### Safety Pharmacology

Safety pharmacology assessments were included in the repeat-dose toxicology study as recommended by International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) S6 (R1) (Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals) and ICH S9 (Nonclinical Evaluation for Anticancer Pharmaceuticals). In the pivotal GLP 4-week repeat-dose toxicity study in cynomolgus monkeys, cardiovascular (electrocardiograms, blood pressure and heart rate); respiratory and neurological function were evaluated (Study PROJECT 11-022 and

Sectio[n 4.3.2.2](#_bookmark51)).

Safety pharmacology findings were as follows:

* No effects of PROJECT 11 on neurological function on day 22 at doses up to 200 mg/kg (highest dose tested).
* No effects of PROJECT 11 on heart rate, respiratory rate or electrocardiography assessment on days 1 and 22 at doses up to 200 mg/kg.
* Mean arterial pressure (MAP), systolic pressure and diastolic pressure reductions on day 1 (postdose) were statistically significant for male animals (but not female animals)

in the 200 mg/kg dose group. 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, but the relationship to PROJECT 11 could not be determined.

* Dose levels were set at 50 (low), 100 (mid) and 200 (high) mg/kg per week; however, the actual dose levels administered for males in low-, mid- and high-dose groups were 102, 165 and 147 mg/kg (day 1), respectively and 105, 178 and 145 mg/kg (day 15), respectively.

In conclusion, no PROJECT 11-related adverse findings were noted for any safety pharmacology parameter assessed at doses up to 200 mg/kg per week.

### Pharmacodynamic Drug Interactions

No pharmacodynamic drug interaction studies with PROJECT 11 have been conducted to date.

## Toxicology

The safety of PROJECT 11 has been evaluated in 1 pivotal GLP 4-week repeated intravenous infusion dose toxicity study in cynomolgus monkeys (Study PROJECT 11-022), 1 tissue

cross-reactivity study (Study PROJECT 11-021) and 1 cytokine release and proliferation assay (Study PROJECT 11-026). In addition, a non–GLP pharmacokinetic, pharmacodynamic and tolerability study was conducted in cynomolgus monkeys (Study PROJECT 11-016).

Recombinant cynomolgus monkey and human GITR exhibited comparable binding to PROJECT 11. This finding is supported by the extracellular domain GITR sequence of cynomolgus monkeys, which was 89.8% homologous to humans. The cross-reactivity of PROJECT 11 to cynomolgus monkey GITR allowed the use of the cynomolgus monkey for toxicity and toxicokinetic studies. PROJECT 11 does not cross-react with mouse or rat GITR. Sequence homology of the mouse and rat extracellular domains to human GITR were 56.9% and 62.7%, which were identical to human, respectively (Study PROJECT 11-002). Based on these binding data, a toxicity assessment was not conducted in rodents.

### Single-dose Toxicity

A single (0.3, 3 mg/kg) and repeat (30 mg/kg/week; 4 doses) dose pharmacokinetic, pharmacodynamic and tolerability intravenous infusion study was conducted in cynomolgus monkeys (Study PROJECT 11-016). There were no changes in any parameters evaluated that were considered toxicologically relevant after a single dose up to 30 mg/kg.

In addition, there were no adverse findings observed in cynomolgus monkeys after the first dose of the 4-week repeat-dose toxicity study at doses up to 200 mg/kg (Study PROJECT 11-022).

### Repeat-dose Toxicity

A 4-week (4-dose) pivotal GLP intravenous toxicity study in cynomolgus monkeys was conducted (Study PROJECT 11-022). A tabulated summary of the findings can be found in [End-of-Text Table 3.2].

In addition, a non–GLP single and 4-week repeated intravenous dose pharmacokinetic and pharmacodynamic study in male cynomolgus monkeys was conducted (Study PROJECT 11-016); tolerability endpoint findings are discussed in [[Section 4.3.2.1](#_bookmark50)].A Non–GLP Single and Repeat Intravenous Infusion Dose Pharmacokinetic, Pharmacodynamic and Tolerability Study of PROJECT 11 in Cynomolgus Monkeys

The objective of this study was to determine the pharmacokinetics, pharmacodynamics and tolerability of PROJECT 11 following single (0.3, 3 mg/kg) or repeat (30 mg/kg per week for 4 weeks) intravenous infusions (i.e., 30-minute infusion) to cynomolgus monkeys

(Study PROJECT 11-016). The pharmacodynamic and pharmacokinetic findings are discussed in [Sections [4.1.1.2.2](#_bookmark29) and [4.2](#_bookmark34)], respectively. The effect of PROJECT 11 on tolerability endpoints is discussed below.

