### Safety Pharmacology

The cardiovascular, respiratory and neurological effects of PROJECT 18 were assessed in the single ascending dose study in cynomolgus monkeys (Study 28895). PROJECT 18 was administered by intravenous bolus injection to 3 male and 3 female cynomolgus monkeys at a dose of 10, 30 or 100 mg/kg. Each administration was separated by a 14-day interval.

No PROJECT 18-related effects on the cardiovascular, respiratory or central nervous system were observed [[Table 2](#_bookmark28)].

### Table 2 Overview of Nonclinical Safety Pharmacology Study

|  |  |  |  |
| --- | --- | --- | --- |
| **Type of Study** | **Dose (mg/kg)** | **Noteworthy Findings** | **GLP** |
| Cardiovascular, respiratory and CNS (SAD study in cynomolgus monkeys) | 10  30  100  (iv bolus) | **Parameters:**  Heart rate, ECG, blood pressure and respiratory- and CNS-related clinical signs **Outcome:**  No PROJECT 18-related findings | Yes† |

CNS: central nervous system; ECG: electrocardiogram; GLP: Good Laboratory Practice; SAD: single ascending dose

† Toxicokinetic analysis performed under non-GLP conditions. Source: Study 28895

### Pharmacodynamic Drug Interactions

No specific pharmacodynamic drug interaction studies with PROJECT 18 have been completed thus far.

## 

## Toxicology

The nonclinical toxicology testing battery for PROJECT 18 referred to existing regulatory guidelines: ICH S6 (R1) biotechnology-derived pharmaceuticals and ICH S9 anticancer pharmaceuticals. A list of the studies conducted is presented in [[Table 4](#_bookmark45)].

### Table 4 Overview of Nonclinical Toxicity Studies

|  |  |  |  |
| --- | --- | --- | --- |
| **Type of Study** | **Study Title** | **GLP** | **Study Number** |
| Single-dose | Pharmacokinetic study of PROJECT 18 after multiple intravenous injections in cynomolgus monkeys | Yes† | 28895 |
| Repeat-dose | PROJECT 18 28-days repeated dose study in mice‡ | No | GP\_P0081 |
| Tissue Cross- reactivity | Tissue cross reactivity study with five animal species | No | GP\_P0077 |
| Tissue Cross- reactivity | TCR-study of PROJECT 18 with a human tissue panel | No | GP\_P0082 |
| Tissue Cross- reactivity | A cross-reactivity study with human tissues selected according to FDA and EMA guidelines | Yes | 00468 |
| Cytokine Release Assay | Cytokine release assay of PROJECT 18 using human peripheral blood | No | Project 18-TX-1001 |
| Toxicokinetics | Preliminary single intravenous dose toxicokinetics study of PROJECT 18 in mice | No | Project 18-TX-1002 |
| Toxicokinetics | Preliminary single intravenous dose toxicokinetics study of PROJECT 18 in cynomolgus monkeys | No | Project 18-TX-1003 |

GLP: Good Laboratory Practice.

† Toxicokinetic analysis performed under non-GLP conditions.

‡ Study drug was administered on days 1, 8, 15, 22 and 29.

### Single-dose Toxicity

### Single Ascending Dose Study in Cynomolgus Monkeys (GLP)

A single ascending dose study of PROJECT 18 was performed in non-naïve cynomolgus monkeys (Study 28895) [[Table 5](#_bookmark48)]. PROJECT 18 in buffer (25 mmo/L histidine, 8% trehalose, 0.01% polysorbate 20, pH5.7) was administered by intravenous bolus injection to 3 males and 3 females at doses of 10, 30 or 100 mg/kg. A 14-day interval was placed between each administration. The animals were returned to the stock colony on day 60.

The following investigations were performed: local tolerance of injection sites, mortality, clinical signs, body weight, food and water consumption, ECG (including heart rate and blood pressure), hematology, clinical biochemistry, urinalysis, ophthalmology, auditory function and toxicokinetics. Postmortem examination was not conducted.

The study did not show any PROJECT 18-related findings at any dose levels.

### Table 5 Findings of Single Ascending Dose Study in Cynomolgus Monkeys (Study 28895)

|  |  |
| --- | --- |
| **Parameter** | **Findings** |
| Local tolerance | No PROJECT 18-related changes at any dose. |
| Mortality |
| Clinical signs |
| Body weight |
| Food and water consumption |
| Electrocardiography |
| Peripheral arterial systolic and diastolic blood pressure |
| Hematology |
| Clinical biochemistry |
| Urinalysis |
| Ophthalmology |
| Auditory function test† |
| Toxicokinetics† | Cmax (g/mL) at 10 mg/kg male: 339  female: 315  Cmax (g/mL) at 100 mg/kg male: 3356  female: 3180  AUC168 (g·h/mL) at 10 mg/kg male: 21851  female: 16924  AUC168 (g·h/mL) at 100 mg/kg male: 181500  female: 167306 |

† non-Good Laboratory Practice Source: Study 28895

### Repeat-dose Toxicity

### 29-Day, Repeat-dose Study in Mice (Non-GLP)

A 29-day repeat-dose toxicity study of PROJECT 18 was performed in mice (Study GP\_P0081). Vehicle (25 mmol/L histidine, 8% trehalose, 0.01% polysorbate 20, pH 5.7) or PROJECT 18 was administered once weekly by intravenous bolus injection to groups of 5 male and 5 female NMRI mice at a dose level of 400 mg/kg for 29 days; 5 applications in total. Necropsies were performed within 48 hours or 4 weeks after the last injection. A further 3 mice per sex were assigned to control and PROJECT 18 400 mg/kg to assess toxicokinetics.

