential as a treatment of SLE, where B cells play a pathogenic role.

### Toxicology

* + - 1. **Rationale for Animal Selection**

The nonclinical safety profile of PROJECT 9 has been evaluated according to ICH S6(R1); and all findings were evaluated for relevance to human risk. PROJECT 9 binds to the Igβ from humans and cynomolgus monkeys, but did not bind to rodent Igβ. In addition, PROJECT 9 showed pharmacological activity in the cynomolgus monkey as evidenced by a drug- associated decrease in circulating CD20+ cells. Based on these findings the cynomolgus monkey was chosen as the toxicology test species and no toxicity studies were conducted in rodents.

### Exposure Ratios

Exposure ratios were calculated based on a simulated human exposure at a predicted efficacious dose [[Table 1](#_bookmark54)].

### Table 1 Exposure Ratios Based on Animal Cmax/AUC and Predicted Human Cmax/AUC of PROJECT 9

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study Type (Study No.)** | **Dose† (mg/kg)** | **Sex/ No. of Animals** | **Cmax (µg/mL)** | | | | **AUC168 (µg·h/mL)** | | | |
| **First Dose** | **Last Dose** | **Exposure Ratios‡** | | **First Dose** | **Last Dose** | **Exposure Ratios‡** | |
| **First** | **Last** | **First** | **Last** |
| Monkey/ 4-week, iv  (Project 9-TX-0001) | 0.3 | M/4 | 7.65 | 2.99 | 0.7 | 0.3 | 626 | 135 | 1.3 | 0.3 |
| F/4 | 7.99 | 10.1 | 0.8 | 1.0 | 704 | 1010 | 1.5 | 2.2 |
| 1 | M/4 | 25.3 | 27.4 | 2.5 | 2.7 | 2230 | 2370 | 4.8 | 5.1 |
| F/4 | 24.6 | 15 | 2.4 | 1.5 | 2160 | 1340 | 4.7 | 2.9 |
| 3 | M/7 | 74.5 | 108 | 7.2 | 10 | 6790 | 10800 | 15 | 23 |
| F/7 | 82.6 | 129 | 8.0 | 13 | 7510 | 12900 | 16 | 28 |
| 30 | M/7 | 824 | 1630 | 80 | 158 | 71500 | 177000 | 154 | 382 |
| F/7 | 795 | 1540 | 77 | 150 | 72000 | 165000 | 155 | 356 |

F: female; M: male; NOAEL: no-observed-adverse-effect level.

† The underlined dose represents the NOAEL.

‡ The exposure ratios were calculated as [AUC168 x4] or [Cmax] / [estimated human systemic exposure level at the efficacious dose]. The estimated human systemic exposure level of Cmax or AUC672 was 10.3 µg/mL or 1855.2 µg∙h/mL, respectively, at the estimated efficacious dose of 0.4 mg/kg, which would be needed to maintain the target trough concentration of 0.21 μg/mL for 28 days [Study Project 9-ME-9001].

A summary of potential risks is listed in [[Table 2](#_bookmark63)] and discussed further below. In the first in human (FIH) study, these risks will be managed through selection of inclusion/exclusion criteria, use of sentinel dosing, use of pre-defined stopping rules and dose escalation rules, adverse event assessment, and monitoring of clinical laboratory tests.

### Effects on Blood Cells and Hematopoietic System

In the 4-week repeated dose study, hematologic changes including increased reticulocyte ratio, increased eosinophil count, and decreased platelet count, and possibly relevant histopathological finding of increased hematopoiesis in the femoral bone marrow were observed at the 30 mg/kg dose level. Similar changes were observed in the preliminary non- GLP 4-week dose study. The mechanism of these changes has not been identified, however, it is possible that these hematologic findings may be associated with the immunogenicity of PROJECT 9 (i.e., ADA-related changes). It has been reported in the literature that immune complex clearance can be accompanied by changes in erythrocyte, neutrophil and platelet counts [Rojko et al, 2014] as were noted in the 4-week study with PROJECT 9. These changes were reversible by the end of 6-week recovery period and were monitorable by a standard assessment of hematology parameters; and therefore, these findings are considered to be of minimal clinical concern.

Additionally, a reduction of B-cell (CD3-CD20+) counts was also observed at doses equal to or greater than 0.3 mg/kg. This was considered to be a pharmacological effect of PROJECT 9, and there was a trend toward reversibility by the end of the 6-week recovery period. These data suggest B cell counts should be monitored in the clinical study.

### Effects on the Phagocytic System

In the 4-week repeated dose study, enlargement of the spleen and increased splenic organ weight with congestion and hyperplasia of the red pulp were noted. In addition, hypertrophy of liver Kupffer cells and hypertrophy of the submandibular lymph node sinus histiocytes were observed at the 30 mg/kg dose level. These changes were reversible by the end of the 6-week recovery period. The mechanism of these changes has not been identified; however, since almost all of these changes were seen in the animals that were ADA positive, these findings were considered to be secondary to the processing of immune complexes.

### Effects on the Thymus

In the 4-week repeated dose study, 3 male animals showed slight to moderate atrophy in the thymus at the 30 mg/kg dose level. This histological finding was reversible by the end of the 6-week recovery period. The cause of this finding has not been identified, however, it was speculated that it might be associated with prolonged inhibition of B cell mediated immune responses by PROJECT 9, since similar changes have been observed with other immuno- suppressive drugs. In addition, the thymic atrophy occurred at systemic exposures that are estimated to be 154-fold higher than the estimated human systemic exposure at the clinically efficacious dose. Accordingly, the risk of adverse effect on the thymus in the clinical study is considered minimal.

