Supplemental Information

Supplemental methods, TDM4373g:

Patient inclusion criteria: patients included in the Phase Ib portion of this study presented with histologically-documented locally advanced or metastatic HER2-positive breast cancer, determined as fluorescence in situ hybridization (FISH)-positive, chromogenic in situ hybridization (CISH)-positive or immunohistochemical (IHC) score of 3+ by local laboratory assessment and were required to have measurable disease. All patients were required to have received prior HER2-directed therapy (trastuzumab, lapatinib) but no prior T-DM1 or pertuzumab therapy. Patients were required to have adequate organ function (serum aspartate transaminase and alanine transaminase $\leq$ 2.5 x upper limit of normal (ULN) except in the case of liver metastases when both transaminases must have been $\leq$5 x ULN, total bilirubin $\leq$ 1.5 x ULN, creatinine $\leq$ 1.5 g/dL or creatinine clearance $\geq$ 50 mL/min, absolute neutrophil count $\geq$ 1500/mm^3, platelet count $\geq$ 100,000/mm^3, hemoglobin $\geq$ 9 g/dL) and cardiac ejection fraction > 55% as assessed by either echocardiogram (ECHO) or multiple gated acquisition (MUGA). Exclusion criteria included: peripheral neuropathy $\geq$ Grade 3, history of exposure to cumulative doses of anthracyclines > 500 mg/m^2, or a history of cardiac dysfunction.

Study assessment: Patients were monitored for safety and tolerability of treatment weekly during the first 3 cycles, every cycle thereafter, at the treatment termination visit, and during the follow-up period (30 days after ending study treatment). Adverse events were assessed according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 3.0. Radiographic assessment of disease status was conducted at screening and then every 2 cycles thereafter throughout the duration of the study based on modified RECIST (Response Evaluation Criteria in Solid Tumors). Patients evaluable for both safety and efficacy were those receiving any study treatment. Objective response rates (ORR) were determined using modified RECIST v1.0 criteria from complete or partial responses on 2 consecutive occasions at least 4 weeks apart, through at least 4 cycles or disease progression or death. Any

patient with a significant decline in ejection fraction or with symptomatic congestive heart failure was withdrawn from study treatment. Patients whose disease progressed or who died prior to the first tumor assessment were considered efficacy evaluable and treated as non-responders.

Supplemental Figure 1. Combination Index as a function of fractional effect (relative growth inhibition) for T-DM1 combined with pertuzumab in MDA-175 (top), Calu-3 (middle) and BT-474 (bottom) cells. Data points below 1.0 indicate synergy between T-DM1 and pertuzumab.

Supplemental Figure 2. Kaplan-Meier analysis of time-to-tumor volume doubling for MDA-MB-175-VII xenograft study from figure 1. Time-to-tumor progression definition: time-to-tumor volume doubling (2x day 0 volume) or survival time if no tumor volume progression or doubling. All treatment groups were significantly different from control group and the combination treatment group was significantly different from single agent treatment groups.

Supplemental Figure 3. Effects of NRG-1β on T-DM1-induced apoptosis in ZR-75-30 and HCC1954 breast cancer cells.

Supplemental Fig. 4. The presence of NRG-1β reduces the cytotoxic effects of free DM1, docetaxel and vinorelbine in BT-474 (left panel), but not KPL-4 (right panel) breast cancer cells, similar to observations with T-DM1.

Supplemental Figure 5. Kaplan-Meier analysis of time-to-tumor volume doubling. A. Calu-3 lung tumor xenograft study from figure 5. Single agent treatment groups, with the exception of 1 mg/kg T-DM1, were significantly different from the control group. Tumor growth reduction in all combination treatment groups was significantly different than the corresponding single agent treatment arms for each combination. B. KPL-4 breast cancer xenograft study from figure 5. All

combination groups showed statistically significant delays in time-to-tumor doubling compared to the corresponding single agent treatment arms.

Supplemental Figure 6. TDM4373g clinical study design. In this 3+3 study design, patients received standard dose pertuzumab (840 mg, cycle 1; 420 mg, cycle 2 and beyond). Cohort 1 patients received 3.0 mg/kg T-DM1 plus pertuzumab every 3 weeks; Cohort 2 patients received 3.6 mg/kg T-DM1 plus pertuzumab. Data from the expansion phase are not reported here.

Supplemental Table 1. TDM4373g Phase Ib Patient Demographics and Baseline Characteristics

Supplemental Table 2. Prior Therapies

Supplemental Table 3. TDM4373g Phase Ib Adverse Events, Any Grade, in at Least 2 Patients