No mortality was observed during the study. Clinical signs were limited to a single animal in the high dose group (Group 3, 30 mg/kg, once weekly for 4 weeks) who exhibited mild tremors on day 15 (third dose) and showed signs of a mild infusion reaction (including reddening of the face with slight swelling around eyes) during and after the final (fourth) dose. The animal was given diphenhydramine (5 mg/kg, intramuscularly) about 5 minutes prior to the end of the infusion. Some redness was still evident the following day, but it had disappeared by day 24.

The administered doses of PROJECT 11 did not result in significant changes of IFN-γ, TNF,

IL-1β, IL-2, IL-6, IL-8 and IL-10 concentrations in the serum over time. Additionally, no noteworthy sustained changes in the frequencies of multiple immune cell subsets attributed to PROJECT 11 dosing were observed in this study. Minor changes in some hematology and clinical chemistry parameters were observed but the relationship to PROJECT 11 could not be determined.

All animals from the single dose groups (0.3 and 3 mg/kg) developed a positive ADA response at day 29, while a positive ADA response was noted on day 15 for 2 out of the

3 animals in the repeat dose group (30 mg/kg) (including the animal that showed the infusion reaction).

On day 1, mean Cmax was 5.45 µg/mL and AUC168 was 11.37 µg∙day/mL for the 0.3 mg/kg single dose group; mean Cmax was 67.85 µg/mL and AUC168 was 115.18 µg∙day/mL for the 3 mg/kg single dose group; and mean Cmax was 594 µg/mL and AUC168 was

1084 µg∙day/mL for the repeat dose group (i.e., 30 mg/kg once weekly for 4 weeks).

In conclusion, administration of PROJECT 11 by a single intravenous infusion or repeat intravenous infusions was well-tolerated in male cynomolgus monkeys.

### A 4-week Intravenous Infusion GLP Study of PROJECT 11 in Cynomolgus Monkeys, With a 1-month Recovery Period

Cynomolgus monkeys (3 males and 3 females per group) were administered PROJECT 11 by intravenous infusion (1-hour infusion) at doses of 0, 50, 100 and 200 mg/kg once weekly for 4 weeks (Study PROJECT 11-022). The animals were dosed on days 1, 8, 15 and 22 and euthanized on day 30. On day 1 and day 15 in males only, the intended dose levels were not delivered; the actual dose levels were 102, 165 and 147 mg/kg (day 1) and 105, 178 and 145 mg/kg (day 15), respectively. In addition, 2 males and 2 females were added to the control and 200 mg/kg groups to assess the reversibility of toxicity findings following a 4-week recovery period.

Infusion reactions occurred in 5 animals (4 males, 1 female) during the fourth dose on day 22 within approximately 16 minutes after the start of the infusion. No infusion reactions were observed upon dosing on days 1, 8 or 15. The infusion reactions were not dose-related (i.e., the reactions were observed in at least 1 animal in each PROJECT 11 dose group) and were characterized during the infusion by loss of consciousness, pale skin, shallow or labored breathing, cold to touch, decreased heart rate and/or decreased activity. The infusion reactions led to mortality in 1 male (in the 50 mg/kg dose group) and to euthanasia of a moribund male (in the 200 mg/kg dose group). No significant gross or microscopic changes were observed in either animal.

Due to unexpected infusion reactions in 4 males on day 22, infusions were stopped early for all males with only partial dose levels administered. Female animals were pretreated with 5 mg/kg intramuscular injection diphenhydramine on day 22 and all received their intended dose of PROJECT 11, with the exception of 1 female in the 100 mg/kg dose group who exhibited loss of consciousness 12 minutes after the start of the infusion, but recovered quickly after the infusion was discontinued. Except for the 2 animals that died or were euthanized on day 22, all other animals displaying infusion reactions and/or clinical signs fully recovered within an hour 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 ADA-mediated response) on body weights, qualitative food evaluation, ophthalmology, 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 and/or clinical signs that survived to their scheduled euthanasia on day 30 (main group) or day 57 (recovery group).

