Trastuzumab Emtansine: A Unique Antibody-Drug Conjugate in Development for Human Epidermal Growth Factor Receptor 2–Positive Cancer

Patricia M. LoRusso1, Denise Weiss1, Ellie Guardino2, Sandhya Girish2, and Mark X. Sliwkowski2

Abstract

Trastuzumab emtansine (T-DM1) is a human epidermal growth factor receptor (HER2)–targeted antibody-drug conjugate, composed of trastuzumab, a stable thioether linker, and the potent cytotoxic agent DM1 (derivative of maytansine), in phase III development for HER2-positive cancer. Extensive analysis of T-DM1 in preclinical studies has shown that T-DM1 combines the distinct mechanisms of action of both DM1 and trastuzumab, and has antitumor activity in trastuzumab- and lapatinib-refractory experimental models. Clinically, T-DM1 has a consistent pharmacokinetics profile and minimal systemic exposure to free DM1, with no evidence of DM1 accumulation following repeated T-DM1 doses. Although a few covariates were shown to affect interindividual variability in T-DM1 exposure and clearance in population-pharmacokinetics analyses, the magnitude of their effect on T-DM1 exposure was not clinically relevant. Phase I and phase II clinical trials of T-DM1 as a single agent and in combination with paclitaxel, docetaxel, and pertuzumab have shown clinical activity and a favorable safety profile in patients with HER2-positive metastatic breast cancer. Two randomized phase III trials of T-DM1 are recruiting patients: EMILIA (NCT00829166) is evaluating T-DM1 compared with lapatinib plus capecitabine, and MARIANNE (NCT01120184) is evaluating T-DM1 plus placebo versus T-DM1 plus pertuzumab versus trastuzumab plus a taxane. Additional combinations of T-DM1 (for example, with GDC-0941) and additional disease settings (early-stage HER2-positive breast cancer) are also under investigation. Data from the phase III trials and other studies of T-DM1–containing agents are eagerly awaited. Clin Cancer Res; 17(20); 6437–47. ©2011 AACR.

Introduction

Chemotherapies are limited by systemic toxicity and lack of tumor selectivity, and thus they have a narrow therapeutic index. Antibody-drug conjugates (ADCs) are a therapeutic class comprising a tumor antigen-specific targeting antibody linked to a cytotoxic drug. ADCs may improve the therapeutic index because they are designed to specifically deliver cytotoxic agents to tumor cells and limit collateral damage to normal cells. The concept of ADCs has existed for many years; however, it is only recently that advances in this technology have resulted in clinically useful therapeutic agents. A review of the key challenges in the development of these agents and ADCs currently in clinical development is included in this CCR Focus section (1). To date, the only ADC to have received approval from the U.S. Food and Drug Administration (FDA) is gemtuzumab ozogamicin (Mylotarg), which was approved for the treatment of relapsed CD33-positive acute myeloid leukemia in older patients. However, it was recently withdrawn from use because postmarketing studies showed a lack of clinical benefit [reviewed in this CCR Focus section by Ricart (2)]. A number of other ADCs, however, are currently in clinical development for hematological malignancies. These include antibodies conjugated to microtubule polymerization inhibitors (3, 4), DNA intercalaters (2), and protein synthesis inhibitors (i.e., protein toxins; ref. 5). Antibody-radiouclide conjugates have also been approved for the treatment of hematologic malignancies (6). Trastuzumab emtansine (T-DM1), a human epidermal growth factor receptor (HER2)–targeted ADC composed of the microtubule polymerization inhibitor DM1 (derivative of maytansine) linked to trastuzumab, is in phase III development for HER2-positive breast cancer. As such, it is the only ADC in late-stage clinical development for a solid tumor.

Breast cancer accounts for ~28% of all new cases of cancer in women (7), and 15% to 25% of these new cases contain gene amplifications or protein overexpression of HER2 (8–10). HER2-positive disease is an aggressive form of breast cancer that typically is associated with a higher risk of distant recurrence with a shorter time to relapse, lower disease-free and overall survival rates, and greater therapeutic resistance compared with HER2-normal disease (8–14). Despite treatment advances, including the humanized anti-HER2 antibody trastuzumab and the dual epidermal growth factor receptor (EGFR)/HER2 tyrosine kinase

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inhibitor lapatinib, HER2-positive breast cancer will eventually progress in most patients, highlighting the need for novel, alternative therapies. In addition, currently available HER2-targeted therapies are rarely given as monotherapy but are generally given in combination with other agents (e.g., chemotherapy or hormonal therapy). Because toxicities associated with chemotherapy can be a significant source of comorbidity for patients with cancer, ADCs are a promising therapeutic approach for this patient population.

