### Safety Pharmacology

Safety pharmacology parameters were measured in the 5-week intravenous repeat-dose toxicity study in cynomolgus monkeys (Study Project 13-TX-0002/PROJECT 13-[018) [Section 4.3.2](#_bookmark39)]. PROJECT 13 at doses of ≤ 200 mg/kg per week had no discernible effect on the assessed safety pharmacology parameters (neurological evaluation, electrocardiogram and respiratory function).

### Pharmacodynamic Drug Interactions

No pharmacodynamic drug interaction studies of PROJECT 13 have been conducted to date.

## Toxicology

PROJECT 13 has been assessed in 1 pivotal repeat-dose toxicity study (Project 13-TX-0002/ PROJECT 13-018), 1 non-GLP and 1 GLP tissue cross-reactivity study (Project 13-TX-0004/

PROJECT 13-019 and Project 13-TX-0001/PROJECT 13-020, respectively) and 1 cytokine release assay (Project 13-TX-0003/PROJECT 13-044).

### Single-dose Toxicity

No single dose toxicity study of PROJECT 13 has been conducted. A single dose pharmacokinetic/pharmacodynamic study was conducted in the cynomolgus monkey with tolerability endpoints of mortality, clinical observations, body weights and clinical pathology (Study Project 13-ME-9001/PROJECT 13-021).

In this study, male cynomolgus monkeys were given a single intravenous dose of PROJECT 13 of 0.3, 3.0 and 30 mg/kg (60 min infusion). Animals were administered PROJECT 13 on day 1 and observed for 42 days (1008 h). All animals in this study survived to termination. There were no drug-related effects on clinical observations, body weight or clinical pathology (clinical chemistry, hematology and urinalysis).

### Repeat-dose Toxicity

A tabulated summary of the results of the repeat-dose toxicity study

(Study Project 13-TX-0002/PROJECT 13-018) is presented in [End-of-Text Table 3.2]. The safety of PROJECT 13 was evaluated following weekly intravenous administration (0, 50, 100 and 200 mg/kg) to cynomolgus monkeys (3 males and 3 females per group) for 5 weeks. The animals were sacrificed 24 hours after the last (5th) dose. In addition, 2 males and 2 females were added to the control and 200 mg/kg group to assess the reversibility of toxicity finding following a 4-week recovery period. Local irritation was also evaluated.

No animals died or were euthanized due to moribundity. There were no test article-related changes noted for any of the parameters measured (clinical signs, general behavior and neurobehavioral function, body weight, food consumption, ophthalmology, electrocardiography, respiration rate, urinalysis, hematology, blood chemistry, immunophenotyping, cytokine analysis, organ weights, gross pathology or histopathology).

Systemic exposure, as defined by Cmax and AUC168h), increased with dose in a dose proportional manner. No striking sex differences were observed in exposure. ADA was detected in 2 female animals in the 50 mg/kg group during the dosing period. No ADA was detected in the other animals administered PROJECT 13.

In conclusion, weekly intravenous administration of PROJECT 13 over 5 weeks was well tolerated in both male and female monkeys at doses of ≤ 200 mg/kg. There were no treatment-related changes in the parameters evaluated. Based on these results, the NOAEL was considered to be 200 mg/kg (Cmax 7365 µg/mL and AUC168h 786000 µg∙h/mL, sex combined).

### Genotoxicity

The genotoxicity studies routinely conducted for pharmaceuticals are not applicable to biotechnology-derived pharmaceuticals (ICH S6 and S9).

### Carcinogenicity

No carcinogenicity studies with PROJECT 13 have been conducted and are not considered necessary for anti-cancer therapies (ICH S9).

### Reproductive and Developmental Toxicity

No reproductive and development toxicity studies of PROJECT 13 have been conducted to date.