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 and/or clinical signs were ADA-mediated. An ADA-mediated mechanism is supported by the observed transient activation of complement (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 cytokines (i.e., increased IL-6, IL-10 and/or TNF) that were seen in serum or blood collected from animals with infusion reactions and/or clinical signs approximately 1 hour after dosing on day 22 (unscheduled blood collection).

No effects of PROJECT 11 on cardiovascular (electrocardiograms, blood pressure and heart rate); respiratory and neurological function were observed at doses up to 200 mg/kg. MAP, systolic pressure and diastolic pressure reductions on day 1 (postdose) were statistically significant for male animals (but not female animals) in the 200 mg/kg dose group. 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, but the relationship to PROJECT 11 could not be determined.

In conclusion, intravenous infusion of PROJECT 11 once weekly for 4 weeks (total of 4 doses) to cynomolgus monkeys at doses of 50, 100 or 200 mg/kg resulted in adverse infusion reactions in 5 animals on day 22 that were unrelated to dose, with mortality/moribundity occurring immediately after dosing in 1 male in the 50 mg/kg dose group and 1 male in the 200 mg/kg dose group. Infusion reactions and/or clinical signs were typical of an ADA-mediated hypersensitivity response, occurred in 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 proinflammatory cytokines. No toxicities attributed directly to PROJECT 11 were observed. The NOAEL for non-ADA-mediated toxicity is the highest dose tested (i.e., 200 mg/kg dose group). In females on day 1, this dose generated mean AUC168 value of 9630 µg∙day/mL and mean Cmax value of 5380 µg/mL. Males on day 1 were mis-dosed and instead of 200 mg/kg a dose of 147 mg/kg was administered, which generated a mean AUC168 value of 8460 µg∙day/mL and mean Cmax value of 5650 µg/mL.

### Genotoxicity

No in vitro or in vivo genotoxicity studies of PROJECT 11 have been conducted as they are not considered relevant for a monoclonal antibody product (ICH S6 [R1]).

### Carcinogenicity

No carcinogenicity studies of PROJECT 11 have been conducted as they are not considered necessary for anticancer therapy (ICH S1A [Guideline on the Need for Carcinogenicity Studies of Pharmaceuticals] and ICH S9).

### Reproductive and Developmental Toxicity

No reproductive and developmental toxicity studies of PROJECT 11 have been conducted at this time.

### Local Tolerance

No local tolerance studies of PROJECT 11 have been conducted as of the preparation of the IND. However, local irritation was evaluated as part of the 4-week repeated intravenous toxicity study in cynomolgus monkeys (Study PROJECT 11-022). There were no apparent differences observed in animals given PROJECT 11 or vehicle either by pathological evaluation or clinical assessment of the injection site.

### Other Toxicity Studies

* + - 1. **Tissue Cross-reactivity**

PROJECT 11 at 2 concentrations (25 and 2 µg/mL) was applied to cryosections of 36 different cynomolgus monkey tissues (at least 2 donors per tissue, where available) and normal human tissues (at least 3 donors per tissue) (Study PROJECT 11-021). PROJECT 11 bound to cytoplasmic elements in select human and cynomolgus monkey epithelial tissues. This type of PROJECT 11 binding is unlikely to have biological consequences. The study also showed that the potential for off-target toxicity is low.

Reactivity with PROJECT 11 was primarily cytoplasmic in nature. Due to the inability of antibody drugs to access the cytoplasmic compartment in vivo, monoclonal antibody binding to cytoplasmic sites in tissue cross-reactivity studies generally is considered of little to no toxicologic significance [Leach et al, 2010; Hall et al, 2008].

