**PROJECT 18 28-days Repeated Dose Study in Mice**

**11 SUMMARY**

The novel investigational medicinal product PROJECT 18 is a monoclonal antibody that targets claudin 6 (CLDN6). As a family member of tetraspan transmembrane proteins, CLDN6 is involved in the formation of tight junctions (Krause et al. 2008). The expression of CLDN6 is regulated during embryogenesis (Anderson et al. 2008; Turksen and Troy 2004; Ben-David et al. 2013), but in normal adult tissue CLDN6 expression is undetectable. In human cancers, CLDN6 is frequently expressed or aberrantly activated on the surface of tumor cells of the ovary, uterus, testis, lung, as well as in other cancers (Ganymed study number GP\_P0074). The restricted expression of CLDN6 in human cancer tissue precludes the use of an appropriate relevant species to test on-target effects in safety pharmacology studies in normal tissue (Scientific Advice at the Paul-Ehrlich-Institute, November 02, 2011).

A 28-day repeated dose study using high doses of PROJECT 18 in NMRI mice was conducted to assess potential drug-related adverse effects. The following points regarding CLDN6 expression, structure, and function were taken into consideration:

* + 1. Differences in mouse and human Cldn6 distribution in normal tissues (Ganymed study number GP\_P0073).
    2. Differences in mouse and human CLDN6 protein structure: variation of one amino acid in the first extracellular loop; variation of 3 amino acids in the second extracellular loop resulting in ~10-fold lower binding affinity of PROJECT 18 to murine Cldn6 compared to human CLDN6 (Ganymed study number GP\_P0073).
    3. PROJECT 18-induced immune effector functions on CLDN6-positive cells differ between human and mouse because of species-specific differences of Fc-signaling (Ganymed study number GP\_P0073).

In the 28-day repeated dose study, mice were given 5-weekly bolus i.v. injections of 400 mg/kg b.w. PROJECT 18. PROJECT 18 was administered to mice using the human equivalent dose of 1200 mg/m2, which was planned to determine the maximum tolerated dose in a first-in-human phase I/II study (ClinicalTrials.gov Identifier: NCT02054351). In the vehicle control groups, animals received drug substance buffer only. Pharmacokinetic properties of PROJECT 18 were analyzed in 4 treatment groups of 10 animals (5 male and 5 female) per group, and in two satellite groups with 6 animals (3 male and 3 female) per group. Acute toxicity was assessed after 28 days in animals treated with vehicle or PROJECT 18, and in a recovery group in animals following a 4-week recovery period animals were further analyzed. Mice received PROJECT 18 once per week on test days 1, 8, 15, 22, and 29. The dose of PROJECT 18 was adjusted to the body weight of individual animals. Within 48 hours or 4 weeks after the last injection animals were euthanized.

This study was performed under non-GLP conditions, however GLP guidelines were followed.

The study demonstrates good tolerability in mice using 5-weekly doses of 400 mg/kg b.w. PROJECT 18 bolus i.v. injections. During the course of the study none of the animals died prematurely, all animals showed normal behavior, and no apparent signs of clinical toxicity were observed. Additionally, animal body weight and drinking water consumption was not affected during course of the test item treatment. Blood samples were taken from all animals to determine hematological and clinical biochemistry parameters. Poor sampling quality (most likely due to hemolysis) resulted in large measurement variations between groups, which prevented clear interpretations of the data. Following the final dose of the test item, moderate accumulation was observed in both male and female mice. Four weeks after treatment, the recovery phase, low levels of PROJECT 18 in the serum resumed in male and female mice. Signs of systemic or organ-specific toxicity were not evident throughout the study. *Postmortem* analysis revealed an absence of test item-related morphological or histological abnormalities.