### Local Tolerance

Local irritation was evaluated as part of the 5-week repeated intravenous dose toxicity study in cynomolgus monkeys (Study Project 13-TX-0002/PROJECT 13-[018) [Section 4.3.2](#_bookmark39)]. The gross and microscopic findings were considered incidental, as they are commonly observed in cynomolgus monkeys, following intravenous infusion.

### Other Toxicity Studies

### Tissue Cross-reactivity

A non-GLP tissue cross-reactivity study was performed in limited monkey and human tissues (Study Project 13-TX-0004/ PROJECT 13-019). A GLP tissue cross-reactivity study confirmed the results of this non-GLP study (Study Project 13-TX-0001/PROJECT 13-020). PROJECT 13 was applied to cryosections of 36 different normal human tissues (3 donors per tissue, where available) and cynomolgus monkey tissues (2 donors per tissue) at 2 concentrations (2 and 0.5 µg/mL). In addition, the test article was substituted with an isotype control (monoclonal human IgG4 with hinge stabilizing mutation). Other controls were produced by omission of the test or control articles from the assay (assay control).

PROJECT 13 stained the membrane and cytoplasm of mononuclear cells in human and cynomolgus monkey lymphoid tissues and select nonlymphoid tissues. This staining represented expected reactivity of the test article as TIGIT is reported to be expressed by mononuclear cell types, such as T cells and NK cells [Johnston et al, 2014; Levin et al, 2011; Stanietsky et al, 2009; Yu et al, 2009].

PROJECT 13 also produced cytoplasmic staining of select epithelia, retinal cells in the eye, glial cell processes in the brain, ovarian granulosa cells, and testicular interstitial cells in human tissues, as well as renal tubular epithelium and ovarian theca cells in cynomolgus monkey tissues. No literature describing the expression of TIGIT by the other cell types stained with PROJECT 13 in the current study was found; thus, the staining of these tissue elements might

represent either previously unreported sites of TIGIT expression or unexpected tissue cross-reactivity of the test article. However, this unexpected staining was cytoplasmic in

nature and monoclonal antibody binding to cytoplasmic sites generally is considered of little to no toxicologic significance [Hall et al, 2008; Leach et al, 2010]. Tissue cross-reactivity showed comparable tissue distribution of PROJECT 13 in both humans and cynomolgus monkeys supporting the position that the cynomolgus monkey is a relevant species for use in safety assessments, and suggesting the potential for off target binding is low.

### Cytokine Release and Proliferation

The in vitro effects of PROJECT 13 on human PBMC cytokine release and proliferation was assessed in soluble and wet-coated immobilized assay formats following a 46 ± 2 h period (Study Project 13-TX-0003/PROJECT 13-044). Samples from 10 healthy human donors were collected. Four concentrations of PROJECT 13 were used for the human PBMC cytokine release and proliferation assays. Note that the 4 PROJECT 13 concentrations tested were equivalent in terms of µg/well, between the soluble (0.3, 3.0, 30, and 300 µg/mL) and the wet-coated immobilized (0.06, 0.6, 6.0, and 60 µg/well) stimulation assay formats. For each assay, a negative control, an isotype control, and an anti-human CD3 positive control (and phytohemagglutinin for soluble format) were included.

Cytokine levels were measured using a Luminex multiplex method for IL-1β, IL-2, IL-6, IL-10, IL-12(p70), IFNγ, TNFα and G-CSF and a singleplex method for IL-8.

In summary, in vitro stimulation of human PBMCs with PROJECT 13 in the soluble format (0.3, 3.0, 30 and 300 µg/mL) and the wet-coated immobilized format (0.06, 0.6, 6.0 and

60 µg/well) did not induce the secretion of IL-1β, IL-2, IL-10, IL-12(p70), IFNγ or G-CSF.