### Cytokine Release and Proliferation

In vitro stimulation of human PBMCs with PROJECT 11 in the soluble format (at concentrations of 0.01, 0.1, 1, 10 and 100 µg/mL) resulted in an PROJECT 11-related dose-dependent release of IL-6, IL-8 and TNFα at concentrations ≥ 10 µg/mL compared to the isotype control at

100 µg/mL. At 1 µg/mL PROJECT 11, the levels of IL-6, IL-8 and TNFα were generally similar to the isotype control at 100 µg/mL, but were higher than the negative control. No induction of the secretion of IL-1β, IL-2, IL-10, IL-12(p70), IFN-γ and granulocyte colony-stimulating factor (G-CSF) was noted (Study PROJECT 11-026).

In the wet-coated immobilized format (at concentrations of 0.01, 0.1, 1, 10 and 100 µg/mL), no PROJECT 11-related increases in IL-1β, IL-2, IL-6, IL-10, IL-12(p70), IFN-γ and G-CSF were observed when compared to the negative and isotype controls. Although no

PROJECT 11-related increase in IL-8 and TNFα was observed when compared to the isotype control, PROJECT 11 concentration-related release of TNFα was observed at ≥ 10 µg/mL and an increase in IL-8 was seen at 100 µg/mL when compared to the negative control.

In vitro stimulation of human PBMCs for 3 days with PROJECT 11 (at concentrations of 0.01, 0.1, 1, 10 and 100 µg/mL) in the soluble format and in the wet-coated format did not induce proliferation in any donors.

In conclusion, minor increases in the proinflammatory cytokines IL-6, IL-8 and TNFα were observed relative to the isotype control in the soluble format, with the highest concentration tested (100 µg/mL) generating increases ≤ 6.2-fold. No increases in cytokines relative to the isotype control were observed in the wet-coated format and no proliferation of PBMCs was observed with either format.

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

### 4.4.1 Summary of Nonclinical Data Package

PROJECT 11 is a high affinity, fully human IgG4 (with S228P hinge stabilization) anti–GITR agonistic antibody in a TM format. PROJECT 11 is being developed for the treatment of patients with advanced solid tumors as a monotherapy or in combination with other

immune-modulating agents such as checkpoint therapies. PROJECT 11 has a high affinity for human and cynomolgus monkey recombinant GITR and binds the receptor with high affinity on primary cells of both species. PROJECT 11 does not cross-react with mouse or rat GITR [[Section 4.1.1.1.1.1](#_bookmark7)]. Based on these results, safety studies with PROJECT 11 were performed only in cynomolgus monkeys, who have an extracellular domain GITR sequence 89.8% homologous to humans. PROJECT 11 demonstrates robust in vitro cell activity using both engineered cell lines as well as human primary T cells. PROJECT 11 is more active than traditional bivalent antibodies without FcγR-mediated crosslinking and is expected to reduce the risk of unwanted FcγR-mediated cytokine release or elimination of key effector cells [Jefferis, 2012; Brüggemann et al, 1987].

PROJECT 11 is structured as a TM antibody with the ability to effectively engage the receptor and produce an efficacious costimulation signal better than the one delivered by a traditional bivalent antibody. This was confirmed by comparing PROJECT 11 to a bivalent antibody containing the same variable domains in an assay using TILs from patients, where PROJECT 11 was more effective in inducing costimulation of CD4+ and CD8+GITR+ T cells.

Additionally, PROJECT 11 activated human GITR with potency and efficacy comparable to human trimeric GITRL in assays using primary and engineered cells. In humans, the native trimeric form of GITRL and higher order oligomeric forms are required for optimal activation of GITR [Zhou et al, 2008a; Chattopadhyay et al, 2007].

GITR expression was found to be low on peripheral T cells, including on peripheral blood mononuclear cells (PBMCs) isolated from cancer patients; however, GITR expression was upregulated on TILs isolated from cancer patients. Expression was high on Teff cells in TILs, and was highest on the surface of Tregs isolated from patient tumors. Interestingly, the same TILs that expressed high levels of GITR also expressed high levels of PD-1, a key immune checkpoint molecule already successfully targeted in clinical settings, indicating that possible combination strategies could be beneficial to improve efficacy [[Section 2](#_bookmark1)].

Because PROJECT 11 does not bind to mouse GITR, surrogate antibodies (based on the sequence of agonistic antibody DTA-1 and including a TM format) were used to demonstrate in vivo tumor efficacy when combined with an anti–PD-1 antibody in a setting where none of the antibodies showed efficacy when given as a monotherapy [Section [4.1.1.2.1](#_bookmark17)].