Toxicity to normal cells can occur by both target-dependent and target-independent mechanisms. Perhaps the most important consideration for target-independent cytotoxicity in an ADC is the chemical nature of the linker moiety. ADCs containing maytansines were originally designed with linkers that contained disulfide bonds (15, 16). This strategy assumed that once the ADC engaged the cell surface receptor, the complex between the ADC and the receptor would be internalized and trafficked to an endocytic compartment that was sufficiently reducing to release the maytansine. Experimental data disproved this hypothesis when it was shown that the oxidizing potential of endosomes and lysosomes limits the intracellular cleavage of disulfide-containing ADCs (17). These and other observations regarding improved pharmacokinetics and tolerability guided the choice of incorporating a thioether linker containing a cyclohexane carboxylate spacer into the trastuzumab ADC (18). Additional studies indicated that once T-DM1 is internalized, proteolytic digestion of the conjugate occurs, releasing the active metabolite lysine-N\(^{-}\)-maleimidomethyl cyclohexane-1-carboxylate (MCC)-DM1. Because it is a zwitterion, lysine-N\(^{-}\)-MCC-DM1 does not readily cross the plasma membrane of neighboring normal cells. This likely contributes to the overall safety profile of T-DM1 (19).

The nonclinical activity of T-DM1 was initially assessed in experimental models that were refractory to trastuzumab or lapatinib (18, 20, 21), because trastuzumab and lapatinib are established for the treatment of HER2-positive metastatic breast cancer (MBC). To date, meaningful antitumor activity has been observed in all of these models. To gain further insight into these findings, we conducted studies to assess the activity of trastuzumab relative to T-DM1. Multiple lines of evidence, including the direct release of adenylate kinase, PARP cleavage, caspase 3/7 activation, and cell cycle analysis, indicate that T-DM1 induces a direct cytotoxic effect against cells that overexpress HER2 (18). The mechanisms of action for trastuzumab include inhibition of the HER3/phosphoinositide 3-kinases (PI3K)/AKT signaling pathway, inhibition of HER2 shedding, and Fcy receptor–mediated engagement of immune cells, which may result in antibody-dependent cellular cytotoxicity (22). Of importance, T-DM1 retains these same mechanisms of action of unconjugated trastuzumab (20).

**Clinical Efficacy of Single-Agent T-DM1**

T-DM1 was initially evaluated as a single agent in a dose escalation phase I trial in patients with HER2-positive MBC who previously received a trastuzumab-containing chemotherapy regimen. T-DM1 was given at various doses on a weekly (23) or every 3 weeks schedule (ref. 24; Table 1). The maximum tolerated dose (MTD) was 3.6 mg/kg every 3 weeks, based on the dose-limiting toxicity (DLT) of grade 4 thrombocytopenia at 4.8 mg/kg every 3 weeks. In a group of 15 patients receiving 3.6 mg/kg every 3 weeks, the clinical benefit rate (CBR; objective response rate [ORR] plus stable disease at 6 months) was 73% (ref. 24; Table 1). Interim results for patients receiving weekly T-DM1 showed 9 partial responses (PR; 8 were confirmed) in 15 patients evaluable for response (ORR 53%; ref. 23). On the basis of its clinical activity and dosing convenience, T-DM1 3.6 mg/kg every 3 weeks was selected for further clinical development.

Two large multicenter, single-arm, phase II studies evaluating single-agent T-DM1 3.6 mg/kg every 3 weeks in pretreated patients with locally assessed HER2-positive MBC following progression on previous chemotherapy and HER2-directed therapy have been completed (refs. 25, 26; Table 1). In the first study, the ORR by independent review was 25.9% [95% confidence interval (CI), 18.4–34.4%] and 37.5% by investigator assessment, including 4 complete responses (CR; see Table 1). The median progression-free survival (PFS) was 4.6 months (95% CI, 3.9–8.6 months; ref. 25). In the second study, patients had been previously treated with an anthracycline, a taxane, and capecitabine, as well as lapatinib and trastuzumab with a median of 8.5 agents (range: 5–19) in all settings and 7.0 agents (range: 3–17) for metastatic disease (26). An interim report indicated that the ORR was 34.5% (all PRs; 95% CI, 26.1–43.9%) and the CBR was 48.2% (95% CI, 38.8–57.9%) by independent review. The median PFS was 6.9 months (95% CI, 4.2–8.4 months; Table 1).