Stimulation with PROJECT 13 at 30 and 300 µg/mL in the soluble format resulted in some increases in IL-6, IL-8 and TNFα (1.1- to 6.3-fold compared to negative and isotype control), while stimulation at 6.0 and 60 µg/well resulted in an increase in TNFα levels in the

wet-coated format. These changes were not dose-dependent. The assay positive control (anti-CD3 antibody, 10 µg/mL) resulted in increases of > 21-fold for the aforementioned 3 cytokines demonstrating that the PBMCs used in the assay had the potential to release much higher levels of cytokines, when strongly stimulated, than the levels of cytokine released after treatment with PROJECT 13.

In vitro stimulation of human PBMCs for 3 days with PROJECT 13 in soluble format (0.3, 3.0, 30 and 300 µg/mL) and in wet-coated format (0.06, 0.6, 6.0 and 60 µg/well) did not induce proliferation in any of the 10 donors tested.

## Integrated Nonclinical Overview and Conclusion: Potential Clinical Relevance

### Rationale for Species Selection

Binding studies were conducted to assess if PROJECT 13 recognizes rodent andcynomolgus monkey TIGIT in order to determine the appropriate species for safety studies. Results of these studies indicate that there is low binding of PROJECT 13 to mouse TIGIT in vitro and no

binding to rat TIGIT, while binding to cynomolgus monkey TIGIT was similar to that of human TIGIT. Therefore, cynomolgus monkey was chosen as the appropriate species for safety evaluation of PROJECT 13.

### Summary of Nonclinical Data Package

*Pharmacology*

PROJECT 13 is a fully human monoclonal IgG4 antibody that binds to TIGIT with high affinity to block interaction with its ligands. PROJECT 13 contains the S228P hinge modification to ablate IgG4 arm exchange and is expressed and purified from Chinese hamster ovary cells.

PROJECT 13 has a high affinity for recombinant human and cynomolgus monkey TIGIT. PROJECT 13 induced IL-2 production in human TIGIT Jurkat/anti-CD3 antibody HT-1080 co-culture assay. PROJECT 13 also increased IFN-γ production in sub-optimally stimulated PBMCs as well as in CD4+ T cells isolated from human PBMCs. PROJECT 13 enhanced the increase in IL-2, IFN-γ and TNF in a CMV-specific T cell recall response assay. The

combination of PROJECT 13 with an anti-PD-1 antibody enhanced the TNF induction observed with either PROJECT 13 or anti-PD-1 antibody alone. A surrogate anti-TIGIT antibody (SEC1) demonstrated anti-tumor activity in 2 syngeneic mouse tumor models, either as a single agent or in combination with an anti-PD-1 antibody.

*Pharmacokinetics*

PROJECT 13 exhibited non-linear behavior after single-doses of 0.3 to 30 mg/kg, while exhibiting approximately linear behavior after repeat-doses of 50 to 200 mg/kg. The mean serum t1/2 in the recovery period after repeated-dosing at 200 mg/kg was 343 h. No striking sex differences were observed in exposure. The exposure of PROJECT 13 on day 22 and day 29 (in the recovery period) was higher when compared to day 1.

*Toxicology*

PROJECT 13 at a dose of ≤ 200 mg/kg, which is at least 34.4-fold higher than the projected human exposure at the predicted clinically efficacious dose for in cynomolgus monkeys, did not result in any discernible toxicity.

Human PBMCs stimulated in vitro at clinically relevant concentrations of PROJECT 13 showed increases in IL-6, IL-8 and TNFα compared to negative and isotype controls in unstimulated human PBMCs in vitro at 30 and 300 μg/mL; these increases were not dose-dependent and were of lower magnitude than that observed with the positive control (1.1- to 6.3-fold vs

> 21-fold). Data in the literature, as well as internal data (Study Project 13-PH-9004/

PROJECT 13-005) suggest TIGIT antagonism can result in increases in some cytokines, although in general these effects are weak in the absence of TCR activation, consistent with its function as a coinhibitory checkpoint molecule. Since the ability to define the clinical risk of cytokine release syndrome (CRS) from in vitro cytokine release assays is limited [Finco et al, 2014], it cannot be ruled out that the observed increases may reflect the possibility for cytokine release, and cytokine release syndrome should be considered a potential risk as it is for any monoclonal antibody [Bugelski et al, 2009].