Antitumor activity for the mSEC12-TM mouse surrogate of PROJECT 11 was observed at doses

≥ 75 µg/animal and appeared to be associated with a Cmax exposure after the first dose of

> 100 µg/mL when combined with the anti–PD-1 treatmen[t [Section 4.1.1.2.1.1](#_bookmark23)]. Therefore, mSEC12-IgG1 was used in combination with an anti–PD-1 antibody in a subsequent dose scheduling study. Maximum comparable efficacy was obtained when mSEC12-IgG1 was dosed once at 750 µg or twice at 250 µg, in combination with 100 µg of anti–PD-1 antibody [Section [4.1.1.2.1.2](#_bookmark26)].

Safety pharmacology assessments of cardiovascular (electrocardiograms, heart rate and blood pressure), respiratory and neurological function were conducted as part of a pivotal GLP

4-week intravenous infusion study of PROJECT 11 in cynomolgus monkeys. RO was monitored on peripheral Tregs. The administered doses of PROJECT 11 did not result in significant changes of IFN-, TNF, IL-1β, IL-2, IL-6, IL-8 and IL-10 concentrations in the serum over time. Additionally, no significantly sustained changes in the frequencies of multiple immune cell subsets that could be attributed to PROJECT 11 dosing were observed in this study. All doses resulted in full RO 24-hours postinfusion [Section [4.3.2.1](#_bookmark50)].

The pharmacokinetics and toxicokinetics of PROJECT 11 were characterized in cynomolgus monkeys in single-dose and repeat-dose studies. PROJECT 11 exhibited an approximately dose-proportional increase in exposure after a single dose of 0.3 to 200 mg/kg [Section

[4.2.1.1](#_bookmark36) and Section [4.2.1.2](#_bookmark39)]. No sex differences were observed in exposure on day 1. The elimination of PROJECT 11 was faster than expected for bivalent IgG4 molecules [Morell et al, 1976]. Because almost all animals tested positive for ADAs after PROJECT 11 administration, the pharmacokinetics of PROJECT 11 after repeated doses could not be evaluated appropriately.

The safety of PROJECT 11 has been evaluated in 1 pivotal GLP 4-week intravenous infusion repeat-dose toxicity study in cynomolgus monkeys, 1 tissue cross-reactivity study and 1 cytokine release assay. Additionally, the tolerability endpoints from a non–GLP pharmacokinetic, pharmacodynamic and tolerability study conducted in cynomolgus monkeys were evaluated.

No mortality was noted in a non–GLP pharmacokinetic, pharmacodynamic and tolerability study in cynomolgus monkeys. One animal from the 30 mg/kg dose group exhibited mild tremors on day 15 (during infusion of the third dose) and showed signs of a mild infusion reaction (including reddening of the face with slight swelling around eyes) on day 22 (during and after infusion of the fourth dose). The animal was treated with diphenhydramine (5 mg/kg, intramuscularly) about 5 minutes prior to the end of the infusion. Some redness was still evident the following day, but had disappeared by day 24. After a single dose of PROJECT 11, all animals from the single dose groups (0.3 and 3 mg/kg) had developed positive ADA responses at day 29. In the repeat dose group (30 mg/kg), a positive ADA response was noted at day 15 for 2 out of the 3 animals, including the animal that showed a mild infusion reaction. Minor changes in some hematology and clinical chemistry parameters were observed, but the relationship to PROJECT 11 could not be determined [Section [4.3.2.1](#_bookmark50)].

In the pivotal GLP 4-week toxicity study of PROJECT 11 in cynomolgus monkeys, intravenous administration of PROJECT 11 resulted in approximately dose-proportional increases in Cmax and AUC values on day 1 of the study (PROJECT 11-[022) [Section 4.3.2.2](#_bookmark51)]. Exposure ratios, calculated based on day 1 animal exposures in the 4-week GLP toxicity study and the predicted steady-state human exposure of PROJECT 11 are shown in [[Table 8](#_bookmark61)]. The exposure ratios were calculated using day 1 data because most of the animals in the 4-week study developed ADA and subsequently showed reduced exposures and high variability after repeated dosing.