To examine the relationship between HER2-positive status and response to T-DM1 (Fig. 1) and identify associated biomarkers, LoRusso and colleagues (28) performed a retrospective analysis using archival tumor tissue from these 2 phase II studies. Confirmed HER2-positive status [immunohistochemistry (IHC) 3+ or fluorescence in situ hybridization (FISH)+ by central retesting] was associated with a higher ORR than HER2-normal status (TDM4258g: 33.8% in the 74 confirmed HER2-positive patients vs. 4.8% in the 21 HER2-normal patients; TDM4374g: 40.8% in the 76 confirmed HER2-positive patients vs. 20.0% in the 15 HER2-normal patients). Analysis by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) showed that levels of HER2 mRNA expression equal to or above the median were also associated with a higher ORR than levels below the median [TDM4258g: 36.0% (n = 25) vs. 28.0% (n = 25); TDM4374g: 50.0% (n = 26) vs. 33.3% (n = 19); Table 2]. These results support the specificity of the effect of T-DM1 on HER2-positive MBC. They further suggest that tumor response to T-DM1 may be dependent on HER2 quantity, even among tumors that are already deemed HER2-positive by standard methods. It is important to note, however, that these data are from exploratory
analyses in a small number of patients; additional studies are necessary to adequately test these hypotheses.

Patients with wild-type PI3K mutation status and normal PTEN expression appeared to achieve a better response in TDM4258g. This association, however, was not observed in patients in TDM4374g (see Table 2). Thus, although no consistent trend in T-DM1 activity was observed in patients with activating PI3K mutations and/or decreased PTEN expression, it should be noted that this analysis was limited because of the exclusive use of archival tissue from the patients’ initial diagnoses (28).

A randomized, open-label phase II study (TDM4450g; ref. 29) is investigating single-agent T-DM1 compared with trastuzumab plus docetaxel in the first-line treatment of HER2-positive recurrent, locally advanced breast cancer or MBC (ref. 27; Table 1). Enrollment was completed in December 2009, and safety and ORR data as of April 2, 2010, were included in an interim analysis. Thirteen patients in the T-DM1 arm (19.4%) and 18 patients in the trastuzumab-plus-docetaxel arm (25.7%) had previously received trastuzumab. The ORR by investigator assessment was 47.8% (n = 32; 95% CI, 35.4–60.3%) for T-DM1 and 41.4% (n = 29; 95% CI, 30.2–53.8%) for trastuzumab plus docetaxel. There were 3 CRs (4.5%) and 1 CR (1.4%), respectively. Final analysis of the primary endpoint, PFS, is eagerly awaited.

Clinical Safety of Single-Agent T-DM1

The most common adverse events (AE) of all grades for T-DM1 seen to date include fatigue (range: 37.5–65.2%), anemia (10.4–29.2%), nausea (25.0–50.9%), and hypokalemia (4.2–24.1%). Among these, the incidence of grade 3 or 4 AEs was < 5%, with the exception of grade 3 or 4 hypokalemia in one study (TDM4258g, 8.9%; refs. 24–27; Table 3). T-DM1 also had a favorable safety profile relative to standard-of-care treatment in the first-line setting (27), with fewer grade 3 or 4 AEs (37% with T-DM1 vs. 75% with trastuzumab plus docetaxel). In addition, many of the AEs associated with traditional chemotherapies (e.g., diarrhea, neutropenia, rash, neuropathy, and alopecia) were observed at much lower rates with T-DM1 treatment compared with trastuzumab plus docetaxel (see Table 3; ref. 27).

Thrombocytopenia was one of the most frequently reported grade 3 or 4 laboratory abnormalities across the phase II studies of T-DM1 (range: 7.3–8.0%; refs. 25–27). These reductions in platelet count were generally reversible.

