### Safety Pharmacology

A safety pharmacology assessment was incorporated in the 4-week intravenous toxicity study in cynomolgus monkeys [Study Project 10-TX-0002]. At up to 60 mg/kg, no effects were noted on the cardiovascular, respiratory or central nervous systems.

### Pharmacodynamic Drug Interactions

No nonclinical pharmacodynamic drug interaction studies with PROJECT 10 have been conducted.

### Other Pharmacology Studies

No other nonclinical pharmacology studies with PROJECT 10 have been conducted.

## Toxicology

Toxicology studies consisted of 4-week intravenous repeated dose toxicity studies in rats and cynomolgus monkeys, 13-week subcutaneous repeated dose toxicity studies in rats and cynomolgus monkeys, an embryo-fetal development study in rats, and in vitro human, cynomolgus monkey and rat tissue cross-reactivity studies. Toxicology studies were conducted under appropriate guidelines/guidance including ICH guidelines and in accordance with Good Laboratory Practice standards.

### Single-dose Toxicity

No independent single-dose toxicity studies have been conducted with PROJECT 10. Effects after a single intravenous dose of PROJECT 10 were evaluated in the repeated dose toxicity studies.

### Repeat-dose Toxicity

* + - 1. **4-Week Intravenous Repeated Dose Toxicity Study in Rats [Study Project 10-TX-0001]**

PROJECT 10 was dissolved in 20 mmol/L citrate solution (pH 4.9) containing 240 mmol/L

D-sorbitol and 0.02% (wt/vol) polysorbate 80 and was intravenously administered at doses of 0 (vehicle), 6, 20 and 60 mg/kg once every other day for 4 weeks to male and female Crl:CD Sprague-Dawley (SD) rats in order to investigate its toxicity. Five male and 5 female rats were added to the vehicle (control) and 60 mg/kg groups to evaluate reversibility of the changes following a 4-week recovery period. Systemic exposure to PROJECT 10 and

anti-PROJECT 10 antibody levels was also assessed.

No animal, at any dose level, died or became moribund during the dosing or recovery period. No test article-related effects on body weight, food consumption or ophthalmology were noted.

In all PROJECT 10-treated groups, lesions and crust in the dorsal cervical skin occurred in a

dose-dependent manner. As a result, some clinical pathology changes, including occult blood and protein in urine, decreases in erythrocytes, hematocrit value and hemoglobin concentration, increased reticulocyte ratio, platelet, leukocyte, neutrophil and monocyte counts were noted at the end of the dosing period. Associated with the lesion, spleen weight increased and macroscopic changes at necropsy were observed. Histopathologic findings revealed skin wounds with inflammatory and repair responses and enhanced extramedullary hematopoiesis in the spleen. The occurrence of skin lesion was higher in males than in females. In addition, histopathology revealed vacuolation of macrophages, which was considered to be associated with administration of a large amount of polyethylene glycol, in the spleen, lymph nodes, pituitary, ovary, vagina and/or uterus in males and females at

20 mg/kg and 60 mg/kg. Other than these changes, there were no notable findings in clinical,

macroscopic or microscopic pathology. At the end of the 4-week recovery period, those changes had reversed or tendencies toward recovery were noted.

The concentration of drug in serum at time zero (C0) and AUC48 increased almost dose proportionally from 6 mg/kg to 60 mg/kg and t1/2 was comparable among these doses in both sexes on days 1 and 27 of dosing. Following repeated dosing, C0 increased 1.3-fold to 1.7- fold and AUC48 increased 1.6-fold to 2.0-fold comparing day 1 to day 27. No sex differences in toxicokinetics were noted at any dose level. Although anti-PROJECT 10 antibodies were detected in several animals, effects on drug exposure were minimal.

In conclusion, PROJECT 10 was well-tolerated in rats up to 60 mg/kg. Skin lesions and lesion-associated clinical pathology and histopathology changes were noted in all PROJECT 10-treated groups. Vacuolation of macrophages in various organs at doses of

20 mg/kg and 60 mg/kg were observed, but no vacuoles were observed at the 6 mg/kg dose. Skin and associated clinical pathology changes were considered to be of limited toxicological significance. Skin lesions could be attributed to rat-specific grooming behavior and may reflect PROJECT 10-related pharmacology. The no observed adverse effect level (NOAEL) was concluded to be uncertain in the 4-week rat study, (also see [section 4.3.2.2](#_bookmark33)).

