HER2 positivity is an important predictive factor for treatment with anti-HER2 agents in several cancers. However, currently available monoclonal antibody and tyrosine kinase inhibitor drugs rarely achieve full disease control. We have developed a new HER2-targeting molecule with a unique proxapatic mode of action that may provide additional benefit to patients. The DARPin® MP0274 localizes to HER2-expressing tumors and also to human serum albumin for half-life extension. As previously shown, in vivo, MP0274 induces apoptosis and inhibits proliferation of cells expressing HER3 (HCC1, HCC2), and HER3 and potentially inhibits HER2/HER3 downstream signaling. To support clinical development of MP0274, we tested the pharma of MP0274 in several HER2-expressing patient-derived xenograft (PDx) models and investigated tumor localization. In addition, pharmacokinetics (PK) and analysis was performed in cynomolgous monkeys. DARPin® molecules (designed arylamid repeat proteins) are small genetically engineered proteins that bind specifically to targets with very high affinity and which can be combined in a modular fashion (cartoon shown below with target binding sites in color).

### Methods

**Antitumor activity of MP0274.** MP0274 was tested in breast and gastric cancer models at preclinical level, and the results are consistent with standard of care therapies. Briefly, tumor fragments were obtained from xenografts in serial passage in nude mice. After removal from donor mice, tumors were cut into fragments (4-5 mm) and placed in PBS until subject-specific implantation. Mice were randomized to groups in which tumors reached a volume of approximately 100 - 120 mm3. The day of randomization and treatment initiation was designated as day 0 in each experiment. MP0274 was dosed at 60 mg/kg i.v. (x) i.v. (l), 100 mg/kg (P), trazolactam and penicillin as well as the combination of both at 10mg/kg 3x weekly. Tumor growth was monitored by two-dimensional measurement with a caliper on the day of randomization and then twice weekly. Relative volumes of individual tumors (individual DTx) for Days 2 were calculated using the individual tumor volume on Day 1 (T) by the individual volume of the same tumor on Day 2 (T+0) multiplied by 100. Tumor localization of MP0274 was studied using an Indium-111 (Ind-111) labeled version of MP0274 in a human ovarian adenocarcinoma (SKOV3) xenograft model with whole-body SPECT/CT imaging. Briefly, MP0274 was functionalized with the bifunctional chlauric (I-4-azidophenylboronic acid)-derivative Boc-propargylalcohol amine (s-SCN-Boc-DT) with an average chelator- MP0274 ratio of 1:8. Subsequently, QC releases confirmed the completeness of the reactions. Female C57BL/6 mice were inoculated subcutaneously in right shoulder with 1 × 10^6 SKOV3 cells. Tumors were allowed to grow until D0 (i.e. 10 mm in diameter) and mice were randomized. 4 mg/kg Ind-111 labelled MP0274 (6.5 MBq) was intravenously injected per mouse. Distribution of Ind-111 labelled MP0274-CT was monitored using Schlieren tomography targeting DARPin® fragments. The PK of MP0274 was studied in cynomolgous monkeys using MP0274 with an N-terminal His-tag. MP0274 is cross-reactive to the cynomolgous HER2 receptor. Single animals were intravenously infused once with 1 x 10 mg/kg over 30 minutes. Serum concentrations were then taken at multiple time points over a 10 day period. Serum concentrations were measured using a DARPin®-specific sandwich ELISA method. PK evaluation was performed using non-comparative analysis.

### Preclinical efficacy in patient-derived xenografts

**MP0274 is an efficient inhibitor of tumor growth in in vivo models.**

- **MP0274 inhibits tumor growth in Her2+ 3rd and 2nd PDx models of breast and gastric cancer**
- **MP0274 shows superior efficacy compared to trastuzumab and lapatinib**
- **MP0274 shows equivalent efficacy compared to a combination of trastuzumab and pertuzumab**
- **In contrast to the antibodies, MP0274 cannot induce ADCC**

**Pharmacokinetics of MP0274 in monkeys**

- **MP0274 PK behavior in monkeys is indicative for TMDD as expected for HER3 binding drugs**
- Non-dose-linear PK behavior in the dose range between 1 and 5 mg/kg indicates that MP0274 exhibits targeted-mediated disposition (TMDD) which is in line with a saturable binding process to HER3 receptors associated with a saturable elimination process such as receptor-mediated endocytosis. Approximately dose-proportional PK behavior at dose levels above 5 mg/kg with half-life values of 5 days.

### Pharmacokinetics of MP0274 in monkeys

**MP0274 PK behavior in monkeys is indicative for TMDD as expected for HER3 binding drugs**

- Non-dose-linear PK behavior in the dose range between 1 and 5 mg/kg indicates that MP0274 exhibits targeted-mediated disposition (TMDD) which is in line with a saturable binding process to HER3 receptors associated with a saturable elimination process such as receptor-mediated endocytosis.

- Approximately dose-proportional PK behavior at dose levels above 5 mg/kg with half-life values of 5 days.

**Planned phase I/a clinical trial design**

Plan for phase I design to be a 1-dose-in-human, single-arm, multi-center, multinational, open-label, repeated-dose escalation study in patients with HER2 positive solid cancers who have progressed after standard therapy for advanced disease. The primary objective is to evaluate the safety and tolerability of MP0274. Secondary and exploratory objectives include assessment of pharmacokinetics, antitumor activity and safety tolerability. The primary endpoint is incidence and severity of adverse events, secondary and exploratory endpoints include serum-concentration profiles of MP0274, incidence of anti-drug antibodies, clinical benefit and response rates, progression free and overall survival parameters.

**Major inclusion criteria** are recently documented HER2 positive solid tumor disease after standard therapies, HER2 assessed according published guidelines, measurable disease according to RECIST v1.1, adequate cardiac and other organ function, and additional established phase 1 trial eligibility.

**Trial design:** Dose escalation follows a standard 3+3 study design up to achieving Recommended Dose (RD). At RD additional patients will be included to further evaluate tumor activity in particular.

**The first regulatory submission is planned Q1 2017.**

### Conclusion

MP0274, with its unique proxapatic mode of action, demonstrates excellent activity in HER2-expressing PDx models, first localization to HER2-expressing tumors and a PK profile consistent with a binding and distribution to HER2 receptors. MP0274 was well tolerated in all studies. These results suggest that MP0274 has the potential to provide additional clinical benefit to patients with HER2-expressing tumors. A GLP repeated dose toxicity study in cynomolgous monkeys has been completed and a phase I clinical trial is in preparation.