In conclusion, the nonclinical pharmacology studies and safety package support the investigation of PROJECT 13 in patients with advanced malignancies.

### Exposure Ratios of PROJECT 13 Based on Cynomolgus Monkeys

The mean Cmax and AUC following the first and last dose and exposure ratio to the projected human exposure are shown in [[Table 6](#_bookmark51)].

### Table 6 Exposure Ratios Based on Animal Cmax/AUC and Predicted Human Cmax/AUC of PROJECT 13

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study Type (Study No.)** | **Sex/No. of Animals** | **Dose† (mg/kg)** | **Cmax (µg/mL)** | | | | **AUC168h (µg∙h/mL)** | | | |
| **First Dose** | **Last Dose§** | **Exposure Ratios‡** | | **First Dose** | **Last Dose§** | **Exposure Ratios‡** | |
| **First** | **Last** | **First** | **Last** |
| Monkey/5-week, iv  (Project 13-TX-0002) | M/3 | 50 | 1240 | 1500 | 3.7 | 4.5 | 95600 | 139000 | 4.5 | 6.6 |
| F/3 | 50 | 1320 | 2250 | 4.0 | 6.8 | 97800 | 217000 | 4.6 | 10.2 |
| M/3 | 100 | 2150 | 3100 | 6.5 | 9.4 | 167000 | 356000 | 7.9 | 16.8 |
| F/3 | 100 | 2240 | 3920 | 6.8 | 11.8 | 177000 | 459000 | 8.3 | 21.7 |
| M/5 or 2¶ | 200 | 4260 | 6450 | 12.9 | 19.5 | 359000 | 729000 | 16.9 | 34.4 |
| F/5 or 2¶ | 200 | 3850 | 8280 | 11.6 | 25.0 | 317000 | 843000 | 15.0 | 39.8 |

F: female; M: male; NA: not applicable; NOAEL: no-observed-adverse-effect level;

† The underlined dose represents the NOAEL.

‡ The exposure ratios were calculated as (AUC168h x 3 [or Cmax]) / (estimated human systemic exposure level at the efficacious dose [700 mg]). The estimated human systemic exposure level of Cmax or AUC21d was

331 µg/mL or 63600 µg·h/mL, respectively, at the estimated efficacious dose of 700 mg.

§ Day 22 for 50 and 100 mg/kg (n = 3) and day 29 for 200 mg/kg (n = 2)

¶ A total of 5 animals: 3 animals during the main part of the study and 2 animals during the recovery part of the study

Source: Study Project 13-ME-9002; Study Project 13-TX-0002/PROJECT 13-018

### Starting Dose Rationale

PROJECT 13 has been shown to stimulate the immune system in in vitro pharmacology models [[Section 4.1.1.1](#_bookmark8)]. The FDA recommends that a minimum anticipated biological effect level (MABEL) be considered for selection of a starting dose for biopharmaceuticals with immune agonistic properties (FDA S9). The PROJECT 13 FIH study uses a MABEL approach for starting dose selection.

MABEL calculations were performed for each of the following in vitro studies: receptor occupancy using human whole blood; sub-optimally stimulated human PBMCs; and

sub-optimally stimulated human CD4+ cells [[Table 7](#_bookmark53)]. MABEL was not calculated for the in

vitro study conducted in Jurkat cells, as these were engineered cells.