### 13-Week Subcutaneous Repeated Dose Toxicity Study in Rats [Study Project 10-TX-0006]

PROJECT 10 in the vehicle (20 mmol/L citrate solution containing 240 mM D-sorbitol and 0.02% [w/v] polysorbate 80 [pH = 4.9]) was administered via subcutaneous injection once every other day for 13 weeks (46 doses) at dosage levels of 0 (vehicle), 2, 6 and 20 mg/kg to 10 animals/sex of Crl:CD (SD) rats. Eight animals/sex were added to the control and

20 mg/kg groups to assess the reversibility of toxicity during a subsequent 13-week recovery period. PROJECT 10 was also administered to satellite rats (4 or 7/sex/group) in the same manner to assess the systemic exposure. The following observations were performed: clinical examinations, body weights, food consumption, ophthalmic examinations, functional observational battery (FOB), motor activity, hematology, serum chemistry, urinalysis, gross pathology, organ weights, histopathology, stereology (on a cranial cervical ganglion and dorsal root ganglion) and toxicokinetics including examinations of ADA.

There were no test article-related mortalities observed during this study. There were no test article-related effects on body weights, food consumption, FOB or serum chemistry and urinalysis parameters. There were no test article-related ophthalmic findings or organ weight changes.

Test article-related clinical observations noted for the 6 and/or 20 mg/kg group animals included moist alopecia of the dorsal/ventral neck and dorsal trunk, scabbing of the dorsal trunk, dorsal, ventral, left/right lateral neck and left/right dose sites, hair loss for the males and/or females during the dosing period. This correlated with higher white blood cell and neutrophil counts and bone marrow myeloid hyperplasia and skin ulceration and/or erosion in histopathology. These clinical observations were generally resolved by the recovery necropsy, indicating reversibility.

Test article-related stereological findings of decreased ganglion volume and neuron size in the cranial cervical ganglion were observed at 20 mg/kg females. This correlated with neuronal atrophy observed histopathologically. This change was reversed by the recovery

necropsy. There was no evidence of functional alterations while the potential changes were extensively examined.

Test article-related microscopic findings were noted in the cranial cervical ganglion (neuronal atrophy), pancreas (acinar atrophy), skin (ulceration and vacuolated histiocyte infiltration), axillary lymph nodes (sinus histiocytosis and medullary plasmacytosis) and bone marrow (myeloid hyperplasia) of the 20 mg/kg group males and females, in the skin and bone marrow of a single 6 mg/kg group male, and at the injection sites (erosion, epidermal hyperplasia, vacuolated histiocyte infiltration and lymphocyte infiltration) in all test article-treated groups. These findings were completely reversed or there was evidence of reversibility at the recovery necropsy.

In toxicokinetics, tmax values were 24 or 48 h on study day 0 and after multiple doses were at earlier time points (predose or 1 h) for some groups. A clear difference in average tmax values was not observed between days or sexes. Cmax and AUC48 increased with repeated dosing and were higher in females; the increased exposure with multiple doses was unchanged after study day 44. Single-dose Cmax and AUC48 were approximately dose-proportional over the dose range evaluated (2, 6 and 20 mg/kg) and were less than proportional after multiple doses. The presence of ADA in 5 females and 3 males had no effect on their serum concentration profiles.

In conclusion, subcutaneous administration of PROJECT 10 to male and female SD rats at dosage levels of 2, 6 and 20 mg/kg once every other day for 13 weeks (46 doses) resulted in no test article-related mortality. Changes mainly observed at 20 mg/kg were atrophy of the cervical cranial ganglion, pancreatic acinar atrophy and skin wounds and associated inflammation reactions. Injection site changes and vacuolated histiocytes were associated with all doses.

These changes were reversible and the toxicological significance was considered to be limited.

The NOAEL was concluded to be uncertain in the 13-week rat study, which is also the conclusion in the 4-week rat study. This was due to the appearance of rat-specific changes in these studies (e.g., skin lesions, which are most likely attributed to rat specific grooming behavior, [section 4.3.2.1](#_bookmark32)), and thus, the NOAELs for the rat studies are not indicated. On the other hand, in monkeys, the NOAEL was determined to be 60 mg/kg, which was the highest dose tested and consistent in the 4 and 13 monkey studies, presented in sections [4.3.2.3](#_bookmark34) and [4.3.2.4](#_bookmark35), respectively.