### Table 8 Exposure Ratios Based on Animal Exposure and Predicted Human Exposure of PROJECT 11

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Dose (mg/kg) ‡** | **Sex** | **Number of Animals** | **ADA Formation/Total Number of Animals at the Final Point**  **(Day 22 and/or 29)** | **Mean Toxicokinetic Values – Day 1** | | **Exposure Ratio† – Day 1** | |
| **Cmax (µg/mL)** | **AUC**  **168**  **(μg.day/mL)** | **Cmax**  **Base** | **AUC**  **Base** |
| 50 | M | 3 | 3/3 | 2430 | 4050 | 9.0 | 11.3 |
| F | 3 | 3/3 | 1230 | 2050 | 4.6 | 5.7 |
| 100 | M | 3 | 3/3 | 3340 | 6440 | 12.4 | 18.1 |
| F | 3 | 3/3 | 3620 | 5870 | 13.4 | 16.4 |
| 200 | M | 5 | 4/4 | 5650 | 8460 | 20.9 | 23.7 |
| F | 5 | 4/5 | 5380 | 9630 | 19.9 | 27.0 |

†: The exposure ratios were calculated by the following equations: Cmax Base = Cmax from animal/Cmax of human predicted value (270 μg/mL); AUCBase = AUC168 from animal x 3/AUCtau of human predicted value

(1070 μg.day/mL); human values are predicted from steady-state pharmacokinetic parameters with a dose level at 700 mg/man per 3 weeks.

‡: In males only on day 1 and day 15, the actual dose levels were 102, 165 and 147 mg/kg (day 1) and 105, 178

and 145 mg/kg (day 15) for the 50, 100 and 200 mg/kg dose groups, respectively. Source: PROJECT 11-022

The proposed starting dose (0.07 mg every 3 weeks) for the phase 1 study is estimated to give Cmax and AUC168 values, at steady-state that are approximately 202000-fold and 251000-fold lower, respectively, than the systemic exposures (day 1 Cmax and AUC168 x 3) in the cynomolgus monkey 4-week repeat-dose toxicity study at the NOAEL for non

ADA-mediated toxicity seen in the toxicity studies (200 mg/kg, males and females combined). The proposed highest clinical dose (1400 mg every 3 weeks) is estimated to have Cmax and AUC168 values that are approximately 10-fold and 13-fold lower compared with the NOAEL.

In the 4-week pivotal GLP study in cynomolgus monkeys, the first 3 doses of PROJECT 11 were well tolerated; however, severe ADA–mediated infusion reactions were observed in several animals on day 22 (during infusion of the fourth dose of PROJECT 11). These reactions were unrelated to dose levels. One male animal in the 50 mg/kg dose group died and 1 male animal in the 200 mg/kg dose group was euthanized due to moribundity. Infusion reactions in most of the animals were characterized by clinical signs such as loss of consciousness, pale skin, shallow or labored breathing, cold to touch, decreased heart rate and/or decreased activity. These findings were considered typical of an ADA-mediated hypersensitivity response. Females were pretreated with diphenhydramine prior to dosing on day 22 and the response was attenuated in all but 1 animal [Sectio[n 4.3.2.2](#_bookmark51)].

The infusion reactions were associated with transient elevations in cytokines (increased IL-6, IL-10 and TNFα levels), transient complement activation and stimulation of coagulation and/or fibrinolytic pathways. High ADA levels, associated with lower PROJECT 11 serum levels, were seen in animals with infusion reactions, suggesting these findings were

ADA-mediated [Sectio[n 4.3.2.2](#_bookmark51)]. The findings are similar to those described for other

human monoclonal antibodies that induce ADA–associated infusion reactions in cynomolgus monkeys [Mease et al, 2017; Leach et al, 2014; Rojko et al, 2014].

No toxicity attributed directly to treatment with PROJECT 11 was observed in the 4-week repeat- dose study (PROJECT 11-022) in cynomolgus monkeys and no target organs of toxicity were identified at an exposure ratio that ranged from approximately 24- to 27-fold higher than the estimated human systemic exposure at the projected human efficacious dose of

700 mg/human every 3 weeks. The NOAEL for non-ADA-immune mediated toxicity in this study was identified as 200 mg/kg in male and female animals. In females on day 1, this dose generated mean AUC168 value of 9630 μg∙day/mL and mean Cmax value of 5380 μg/mL. Males on day 1 were mis-dosed and instead of 200 mg/kg, a dose of 147 mg/kg was administered which generated a mean AUC168 value of 8460 μg∙day/mL and a mean Cmax value of 5650 μg/mL [[Section 4.2.1.2](#_bookmark39)].