The following investigations were performed: mortality, clinical signs, body weight, food and water consumption, microscopic examination and toxicokinetics.

The study did not show any PROJECT 18-related findings.

### Table 6 Findings of 29-Day Repeat-dose Toxicity Study in Mice (Study GP\_P0081)

|  |  |
| --- | --- |
| **Parameter** | **Findings** |
| Mortality | No PROJECT 18-related changes. |
| Clinical signs |
| Body weight |
| Drinking water consumption† |
| Microscopic examination |
| Toxicokinetics | Cmax (g/mL) of 400 mg/kg at fifth administration male: 10417  female: 9367 |

† Drinking water consumption was monitored every 3 to 4 days for each animal per cage; food consumption was not monitored during the study.

Source: Study GP\_P0081

### Genotoxicity

PROJECT 18 is an antibody. Therefore, PROJECT 18 would not react directly with DNA or other chromosomal material and no genotoxicity studies have been conducted or are planned, in accordance with ICH S6(R1).

### Carcinogenicity

No carcinogenicity studies are planned in accordance with ICH S9.

### Reproductive and Developmental Toxicity

No reproductive and developmental toxicity studies have been conducted in accordance with ICH S9.

### Local Tolerance

No dedicated local tolerance studies have been conducted. In the single ascending dose study in cynomolgus monkeys (Study 28895), no PROJECT 18-related local intolerance reactions were noted during daily inspections of the injection sites.

### Other Toxicity Studies

### Tissue Cross-reactivity

Cross-reactivity of PROJECT 18 was studied in 5 animal species: cynomolgus monkey, Himalayan rabbit, guinea pig, rat and NMRI mouse tissues (Study GP\_P0077). No specific staining was noted in monkey, guinea pig or rabbit tissues. In the rat and mouse tissues, weak to moderate staining was noted in placenta [[Table 7](#_bookmark58)].

Cross-reactivity of PROJECT 18 was also studied in human tissues (Studies GP\_P0082 and 00468) [[Table 7](#_bookmark58)]. In a preliminary non-GLP study (Study GP\_P0082), PROJECT 18 bound to testicular and ovarian cancer tissues. In the definitive GLP study, the tissue cross-reactivity pattern of PROJECT 18 on a panel of normal human tissues was studied (Study 00468). The results showed that PROJECT 18 did not bind to any normal adult human tissues except for intercalated duct cells of the pancreas and blood platelets. Immunohistochemistry with PROJECT 18 resulted in specific membrane binding in a few cells for a few intercalated ducts of

the pancreas in 1 donor. This specific binding was not recorded in the other 2 donors. Specific binding with PROJECT 18 was also noted in blood platelets from all 3 donors. Since platelets are small cells, it was difficult to determine if this binding was cytoplasmic or membrane.

### Table 7 Overview of Tissue Cross-reactivity Studies

|  |  |  |  |
| --- | --- | --- | --- |
| **Antibody** | **Tissue Source** | **Noteworthy Findings** | **Study Number** |
| FITC conjugated-PROJECT 18 | Cynomolgus monkey | No staining on the tissues examined | GP\_P0077 |
| FITC conjugated-PROJECT 18 | Rabbit | No staining on the tissues examined | GP\_P0077 |
| FITC conjugated-PROJECT 18 | Guinea pig | No staining on the tissues examined | GP\_P0077 |
| FITC conjugated-PROJECT 18 | Rat | Weak to moderate membranous staining of placenta | GP\_P0077 |
| FITC conjugated-PROJECT 18 | Mouse | Weak to moderate membranous staining of placenta | GP\_P0077 |
| FITC conjugated-PROJECT 18 | Human | No staining on the adult normal tissues  Specific staining for testicular and ovarian cancer tissues | GP\_P0082 |
| FITC conjugated-PROJECT 18 | Human | Membrane binding in a few intercalated duct cells of pancreas for one donor out of three donors  Specific binding for blood platelets from all 3 donors  No staining for the other tissues | 00468 |

FITC: fluorescein isothiocyanate

### Cytokine Release Assay Using Human Peripheral Blood

The objective of the study (Study Project 18-TX-1001) was to evaluate the cytokine release potential of PROJECT 18 at higher doses of PROJECT 18 than those tested in the previous study (Study GP\_P0073).

Human peripheral blood samples obtained from 10 healthy volunteers were treated with PROJECT 18 at concentrations of 2, 20, 200 and 2000 µg/mL. After incubation for 24 hours, the concentrations of cytokines (IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ and TNF-α) in the supernatant were determined using the Luminex method.