### 4-Week Intravenous Repeated Dose Toxicity Study in Monkeys [Study Project 10-TX-0002]

In order to investigate its toxicity, PROJECT 10 was dissolved in a 20 mmol/L citrate solution (pH 4.9) containing 240 mmol/L D-sorbitol, 0.02% (wt/vol) polysorbate 80 and was intravenously administered once weekly for 4 weeks at doses of 0 (vehicle), 6, 20 and

60 mg/kg to male and female cynomolgus monkeys. Three male and 3 female monkeys were added to the 60 mg/kg dose group to evaluate reversibility of the changes following a 4-week recovery period. Systemic exposure to PROJECT 10 and anti-PROJECT 10 antibody levels was also assessed.

No deaths occurred during the dosing or recovery period. Animals tolerated the test compound well and there were no effects on the cardiovascular and respiratory systems or CNS clinical signs. There were no test article-related changes in clinical signs, body weight, food consumption, ophthalmology, electrocardiography, body temperature, blood pressure, respiration rate, urinalysis, hematology, blood chemistry, gross pathology, organ weights or histopathology resulting from doses up to 60 mg/kg.

C0 and AUC168 increased almost dose proportionally from 6 mg/kg to 60 mg/kg and t1/2 was comparable among these doses in both sexes on days 1 and 22 of dosing. Following repeated dosing, C0 and AUC168 increased 1.5 to 2.1 times from day 1 of dosing, although t1/2 was unchanged. No obvious sex differences in toxicokinetics were noted at any dose level. In some animals, ADAs were detected and an increased clearance of PROJECT 10 was noted.

The NOAEL in monkeys was set at 60 mg/kg, the highest dose tested.

### 13-Week Subcutaneous Repeated Dose Toxicity Study in Monkeys [Study Project 10-TX-0007]

PROJECT 10 in the vehicle (20 mmol/L citrate solution containing 240 mM D-sorbitol and 0.02% [w/v] polysorbate 80 [pH = 4.9]) was administered via subcutaneous injection once weekly for 13 weeks (13 doses) at dosage levels of 0 (vehicle), 6, 20 and 60 mg/kg to

8 animals/sex in the control and 60 mg/kg groups and 4 animals/sex/group in the 6 and 20 mg/kg groups of cynomolgus monkeys. Four males and 4 females of each group were

euthanized after the 13-week dosing period and an additional 4 males and 4 females of the control and 60 mg/kg groups were euthanized after the 4-week recovery period. The following observations were performed in this study: clinical examinations, body weights, food consumption (qualitative), ophthalmic examinations, ECG and heart rates, FOB, hematology, coagulation, serum chemistry, urinalysis, gross pathology, organ weights, histopathology, stereology (on a cranial cervical ganglion and dorsal root ganglion) and toxicokinetics including ADA examinations.

There were no test article-related mortalities observed during this study. A single mid-dose monkey (receiving 20 mg/kg) was sacrificed in moribund condition towards the end of treatment. The findings were specific to this animal (hemorrhage in lung, histopathological observation of rectal ulceration) or much more severe than in other animals (injection site observations). A definitive cause of death could not be identified but the death is unlikely related to the (exaggerated) pharmacology of NGF binding by the monoclonal antibody.

ADAs were not found and the involvement of an immune response was not clear. Based on the isolated occurrence, it was concluded that the poor condition/sacrifice of the monkey was not drug related.

Test article-related clinical observations during the dosing period were desquamation of forelimb(s), hindlimb(s), and ventral trunk; scabbing of the forelimb(s), hindlimb(s), dorsal trunk, and facial area; and hair loss of the dorsal head, forelimb(s), hindlimb(s), dorsal trunk and rump area were noted for the 60 mg/kg group males and/or females. Although these skin changes persisted during the recovery period, evidence of recovery was seen.

Test article-related microscopic findings of accumulation of vacuolated histiocytes at the injection sites, spleen and lymph nodes, as well as vacuolated Kupffer cells within the liver were noted for the 20 mg/kg and 60 mg/kg animals. The incidence and severity of vacuolated histiocytes were lower after a 13-week recovery period, which indicated partial

reversibility. These changes were associated with the administration of PEG which was a component of the test article and were considered nonadverse.