In general, ADA-mediated infusion reactions following administration of a human antibody to monkeys are not considered predictive of a clinical immune response or immunogenicity in humans.

An in vitro tissue cross-reactivity study demonstrated that PROJECT 11 bound to cytoplasmic elements in select human and cynomolgus monkey epithelial tissues. This type of PROJECT 11 binding is unlikely to have biological consequences. The study also showed that the potential for off-target toxicity [is low [Section 4.3.7.1](#_bookmark57)].

In the cytokine release and proliferation assay in PBMCs, minor increases in the proinflammatory cytokines IL-6, IL-8 and TNFα were observed relative to the isotype control in the soluble format, with the highest concentration tested (100 µg/mL) generating increases

≤ 6.2-fold. No increases in cytokines relative to the isotype control were observed in the wet- coated format and no proliferation of PBMCs was observed with either format

[[Section 4.3.7.2](#_bookmark58)].

In conclusion, the data from the nonclinical studies suggest that PROJECT 11 may have therapeutic potential as a treatment of advanced solid tumors through T cell activation. From the toxicity point of view, based on the high levels of ADA detected in most animals administered PROJECT 11, the weight of evidence suggests that the observed infusion reactions and/or clinical signs were ADA-mediated. An ADA-mediated mechanism is also supported by the observed 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 cytokines (i.e., increased IL-6, IL-10 and/or TNF) that were seen in serum or blood from select animals with infusion reactions and/or clinical signs.

Similar findings in cynomolgus monkeys have been described in the literature for other human monoclonal antibodies [Mease et al, 2017; Leach et al, 2014; Rojko et al, 2014]. The observed infusion reactions in monkeys with PROJECT 11 were severe and careful management in the clinical study for any ADA-mediated reactions is warranted.

### Starting Dose Rationale for First-in-Human (FIH) Study

PROJECT 11 has been shown to stimulate the immune system in nonclinical pharmacology models. In addition, infusion reactions were observed following the fourth weekly dose in the GLP toxicity study in cynomolgus monkey[s [Section 4.3.2.2](#_bookmark51)]. Although these infusion reactions were considered to be ADA-mediated and similar immunogenicity may not necessarily occur in humans, caution should be exercised when administering PROJECT 11 in the FIH study. Therefore, the starting dose for PROJECT 11 was selected using a minimum anticipated biological effect level (MABEL) approach, and was chosen as 0.07 mg based on the average EC20 from 3 in vitro functional assays (IL-2 production [activated human

T-blasts], IL-8 production [engineered HT-1080 cells] and NF-κB promoter activation [engineered Jurkat cells]). PROJECT 11 is expected to reach < 10% RO of activated human primary cells at this dose level. The predicted steady state Cmax following the administration of 0.07 mg PROJECT 11 is anticipated to be 366-fold less than the lowest observed effect level of 10 μg/mL observed in the in vitro cytokine release assay [[Section 4.3.7.2](#_bookmark58)] and approximately 202000-fold lower than the mean Cmax observed in the 200 mg/kg dosing group (NOAEL) in the GLP toxicity study. Based on these data, the PROJECT 11 starting dose of 0.07 mg is expected to have minimal pharmacological effect.

### Conclusion

Based on the currently available safety data, it was concluded that there were no identified toxicological findings that would preclude initiation of clinical development of PROJECT 11. Monitoring of appropriate parameters in clinical studies should be considered [Section [6.2.5](#_bookmark72)].

### List of References

Brüggemann M, Williams GT, Bindon CI, Clark MR, Walker MR, Jefferis R, et al. Comparison of the effector functions of human immunoglobulins using a matched set of chimeric antibodies. J Exp Med. 1987;166(5):1351-61.