<table>
<thead>
<tr>
<th>Trial and reference</th>
<th>Study design and T-DM1 dose</th>
<th>Study population</th>
<th>Patients, n ORR, %</th>
<th>CR, %</th>
<th>CBRc, %</th>
<th>Median DOR (months)</th>
<th>Median PFS (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDM3569g (24)</td>
<td>Phase I single arm; 0.3–4.8 mg/kg</td>
<td>Previously treated with chemotherapy and progressed on T</td>
<td>24</td>
<td>25.0b</td>
<td>0</td>
<td>73</td>
<td>NR</td>
</tr>
<tr>
<td>TDM4258g (25)</td>
<td>Phase II single arm; 3.6 mg/kg</td>
<td>Previously treated with chemotherapy and progressed on HER2-targeted therapy</td>
<td>112</td>
<td>37.5 (25.9)</td>
<td>3.6 (0)</td>
<td>NR</td>
<td>9.4 (6.2–NE)</td>
</tr>
<tr>
<td>TDM4374g (26)</td>
<td>Phase II single arm; 3.6 mg/kg</td>
<td>Previously treated with anthracycline, a taxane, and capecitabine, plus lapatinib and T for MBC</td>
<td>110</td>
<td>32.7 (34.5)</td>
<td>4.5 (0)</td>
<td>46.4 (48.2)</td>
<td>NR (7.2)</td>
</tr>
<tr>
<td>TDM4450g (27)</td>
<td>Phase II randomized; T-DM1 3.6 mg/kg vs. T + Dd</td>
<td>Recurrent, locally advanced breast cancer or MBC, with no prior chemotherapy for metastatic disease</td>
<td>T-DM1, n = 67</td>
<td>47.8</td>
<td>4.5</td>
<td>55.2</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T + D, n = 70</td>
<td>41.4</td>
<td>1.4</td>
<td>57.1</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

Data shown are by investigator assessment, with independent review results in parentheses (where available).

Abbreviations: CBR, clinical benefit rate; CR, complete response; D, docetaxel; DOR, duration of response; HER2, human epidermal growth factor receptor 2; MBC, metastatic breast cancer; NE, not estimable; NR, not reported; ORR, objective response rate; PFS, progression-free survival; T, trastuzumab; T-DM1, trastuzumab emtansine.

aEfficacy outcomes reported are for patients treated at the MTD (3.6 mg/kg every 3 weeks; n = 15).

bConfirmed ORR among patients with measurable disease who were treated at the MTD (n = 9) was 44%.

cDefined as CR, PR, or stable disease ≥ 6 months.

dTrastuzumab (8 mg/kg loading dose; 6 mg/kg every 3 weeks) + docetaxel (75 or 100 mg/m2 every 3 weeks).
Thrombocytopenia was observed as early as 1 day after T-DM1 treatment. In most patients, platelet counts reached a nadir by day 8 and recovered by day 18 (24, 30). This pattern persisted even after repeated dosing, and it appears to be distinguishable from immune-mediated thrombocytopenia (24, 30). Thrombocytopenia was not typically associated with clinically meaningful bleeding events. For example, in the first phase II study, 9 patients had grade 3 or 4 thrombocytopenia, but only 1 patient had a concurrent grade 3 bleeding event (i.e., epistaxis; Table 2).

Table 2. T-DM1 activity in efficacy-evaluable patients by HER2 qRT-PCR level, PI3K mutation status, and PTEN expression level (28)

<table>
<thead>
<tr>
<th></th>
<th>TDM4374g</th>
<th></th>
<th>TDM4258g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>ORR, % (95% CI)</td>
<td>n</td>
</tr>
<tr>
<td>HER2 qRT-PCR level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥Median&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26</td>
<td>50.0 (29.9–70.1)</td>
<td>25</td>
</tr>
<tr>
<td>&lt;Median</td>
<td>39</td>
<td>33.3 (19.7–50.0)</td>
<td>25</td>
</tr>
<tr>
<td>PI3K mutation&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>48</td>
<td>35.4 (22.2–50.0)</td>
<td>42</td>
</tr>
<tr>
<td>Mutant</td>
<td>11</td>
<td>36.4 (13.5–66.7)</td>
<td>9</td>
</tr>
<tr>
<td>PTEN by IHC&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>38</td>
<td>36.8 (22.6–53.5)</td>
<td>30</td>
</tr>
<tr>
<td>Decreased</td>
<td>3</td>
<td>33.3</td>
<td>6</td>
</tr>
</tbody>
</table>

<sup>a</sup>Median was based on TDM4258g data.
<sup>b</sup>FISH<sup>+</sup> and/or IHC3<sup>+</sup>.