There was no difference in tmax (24 or 72 h) between dose groups. Cmax and AUC168 were approximately dose proportional over the dose range evaluated (6, 20 and 60 mg/kg).

Sex-differences were not observed. The presence of ADAs was limited to a single animal, and the effect of ADA on the serum concentration profile was not observed.

In conclusion, subcutaneous administration of PROJECT 10 to cynomolgus monkeys at doses of 6, 20 and 60 mg/kg once weekly for 13 weeks (total of 13 doses) resulted in no test

article-related mortality. Test article-related changes were limited to histiocyte vacuolation of various organs and desquamation and hair loss on various body surfaces at 20 mg/kg and/or 60 mg/kg. Therefore, the NOAEL was set at 60 mg/kg.

### Genotoxicity

Genotoxicity studies are not required for biotechnology-derived pharmaceuticals, such as monoclonal antibodies.

### Carcinogenicity

No long-term studies for carcinogenicity with PROJECT 10 have been conducted.

### Reproductive and Developmental Toxicity

### 4.3.5.1 Effects on Embryo-fetal Development

**4.3.5.1.1 Embryo-fetal Development Study in Rats [Study Project 10-TX-0003]**

In order to investigate the effects on embryo-fetal development, PROJECT 10 was dissolved in 20 mmol/L citrate solution (pH = 4.9) containing 240 mmol/L D-sorbitol and 0.02% (wt/vol) polysorbate 80 and was intravenously administered to pregnant Crl:CD SD rats once every

2 days from days 7 to 17 of gestation at doses of 0 (vehicle), 6, 20 and 60 mg/kg. Systemic exposure to PROJECT 10 and anti-PROJECT 10 antibody levels was also assessed.

No dams died and no test article-related changes were noted in clinical signs, body weight, body weight gain, food consumption, gross pathological findings, the number of corpora lutea or the number of implantations in dams treated with PROJECT 10 up to 60 mg/kg.

No test article-related changes were noted in the number of live fetuses or embryo-fetal deaths, postimplantation loss rate, fetal body weight, placental weight, sex ratio or external, placental, visceral or skeletal findings in fetuses in the treated groups up to 60 mg/kg.

In dams, mean C0 and AUC48 values increased almost dose proportionally from 6 mg/kg to 60 mg/kg on days 7 and 17 of gestation. Mean t1/2 values on day 7 of gestation were approximately 1.6-fold longer than those on day 17 of gestation. After repeated dosing, C0 and AUC48 values on day 17 of gestation were slightly (1.1-fold to 1.3-fold) increased in comparison with those on day 7 of gestation. Although PROJECT 10 was not detected in any fetus of the 6 mg/kg group, trace amounts of PROJECT 10 were detected in fetuses in the

20 mg/kg and 60 mg/kg dose groups. No anti-PROJECT 10 antibody was detected in any dam on day 19 of gestation.

It is concluded that the NOAEL (in rats) is 60 mg/kg for dams and embryo-fetal development.

### Local Tolerance [Study Project 10-TX-0005]

The objective of this study was to investigate local tolerance to a single intravenous or subcutaneous injection of PROJECT 10 in rabbits.

PROJECT 10 (48.3 mg/mL, 1 mL) was administered once intravenously into the left auricular vein or once subcutaneously into the dorsal subcutis of Japanese White rabbits (3 males). Vehicle control was administered into the right auricular vein or a different site of the dorsal subcutis, in the same manner. In the subcutaneous injection study, physiological saline, which was administered in the same manner to a different site as a negative control, and an untreated site, were also examined. Clinical signs and body weight were examined and for injected sites, macroscopic observations according to the Draize method and histopathology were conducted.

No clinical signs were observed and body weight was unaffected.

No macroscopic or microscopic changes were observed at the intravenous or subcutaneous injection site of PROJECT 10 in any of the animals.

It was concluded that, under the conditions of the present study, PROJECT 10 had no venous or subcutaneous irritancy in rabbits.