The study indicated that PROJECT 18 had no discernable potential to induce cytokine release at concentrations up to 2000 µg/mL in human peripheral blood.

### Single Intravenous Dose Toxicokinetics Study in Mice

PROJECT 18 was administered once by intravenous bolus injection to groups of 15 male and 15 female ICR mice at dose levels of 50, 150 and 450 mg/kg (Study Project 18-TX-1002). Cmax

and AUC168 were calculated by sex for each PROJECT 18 dose level [[Table 8](#_bookmark61)]. During the 7-day observation period, no adverse clinical signs were recorded.

### Table 8 Toxicokinetics of a Single Intravenous Dose Study in Mice (Study Project 18-TX-1002)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Pharmacokinetic Parameter** | **PROJECT 18 Dose (mg/kg)** | | | | | |
| **50** | | **150** | | **450** | |
| **Sex** | **Male** | **Female** | **Male** | **Female** | **Male** | **Female** |
| Cmax (μg/mL) | 894 | 824 | 3290 | 3330 | 6680 | 7310 |
| AUC168 (μg·h/mL) | 19800 | 19200 | 58700 | 43100 | 125000 | 118000 |

Source: Study Project 18-TX-1002

### Single Intravenous Dose Toxicokinetics Study in Cynomolgus Monkeys

PROJECT 18 was administered once by intravenous bolus injection to groups of 1 male and

1 female cynomolgus monkeys at dose levels of 150 and 450 mg/kg (Study Project 18-TX-1003). Cmax and AUC168 were calculated by sex for each PROJECT 18 dose level [[Table 9](#_bookmark63)]. During the 7-day observation period, no adverse effects on clinical sign or body weight were recorded.

### Table 9 Toxicokinetics of a Single Intravenous Dose Study in Cynomolgus Monkeys (Study Project 18-TX-1003)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Pharmacokinetic Parameter** | **PROJECT 18 Dose (mg/kg)** | | | |
| **150** | | **450** | |
| **Sex** | **Male** | **Female** | **Male** | **Female** |
| Cmax (μg/mL) | 1930 | 3480 | 8800 | 8410 |
| AUC168 (μg·h/mL) | 138000 | 210000 | 484000 | 408000 |

Source: Study Project 18-TX-1003

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

CLDN6 is a cancer-specific antigen that is not expressed in normal adult human tissues. Thus, there exists no target organ for testing of on-target toxicity. Furthermore, as the relevant animal species for a nonclinical safety evaluation of PROJECT 18 should be one in which PROJECT 18 is pharmacologically active due to the expression of the epitope, there are no relevant animal species.

Nevertheless, cynomolgus monkeys were chosen as a nonrodent species to assess the toxicity of PROJECT 18 due to the following characteristics:

* The tissue distribution of CLDN6 in cynomolgus monkeys was similar to that in humans, i.e., no expression in any normal adult tissue.
* The human and cynomolgus monkey CLDN6 molecules show high overall sequence similarity.
* The binding affinity of PROJECT 18 to cynomolgus and human orthologs of CLDN6 is identical.
* PROJECT 18-induced immune effector functions (ADCC, CDC) are comparable between both species.

Limited toxicological endpoints were studied in mice as this species was considered less predictive for testing whether PROJECT 18 might affect human safety. Mice were considered less appropriate due to following characteristics:

* The mouse protein, Cldn6, is expressed in several tissues that show no CLDN6 expression in humans (pancreas, thyroid and placenta).
* Overall sequence homology between mouse and human is lower than between cynomolgus monkey and human.
* The binding affinity of PROJECT 18 to the mouse ortholog is 10-fold lower compared to human CLDN6.
* PROJECT 18-induced immune effector functions (ADCC, CDC) differ between mice and humans.

PROJECT 18 induced ADCC and CDC in testicular cancer cell lines expressing human CLDN6. PROJECT 18-directed cell killing was strictly dependent on the presence of CLDN6 and did not act via unspecific immune activation. In in vivo assessments, PROJECT 18 significantly inhibited testicular tumor growth in an immunocompromised xenografted mouse model. A combination of PROJECT 18 with platinum derivatives demonstrated a more potent antitumor effect than PROJECT 18 or chemotherapy alone.

Safety pharmacology and toxicity of PROJECT 18 were assessed in 1 single ascending dose study in cynomolgus monkeys (intravenous administration, up to 100 mg/kg), one 29-day repeated dose study in mice (weekly intravenous administration of PROJECT 18 at doses up to 400 mg/kg) and 2 single dose toxicokinetics studies (intravenous administration, up to 450 mg/kg in mice and cynomolgus monkeys). No target organs of toxicity were identified in these studies.

Two tissue cross-reactivity studies were conducted using human tissues. The results showed that PROJECT 18 did not bind to any normal adult human tissues except for intercalated duct cells of the pancreas (in 1 of 3 donors) and blood platelets (in all 3 donors).

In summary, the nonclinical data outlined above support the clinical development of PROJECT 18 in patients with germ cell tumors.

### List of References

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