Figure 1. Structure of T-DM1 and mechanisms of action. After T-DM1 binds HER2, the HER2/T-DM1 complex undergoes internalization, followed by lysosomal degradation. This process results in the intracellular release of DM1-containing catabolites that bind to tubulin and prevent microtubule polymerization as well as suppress microtubule dynamic instability. T-DM1 has also been shown to retain mechanisms of action of trastuzumab, including disruption of the HER3/PI3K/AKT signaling pathway and Fcγ receptor-mediated engagement of immune effector cells, which leads to antibody-dependent cellular cytotoxicity.
Cardiotoxicity is an infrequent AE linked to HER2-directed agents (31, 32). In the single-arm studies of single-agent T-DM1 reported to date, no dose-limiting cardiotoxicities [grade 3 left ventricular ejection fraction (LVEF) decline or symptomatic congestive heart failure] were observed (24–26). In the randomized comparative phase II study, T-DM1 did not increase the risk of cardiotoxicity relative to trastuzumab plus docetaxel. Absolute decreases in LVEF of 10% to 20% were observed in 7.8% (n = 5) of patients in the T-DM1 arm and 16.4% (n = 11) of patients in the trastuzumab-plus-docetaxel arm (27). However, in all T-DM1 studies, a baseline LVEF of ≥50% was required for study entry.

The potential of T-DM1 to prolong the QT interval was assessed in a dedicated multicenter, phase II study of patients with HER2-positive, recurrent, locally advanced breast cancer or MBC. Multiple analytes, including T-DM1 and DM1-containing catabolites, were monitored in this study. An early report of this study indicated that T-DM1 had a minimal effect on the QT interval that was below the threshold of safety concern (33). In addition, study TDM4874g (NCT01196052) is currently evaluating the cardiac safety of T-DM1 after the administration of doxorubicin plus cyclophosphamide (FEC) in patients with early-stage HER2-positive breast cancer (34). Increased serum concentrations of hepatic enzymes are laboratory abnormalities that have been associated with T-DM1 treatment. In phase I and phase II studies of single-agent T-DM1 in HER2-positive MBC, the overall incidence of grade 3 or 4 elevations of alkaline phosphatase, aspartate transaminase, or alanine transaminase ranged between 0% and 13.4% (24, 26, 27). One patient died of hepatic dysfunction (in TDM4374g; ref. 26), but the relation of the death to the administration of T-DM1 was unclear.

**Pharmacokinetic/Pharmacodynamic Profile of T-DM1**

The pharmacokinetics of T-DM1 has been assessed in nonclinical and clinical studies. Preliminary results showed that T-DM1 exhibits dose-proportional pharmacokinetics in non–trastuzumab-binding species (i.e., mice and rats; refs. 35, 36) and a dose-dependent decrease in clearance associated with increasing dose in trastuzumab-binding species (i.e., cynomolgus monkeys and humans; refs. 36, 37). Results from a preclinical absorption, distribution, metabolism, and excretion study of T-DM1 in rats suggest that T-DM1 nonspecifically distributes to

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**Table 3. Most common AEs and AEs of special interest reported in clinical trials of single-agent T-DM1 in HER2-positive MBC**