### Other Toxicity Studies

**4.3.7.1 In Vitro Tissue Cross Reactivity Study in Human, Cynomolgus Monkey and Rat Tissues [Study Project 10-TX-0004]**

PROJECT 10 was applied to cryosections of normal human (3 donors per tissue, where available), cynomolgus monkey and SD rat tissues (2 donors per tissue, where available) at 2 concentrations (2 µg/mL and 20 µg/mL). The following panel was assessed: adrenal, bladder (urinary), blood cells (evaluated from peripheral blood smears), blood vessels

(endothelium; evaluated from all tissues where present), bone marrow, cerebellum, cerebrum (cerebral cortex), breast, colon (large intestine), eye, fallopian tube, gastrointestinal tract (includes esophagus, small intestine and stomach - including underlying smooth muscle), heart, kidney (glomerulus and tubule), liver, lung, lymph node, ovary, pancreas, parathyroid, peripheral nerve, pituitary, placenta, prostate, salivary gland, skin, spinal cord, spleen, striated muscle (skeletal), testis, thymus, thyroid, tonsil, ureter, uterus cervix and endometrium. In addition, the test article was substituted with a human immunoglobulin G (IgG) Fab’ fragment antibody which has a different antigenic specificity from that of the test article (control article), designated human IgG Fab’. Study controls consisted of recombinant human β-NGF UV-resin spot slides as positive control material and human hypercalcemia of malignancy peptide, amino acid residues 1 to 34, UV-resin spot slides, as negative control material. The acetone-fixed tissue slides and control materials were stained immunohistochemically and examined histopathologically.

Moderate to intense staining of the positive control material was evident at both concentrations of PROJECT 10. PROJECT 10 did not specifically react with the negative control material at either concentration. The control article, human IgG Fab’, did not specifically react with either the positive or negative control materials. There also was no staining of the assay control slides. Therefore, the specific reactivity of PROJECT 10 toward the positive

control material and the lack of specific reactivity toward the negative control material or the control article indicated that the assay was sensitive, specific and reproducible.

Under these conditions, no staining was present with PROJECT 10 in normal human, cynomolgus monkey and SD rat tissue panels.

It was concluded that there was no cross-reactivity of PROJECT 10 with normal human, nonhuman primate (cynomolgus monkey) and SD rat tissues.

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

PROJECT 10 is PEGylated Fab’ fragment human monoclonal antibody which has 1 Fab’ domain connected to a PEG chain.

The binding affinity of PROJECT 10 to rhβ-NGF was evaluated using the Gyrolab system. The Kd for the binding of PROJECT 10 to rhβ-NGF was 11.89 pmol/L indicative of a potent binding affinity.

The binding activities of PROJECT 10 to human neurotrophin family proteins (NGF, BDNF, NT-3 and NT-4) using rh proteins in the ELISA method were also evaluated. PROJECT 10 showed binding activity to rhβ-NGF with an EC50 value of 12.97 nmol/L but did not show binding activities to rhBDNF, rhNT-3 or rhNT-4 even at a concentration of 3000 nmol/L, indicating selective binding to NGF.

Furthermore, cross-reactivity to recombinant rat and mouse NGF for PROJECT 10 has been confirmed with Kd values of 21.07 pmol/L and 12.13 pmol/L, respectively. This result suggests the appropriateness of these species for pharmacology studies of PROJECT 10.

It has been shown previously that NGF exerts its hyperalgesic function through the phosphorylation of TrkA. PROJECT 10 showed concentration-dependent inhibition of rhβ-NGF-induced TrkA phosphorylation with an IC50 of 0.831 nmol/L, indicating a functional inhibition of the NGF signal pathway.

Interference with NGF signaling by PROJECT 10 was evaluated in a Freund’s adjuvant-induced arthritis (AIA) pain model in mice. It is well established that in experimental inflammatory conditions, NGF levels are rapidly increased in the inflamed tissue [McMahon, 1996]. The inflammatory AIA pain model therefore represents aspects of cystitis-related pain in humans. PROJECT 10 at doses of 0.2, 0.6 and 2 mg/kg (intravenous) significantly suppressed AIA- induced pain behavior [Study Project 10-PH-0001].

In vitro findings suggesting interference of NGF signaling by PROJECT 10 were subsequently evaluated on visceral pain-related behavior in rats induced by ip administration of CPA, an antitumor agent known to produce hemorrhagic cystitis. This animal model is believed to represent aspects of cystitis-related pain in humans. PROJECT 10 at doses of 2 mg/kg and

6 mg/kg (intravenous) significantly suppressed CPA-induced bladder pain related behavior.

In addition to pain, patients with BPS/IC report an increase in urinary frequency that may be linked to pain. CPA has been used to induce voiding dysfunction in rats to mimic urinary changes seen in patients. It was suggested that CPA administration causes an elevation of pelvic organ NGF levels [Murray et al, 2004] and a decrease in mean volume voided per micturition by activating primary afferent neurons in rats [Hu et al, 2003; Maggi et al, 1992].