### Effects on the Gallbladder

In the 4-week repeated dose study, 1 male animal showed very slight to moderate edema and mixed inflammatory cell infiltration in the subserosa and lamina propria of the gallbladder at the 30 mg/kg dose level. These histological findings were reversible prior to the end of the 6-week recovery period. The cause of this finding has not been identified, however, it was speculated that these changes may be immunogenicity-related since they were only seen in the animal that was ADA-positive.

### Effects on the Urinary Bladder

In the 4-week repeated dose study, vacuolation in the urothelium was observed at the 30 mg/kg dose level. This histological finding was reversible by the end of the 6-week recovery period. The cause of this finding has not been identified; however, the absence of histological findings in the kidney, coupled with no detectable red blood cells in the urine and no increase in urinary protein suggests that the urothelial barrier was not compromised by PROJECT 9.

### Effects on the Spleen

In the 4-week repeated dose study, one male animal showed very slight histological changes including fibrosis, hemorrhage and brown pigmentation in the capsule in the spleen at the end of the recovery period at the 30 mg/kg dose level. The mechanism of these changes has not been identified. However, it was suggested based on the study pathologist’s experience that the enlarged spleen seen during the dosing phase could have increased capsular tension or friction against the surrounding organs, and this might explain the capsular lesions observed at the end of recovery period. This possible mechanism is also supported by the literature [Fujitani et al, 2004]. While the reversibility of these findings was not confirmed in this study, the findings showed features consistent with repair processes. In addition, the severity and the incidence of this change was minimal and it occurred only in the recovery period and at high systemic exposures (154-fold higher than the estimated human systemic exposure at the clinically efficacious dose). There were no findings in the recovery period in animals dosed at 3 mg/kg. Therefore, it is considered that PROJECT 9 poses a minimal risk of spleen injury to the human subjects.

### Effect on the Vasculature of the Liver and Gallbladder

In the 4-week repeated dose study, very slight to slight histological changes including intimal thickening and perivascular fibrosis in the artery of the liver and in the subserosal artery of the gallbladder were observed in one male animal that was ADA-positive at the end of recovery period. It has been reported that small to medium-sized arteries or arterioles in submucosal or subserosal vessels in multiple organs including the gallbladder are the most common sites for immune complex-related vascular/perivascular lesion in monkeys [Rojko et al, 2014]. Therefore, it is possible that these findings may be the result of immunogenicity (immune complex-related) changes to PROJECT 9 in cynomolgus monkeys. The risk of a severe immunogenic response clinically appears unlikely since the humanized monoclonal antibody will likely be less immunogenic in humans.

### Others

In the in vitro tissue cross-reactivity study in human tissues, unanticipated cross-reactivity of PROJECT 9 was observed in the membrane of ductal epithelium of the salivary gland. This cross-reactivity was not observed in the study using cynomolgus monkey tissues. Monitoring of clinical signs such as excess salivation or dry mouth should be considered in the first-in- human study.

### Table 2 Potential Safety Concerns of PROJECT 9

|  |  |  |
| --- | --- | --- |
| **Key Safety Targets** | **Key Observations** | **Relevance to Human Usage** |
| Immune System | **30 mg/kg:**   * Atrophy in the thymus * Edema and mixed inflammatory cell infiltration in the subserosa and lamina propria in the gallbladder * Enlargement of the spleen, high organ weight with congestion and hyperplasia of the red pulp in the spleen, hypertrophy of Kupffer cells in the liver, and hypertrophy of sinus histiocytes in the submandibular lymph node * Fibrosis, hemorrhage, and brown pigmentation in the capsule in the spleen * Intimal thickening and perivascular fibrosis in the artery of the liver and in the subserosal artery of the gallbladder | Possible   * Potential risk of infection/decreased ability to fight infections potentially associated with B cell reduction/inhibition and changes in thymus.   Possible immunogenicity-related responses:   * Increased globulin * Potential risk of infusion related reactions * Other phagocytic system related findings in spleen, lymph node and Kupffer cells on the liver * Increased serum total IgG and IgM |
| Hematopoietic System | **30 mg/kg:**   * B-cell reduction (likely mediated by a pharmacological effect) * Possible immunogenicity-related changes   + Increased reticulocyte ratio, increased eosinophil count and decreased platelet count   + Increased hematopoiesis in the femoral bone marrow | Possible   * Increased reticulocyte ratio * Increased eosinophil count * Decreased platelet count * Increased hematopoiesis in bone marrow |
| Renal System | **30 mg/kg:**   * Vacuolation in the urothelium in the urinary bladder | Possible   * Findings in urinalysis (including proteinuria, blood urea, etc.) |
| Salivary gland | **Tissue cross-reactivity study in human tissues:**   * Unanticipated cross-reactivity observed in ductal epithelium of the salivary gland. | Possible   * Potential risk of salivary related adverse effect suggested from the unanticipated binding to this site in human tissues |

Ig: immunoglobulin

## Conclusions

The currently available nonclinical pharmacology data and safety data support further clinical development of PROJECT 9. Monitoring for declines in circulating B cell counts, as well as for immunogenic side effects secondary to anti-PROJECT 9 antibody formation, and effects on salivary glands (excess salivation or dry mouth) should be considered in the clinical studies.

### List of References

Davies B, Morris T. Physiological parameters in laboratory animals and humans. Pharm Res.

1993;10(7):1093-5.

Fujitani T, Tada Y, Yoneyama M. Chlorpropham-induced splenotoxicity and its recovery in rats.

Food Chem Toxicol. 2004;42(9):1469-77.

Rojko JL, Evans MG, Price SA, Han B, Waine G, DeWitte M, et al. Formation, clearance, deposition, pathogenicity, and identification of biopharmaceutical-related immune complexes: review and case studies. Toxicol Pathol. 2014;42(4):725-64.