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>TDM3569g (24), n = 24a</th>
<th>TDM4258g (25), N = 112</th>
<th>TDM4374g (26), N = 110</th>
<th>TDM4450g (27)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 3/4 All grades</td>
<td>Grade 3/4 All grades</td>
<td>Grade 3/4 All grades</td>
<td>Grade 3/4 All grades</td>
</tr>
<tr>
<td>Thrombocytopenia 3 (12.5)</td>
<td>13 (54.2)</td>
<td>9 (8.0) NR</td>
<td>8 (7.3) 36 (32.7)</td>
<td>5 (7.5) 15 (22.4)</td>
</tr>
<tr>
<td>Increased hepatic enzymesb</td>
<td>0 10 (41.7)</td>
<td>n/a NR NR</td>
<td>6 (5.5) 56 (50.9)</td>
<td>9 (13.4) 27 (40.3)</td>
</tr>
<tr>
<td>Alopecia n/a NR n/a NR</td>
<td>n/a NR NR</td>
<td>n/a NR NR</td>
<td>1 (1.5) n/a 45 (66.2)</td>
<td></td>
</tr>
<tr>
<td>Neutropenia 0 1 (4.2) NR</td>
<td>0 2 (8.3) NR NR</td>
<td>0 20 (18.2) NR</td>
<td>4 (6.0) NR 12 (17.6)</td>
<td></td>
</tr>
<tr>
<td>Diarrhea NR 0 0 9 (25.9)</td>
<td>0 29 (25.9) NR</td>
<td>0 14 (12.7) NR</td>
<td>7 (10.4) NR 31 (45.6)</td>
<td></td>
</tr>
<tr>
<td>Neutropenia 0 1 (4.2) NR</td>
<td>0 1 (0.9) NR NR</td>
<td>NR NR NR</td>
<td>5 (7.5) NR 39 (57.4)</td>
<td></td>
</tr>
<tr>
<td>Rash NR 0 27 (24.1)</td>
<td>5 (4.2) 3 (2.7) 23 (20.5)</td>
<td>2 (1.8) 22 (20.0) NR</td>
<td>7 (10.4) NR 15 (22.1)</td>
<td></td>
</tr>
<tr>
<td>Vomiting 0 3 (12.5) 1 (0.9)</td>
<td>27 (24.1) 0 18 (16.4) NR</td>
<td>NR NR NR</td>
<td>3 (4.5) 31 (46.3) 3 (4.4) 29 (42.6)</td>
<td></td>
</tr>
<tr>
<td>Fatigue 0 9 (37.5) 5 (4.5)</td>
<td>73 (65.2) 5 (4.5) 68 (61.8)</td>
<td>5 (4.5) 68 (61.8)</td>
<td>3 (4.5) 31 (46.3) 3 (4.4) 29 (42.6)</td>
<td></td>
</tr>
<tr>
<td>Anemia 0 7 (29.2) 2 (2.7)</td>
<td>23 (20.5) 2 (1.8) 22 (20.0) NR</td>
<td>NR NR NR</td>
<td>7 (10.4) NR 15 (22.1)</td>
<td></td>
</tr>
<tr>
<td>Nausea 0 6 (25.0) 1 (0.9)</td>
<td>57 (50.9) 1 (0.9) 41 (37.3) NR</td>
<td>NR NR NR</td>
<td>32 (47.8) NR 27 (39.7)</td>
<td></td>
</tr>
<tr>
<td>Hypokalemia 0 1 (4.2) 10 (8.9)</td>
<td>27 (24.1) 1 (0.9) 23 (20.9) NR</td>
<td>NR NR NR</td>
<td>27 (39.7)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: n/a, not applicable; NR, not reported.

aData are shown for patients treated with 0.3–4.8 mg/kg every 3 weeks.

bIncludes alkaline phosphatase, aspartate transaminase, and alanine transaminase.

ref. 25). No patients discontinued treatment because of hemorrhagic events (25).

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The pharmacokinetics of T-DM1 has been assessed in nonclinical and clinical studies. Preliminary results showed that T-DM1 exhibits dose-proportional pharmacokinetics in non–trastuzumab-binding species (i.e., mice and rats; refs. 35, 36) and a dose-dependent decrease in clearance associated with increasing dose in trastuzumab-binding species (i.e., cynomolgus monkeys and humans; refs. 36, 37). Results from a preclinical absorption, distribution, metabolism, and excretion study of T-DM1 in rats suggest that T-DM1 nonspecifically distributes to
tissues without accumulation. The major elimination routes of DM1-containing metabolites/catabolites are through the feces (~80%) and urine (<10%; ref. 38). After dosing of T-[3H]DM1 in rats, T-DM1 was the predominant species in plasma. More than 95% of radioactivity was protein-bound (in the acetonitrile-precipitable fraction), suggesting that the majority of DM1 remains conjugated as T-DM1. Low levels of DM1- and linker-containing catabolites (MCC-DM1 and Lys-MCC-DM1) were detected. Following T-DM1 administration in patients in TDM4688g, low levels of MCC-DM1, Lys-MCC-DM1, and DM1 were detected in plasma without accumulation (38).

A consistent pharmacokinetics profile of T-DM1 has been observed across 4 clinical studies to date (TDM3569g, TDM4258g, TDM4374g, and TDM4688g refs. 24, 25, 37; Table 4). Plasma DM1 concentrations were low: the highest reported concentration of DM1 was 25 ng/mL, with no observed accumulation over treatment cycles despite repeat dosing. The average maximum concentration (Cmax) of DM1 ranged from 4.6 ± 1.3 ng/mL to 5.42 ± 1.6 ng/mL (24, 25, 37).