PROJECT 10 significantly increased mean voided volume per micturition and decreased micturition frequency at doses of 0.1 mg/kg to 10 mg/kg (intravenous) compared to vehicle, without affecting the total urine excretion rate.

Based on the serum PROJECT 10 concentrations determined by an ECLIA that recognizes Fab’, the t1/2 in monkeys (0.1 mg/kg, intravenous) was calculated to be 209 h and the subcutaneous bioavailability in monkeys was calculated to be 67.9%, which suggests that PROJECT 10 has a favorable pharmacokinetic profile. The nonclinical safety profile of PROJECT 10 has been evaluated according to ICH S6 guideline and all findings were evaluated for relevance to human risk. Rats and cynomolgus monkeys were selected for the toxicology evaluation as PROJECT 10 has comparable binding affinities for rat and cynomolgus monkey NGF. There was no discernible cross-reactivity of PROJECT 10 with human, cynomolgus monkey or rat tissues.

The assessment of safety pharmacology was included in the 4-week repeated dose toxicity study in cynomolgus monkeys with once weekly intravenous administration of doses up to 60 mg/kg. No effects were noted on the cardiovascular (electrocardiography and blood pressure), respiratory (respiration rate) systems or on the CNS (clinical signs).

PROJECT 10 was well tolerated in rats and cynomolgus monkeys following 4-week intravenous repeated doses or 13-week subcutaneous repeated doses. In rats, PROJECT 10 was injected every other day, whereas in monkeys the dosing frequency was once weekly. Noteworthy findings were skin lesions, tissue histiocyte vacuoles and atrophy of the neuronal cells in sympathetic ganglia. Reversibility of all changes following cessation of treatment was demonstrated.

Skin lesions and associated clinical pathology changes were noted as low as 6 mg/kg in both rat studies. In the cynomolgus monkey studies, mild skin changes were noted at 60 mg/kg during 13-week dosing. These skin lesions could be attributed to animal-specific grooming behavior. The skin lesions are not regarded as relevant for humans, but may reflect pharmacological effects of PROJECT 10 on sensory nerve function.

Tissue histiocyte vacuoles containing PEG were observed in several organs of rats at 20 and 60 mg/kg in the 4-week study with intravenous dosing and in the skin and/or injection sites of rats after subcutaneous administration at all doses tested (as low as 2 mg/kg) in the 13-week study. Similar tissue histiocyte vacuoles were observed at the subcutaneous injection sites and in the liver, spleen and lymph nodes of monkeys at 20 mg/kg and/or 60 mg/kg following 13-week treatment. The vacuoles in these tissue macrophages contained drug-derived PEG. Macrophage vacuolation is commonly seen in nonclinical studies of PEGylated proteins and generally not associated with functional changes in the affected organs [Ivens et al, 2015; Kronenberg et al, 2013].

In 13-week studies in rats and monkeys, effects on sensory and sympathetic ganglia were evaluated with stereology and histochemistry. No effects on the dorsal root ganglion were observed in rats and monkeys. On the cranial cervical ganglion, there were no effects in monkeys. Ganglion volume and neuron size were decreased in female rats at a dose of 20 mg/kg based on stereology. Histochemistry revealed atrophy, but absence of cell death; the reduction in neuronal size reversed during the postdosing period. No functional changes were observed in clinical observations and functional observation battery indicating no impairment of the autonomic nervous system. Atrophic effects on sympathetic ganglia were associated with the pharmacology and considered a class effect.

No effects were observed on embryo-fetal development in pregnant rats at up to the highest dose of 60 mg/kg.

ADAs were noted in some individual animals without appearing to affect the toxicological evaluation of the studies. The induction of antibody formation in animals is not regarded predictive of a potential for antibody formation in humans (Leach et al, 2014).

In summary, nonclinical pharmacology study results suggest that PROJECT 10 has a therapeutic potential in patients with BPS/IC. The nonclinical safety and pharmacokinetic profile results support the conduct of a study with PROJECT 10 in patients with BPS/IC with a duration of

13 weeks.