Chattopadhyay K, Ramagopal UA, Brenowitz M, Nathenson SG, Almo SC. Evolution of GITRL immune function: murine GITRL exhibits unique structural and biochemical properties within the TNF superfamily. Proc Natl Acad Sci USA. 2008;105:635-40.

Chattopadhyay K, Ramagopal UA, Mukhopadhaya A, Malashkevich VN, DiLorenzo TP, Brenowitz M, et al. Assembly and structural properties of glucocorticoid-induced TNF receptor ligand: implications for function. Proc Natl Acad Sci USA. 2007;104:19452-7.

Cohen AD, Schaer DA, Liu C, Li Y, Hirschhorn-Cymmerman D, Kim SC, et al. Agonist anti-GITR monoclonal antibody induces melanoma tumor immunity in mice by altering regulatory T cell stability and intra-tumor accumulation. PLoS One. 2010;5:e10436.

Hall W, Price-Schiavi S, Wicks J, Rojko JL. Tissue cross-reactivity studies for monoclonal antibodies: predictive value and use for selection of relevant animal species for toxicity testing. In: Cavagnaro JA, editor. Preclinical Safety Evaluation of Biopharmaceuticals: A

Science-based Approach to Facilitating Clinical Trials. Wiley-Interscience. 2008;208-40.

Jefferis R. Isotype and glycoform selection for antibody therapeutics. Arch Biochem Biophys.

2012;526(2):159-66.

Keizer RJ, Huitema ADR, Schellens JHM, Beijnen JH. Clinical pharmacokinetics of therapeutic monoclonal antibodies. Clin Pharmacokinet. 2010;49:493-507.

Kim IK, Kim BS, Koh CH, Seok JW, Park JS, Shin KS, et al. Glucocorticoid-induced tumor necrosis factor receptor-related protein co-stimulation facilitates tumor regression by inducing IL-9- producing helper T cells. Nat Med. 2015;21:1010-7.

Ko K, Yamazaki S, Nakamura K, Nishioka T, Hirota K, Yamaguchi T, et al. Treatment of advanced tumors with agonistic anti-GITR mAb and its effects on tumor-infiltrating Foxp3+CD25+CD4+ regulatory T cells. J Exp Med. 2005;202:885-91.

Leach MW, Halpern WG, Johnson CW, Rojko JL, MacLachlan TK, Chan CM, et al. Use of tissue cross-reactivity studies in the development of antibody-based biopharmaceuticals: history, experience, methodology, and future directions. Toxicol Pathol. 2010;38:1138-66.

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

Mease KM, Kimzey AL, Lansita JA. Biomarkers for nonclinical infusion reactions in marketed biotherapeutics and considerations for study design. Curr Opin Toxicol. 2017;4:1-15.

Morell A, Skvaril F, Barandun S. Serum concentrations of IgG subclasses. In: Bach FH, Good RA, eds. Clinical Immunobiology. Vol 3. New York: Academic Press. 1976:37-56.

Nishikawa H, Kato T, Hirayama M, Orito Y, Sato E, Harada N, et al. Regulatory T cell-resistant CD8+ T cells induced by glucocorticoid-induced tumor necrosis factor receptor signaling. Cancer Res. 2008;68:5948-54.

Ramirez-Montagut T, Chow A, Hirschhorn-Cymerman D, Terwey TH, Kochman AA, Lu S, et al.

Glucocorticoid-induced TNF receptor family related gene activation overcomes tolerance/ignorance to melanoma differentiation antigens and enhances antitumor immunity. J Immunol. 2006;176:6434-42.

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;42:725-64.

Zhou P, L'italien L, Hodges D, Schebye XM. Pivotal roles of CD4+ effector T cells in mediating agonistic anti-GITR mAb-induced-immune activation and tumor immunity in CT26 tumors. J Immunol. 2007;179:7365-75.

Zhou Z, Song X, Berezov A, Zhang G, Li Y, Zhang H, et al. Human glucocorticoid-induced TNF receptor ligand regulates its signaling activity through multiple oligomerization states. Proc Natl Acad Sci USA. 2008a;105:5465-70.

Zhou Z, Tone Y, Song X, Furuuchi K, Lear JD, Waldmann H, et al. Structural basis for ligand- mediated mouse GITR activation. Proc Natl Acad Sci USA. 2008b;105:641-5.