### Table 7 MABEL Calculations Based on In Vitro Pharmacology Studies

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Evaluation** | **PROJECT 13 Concentration (µg/mL)** | | | **Estimated PROJECT 13 Human Dose**  **(mg)** | | |
| **EC10** | **EC50** | **EC90** | **ED10** | **ED50** | **ED90** |
| Receptor occupancy in human whole blood† (n = 2 donors) | 0.0015 | 0.0081 | **0.046** | 0.0044 | 0.024 | **0.14** |
| Human PBMCs sub-optimal stimulation (n = 2 donors, TNF) | **1.8** | 2.5 | 4.1 | **5.4** | 7.5 | 12 |
| Human CD4+ sub-optimal stimulation (n = 3 donors, IFN-γ) | **0.11** | 0.15 | 0.25 | **0.34** | 0.46 | 0.74 |

EC: effective concentration; ED: effective dose; IFN: interferon; MABEL: minimum anticipated biological effect level; PBMC: peripheral blood mononuclear cell; TNF: tumor necrosis factor

†Average of EC values determined in CD4+, CD8+ and natural killer cell subsets in human whole blood MABEL Flat Dose (mg) = Concentration (µg/mL) \* V (L), assuming V = 3 L in 70 kg human

Concentrations in (µg/mL) were calculated based on those in (nmol/L) in Studies Project 13-PH-9013/PROJECT 13-007 and Project 13-PH-9004/PROJECT 13-005, using a nominal molecular weight for IgG of 150 kDa.

EC10, EC50 and EC90 for receptor occupancy: Concentrations to achieve 10%, 50% and 90% receptor occupancy, respectively.

EC10, EC50 and EC90 for PBMC/CD4+ stimulation: Concentrations to achieve 10%, 50% and 90% maximal effect, respectively.

ED10, ED50 and ED90: Effective dose (ED) in humans expected to achieve the concentration observed at EC10, EC50 and EC90, respectively.

Source: Studies Project 13-PH-9013/PROJECT 13-007 and Project 13-PH-9004/PROJECT 13-005

An PROJECT 13 starting dose of 0.5 mg, based on the MABEL approach, has an expected Cmax that is above the EC90 for receptor occupancy in human whole blood. However, the Cmax of PROJECT 13 at 0.5 mg is expected to be less than the EC10 (1.8 µg/mL) observed in human stimulated PBMCs and approximates the EC50 (0.15 µg/mL) observed in the most sensitive in vitro pharmacological model (CD4+ cell stimulation). Based on these data, an PROJECT 13 starting dose of 0.5 mg is anticipated to have minimal pharmacological effect.

The anti-mouse TIGIT surrogate antibody, SEC1, demonstrated anti-tumor activity as a single agent in the MC38 murine syngeneic tumor model at 106 μg/mL. The minimum efficacious exposure observed with SEC1 corresponds to a projected PROJECT 13 human dose of at least 700 mg, based on the pharmacokinetic simulation. Thus, the PROJECT 13 starting dose of 0.5 mg is 1400-fold lower than the estimated minimum efficacious dose in humans (700 mg) based on in vivo data.

### List of References

Bugelski PJ, Achuthanandam R, Capocasale RJ, Treacy G, Bouman-Thio E. Monoclonal antibody- induced cytokine-release syndrome. Expert Rev Clin Immunol. 2009;5:499-521.

Finco D, Grimaldi C, Fort M, Walker M, Kiessling A, Wolf B, et al. Cytokine release assays: current practices and future directions. Cytokine. 2014;66:143-55.

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. p. 208-40.

Johnston RJ, Comps-Agrar L, Hackney J, Yu X, Huseni M et al. The immunoreceptor TIGIT regulates antitumor and antiviral CD8+ T cell effector function. Cancer Cell. 2014;26:923-37.

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

Leach MW, Halpern WG, Johnson CW, Rojko JL, MacLanchlan TK 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.

Levin SD, Taft DW, Brandt CS, Bucher C, Howard ED et al. Vstm3 is a member of the CD28 family and an important modulator of T-cell function. Eur J Immunol. 2011;41:902-15.

Stanietsky N, Simic H, Arapovic J, Toporik A, Levy O et al. The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity. Proc Natl Acad Sci USA. 2009;106:17858-63.

Yu X, Harden K, Gonzalez LC, Francesco M, Chiang E et al. The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. Nat Immunol. 2009; 10:48-57.