### 4.4.1 Exposure Assessment

The exposure levels in the toxicity studies are presented in [Table 2.](#_bookmark45)

### Table 2 Exposure Levels in Toxicity Studies

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study Type** | **No. of Animals** | **Sex** | **Dose † (mg/kg)** | **Cmax (µg/mL)** | | **AUC48 or 168**  **(µg·h/mL)‡** | | **Study No.** |
| **First dose** | **Last dose** | **First dose** | **Last dose** |
| SD rats: 13-week (sc) dose toxicity study | 3 | M | 2 | 4.45 | 21.7 | 149 | 801 | Project 10-TX-0006 |
| 3 | F | 2 | 6.56 | 41.3 | 209 | 1680 |
| 3 | M | 6 | 6.63 | 24.4 | 177 | 900 |
| 3 | F | 6 | 8.91 | 55.2 | 293 | 2030 |
| 3 | M | 20 | 38.8 | 63.4 | 1300 | 2500 |
| 3 | F | 20 | 68.3 | 177 | 1810 | 6160 |
| Monkey: 13-week (sc) dose toxicity study | 4 | M | 6 | 85.6 | 210 | 11300 | 29700 | Project 10-TX-0007 |
| 4 | F | 6 | 67.2 | 178 | 9320 | 23400 |
| 4 | M | 20 | 191 | 609 | 27400 | 78500 |
| 4 | F | 20 | 202 | 573 | 27500 | 82200 |
| 8 | M | 60 | 710 | 1680 | 98900 | 251000 |
| 8 | F | 60 | 711 | 1680 | 93200 | 230000 |

NOAEL: No observed adverse effect level; SD: Sprague-Dawley; sc: subcutaneous.

† The underlined dose represents the NOAEL.

‡ AUC48 for rats and AUC168 for monkeys

### References:

<Available upon request>

Bergmann I, Reiter R, Toyka KV, Koltzenburg M. Nerve growth factor evokes hyperalgesia in mice lacking the low-affinity neurotrophin receptor, p75. Neurosci Lett.

1998;16;255(2):87-90.

Davies B, Morris T. Physiological Parameters in laboratory animals and humans. Pharm Res.1993;10:1093-95.

Ehlers MD, Kaplan DR, Price DL, Koliatsos VE. NGF-stimulated retrograde transport of trkA in the mammalian nervous system. J Cell Biol. 1995;130 (1):149-56.

Hu VY, Malley S, Dattilio A, Folsom JB, Zvara P, Vizzard MA. COX-2 and prostanoid expression in micturition pathways after cyclophosphamide-induced cystitis in the rat. Am J Physiol Regul Integr Comp Physiol. 2003;284(2):R574-85.

Ivens IA, Achanzar W, Baumann A, Brändli-Baiocco A, Cavagnaro J, Dempster M, et al. PEGylated biopharmaceuticals: current experience and considerations for nonclinical development. Toxicol Pathol 2015;43(7):959-83.

Leach MW, Rottman JB, Hock MB, Finco D, Rojko JL, Beyer JC. Immunogenicity/hypersensitivity of biologics. Toxicol Pathol 2014;42(2):293-300.

Kronenberg S, Baumann A, de Haan L, Hinton HJ, Moggs J, Theil F, et al. Current challenges and opportunities in nonclinical safety testing of biologics. Drug Discov Today. 2013;18:1138-43.

Maggi CA, Lecci A, Santicioli P, Del Bianco E, Giuliani S. Cyclophosphamide cystitis in rats: involvement of capsaicin-sensitive primary afferents. J Auton Nerv Syst.

1992;38(3):201-8.

McMahon SB. NGF as a mediator of inflammatory pain. Philos Trans R Soc Lond B Biol Sci. 1996;351(1338):431-40.

Murray E, Malley SE, Qiao LY, Hu VY, Vizzard MA. Cyclophosphamide induced cystitis alters neurotrophin and receptor tyrosine kinase expression in pelvic ganglia and bladder. J Urol. 2004;172(6):2434-9.

Saitoh C, Yokoyama H, Chancellor MB, de Groat WC, Yoshimura N. Comparison of voiding function and nociceptive behavior in two rat models of cystitis induced by cyclophosphamide or acetone. Neurourol Urodyn. 2010;29:501-5.

Salimi-Moosavi H, Rathanaswami P, Rajendran S, Toupikov M, Hill J. Rapid affinity measurement of protein-protein interactions in a microfluidic platform. Anal Biochem. 2012;426:134-41.