Gupta and colleagues (39) developed a population-pharmacokinetics model using pooled data (n = 273) from the phase I and phase II single-agent trials to determine the effects of demographic and pathophysiologic covariates on the pharmacokinetics of T-DM1 and to identify clinical factors affecting T-DM1 exposure in individual patients. The inter-individual variability in T-DM1 clearance after adjustment for covariates was low (<21%), and although body weight, albumin, tumor burden, and aspartate aminotransferase levels were statistically significant covariates accounting for interindividual variability in T-DM1 exposure or clearance, the magnitude of their effect on T-DM1 exposure was minimal (<25%) and expected to be clinically insignificant (Fig. 2). T-DM1 exposure [Cmax and area under the curve (AUC)] was relatively consistent across the patient weight range (48–103 kg), following body weight–based T-DM1 dosing. The results of this population-pharmacokinetics analysis suggest that no dose adjustments for the evaluated covariates are necessary in patients with heavily pretreated MBC.

The relationships between T-DM1 exposure and clinical response and safety were evaluated in an exploratory analysis of 2 phase II studies (37). Although the analyses were limited to a narrow exposure range following 3.6 mg/kg every 3 weeks, variations in T-DM1 exposure did not correlate with response, and differences among patients in circulating levels of trastuzumab due to prior treatment with trastuzumab and the extracellular domain of HER2 did not affect efficacy. Additionally, there was no obvious relationship between exposure to T-DM1 and the incidence of grade 3 thrombocytopenia or grade 3 increases in serum hepatic enzyme concentrations. Overall, 12 of 278 evaluable patients (4.3%) across the 4 studies developed an antibody response to T-DM1 after being exposed to repeated T-DM1 doses (Genentech, data on file). The clinical significance of antibody development against T-DM1 is unknown; however, there were no obvious changes in the pharmacokinetics, safety profiles, or efficacy outcomes of patients who developed an antibody response to T-DM1 compared with data from patients who tested negative for antibodies to T-DM1.

Preliminary assessments of pharmacokinetics-based drug interactions between T-DM1 and the HER2-targeted monoclonal antibody pertuzumab in the TDM4373g study (40) or T-DM1 and paclitaxel in the TDM4652g study (41) showed that the combination had no effect on the pharmacokinetics of the individual agents and had a low risk for drug interactions.

### Phase III Studies of T-DM1

Based on the efficacy and safety profile of T-DM1 in the phase I and phase II single-agent studies, 2 confirmatory, randomized, international, multicenter, phase III trials (EMILIA and MARIANNE) are recruiting patients. EMILIA (NCT00829166) is a randomized (1:1) study evaluating the safety and efficacy of T-DM1 compared with lapatinib plus capecitabine in patients with HER2-positive, locally advanced breast cancer or MBC following prior trastuzumab-based and taxane-containing chemotherapy (42, 43). The study will enroll ~980 patients. The primary endpoints are PFS and

**Table 4. Serum T-DM1 pharmacokinetic parameters for patients treated with T-DM1 3.6 mg/kg every 3 weeks in studies TDM3569g, TDM4258g, TDM4374g, and TDM4688g (37)**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Study (number of patients)</th>
<th>Cmax (µg/mL)</th>
<th>AUC_{24} (day µg/mL)</th>
<th>t_{1/2} (day)</th>
<th>Vss (mL/kg)</th>
<th>CL (mL/day/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum T-DM1</td>
<td>TDM3569g (n = 15)</td>
<td>83.1 (20.1)</td>
<td>486 (124)</td>
<td>3.61 (0.67)</td>
<td>29.6 (7.88)</td>
<td>7.87 (2.18)</td>
</tr>
<tr>
<td></td>
<td>TDM4258g (n = 101)</td>
<td>80.9 (20.7)</td>
<td>457 (129)</td>
<td>3.53 (0.71)</td>
<td>28.4 (12.9)</td>
<td>8.51 (2.69)</td>
</tr>
<tr>
<td></td>
<td>TDM4374g (n = 105)</td>
<td>79.5 (21.1)</td>
<td>486 (141)</td>
<td>3.96 (0.96)</td>
<td>31.2 (10.9)</td>
<td>8.04 (2.97)</td>
</tr>
<tr>
<td></td>
<td>TDM4688g (n = 51)</td>
<td>75.6 (21.9)</td>
<td>431 (126)</td>
<td>4.02 (0.94)</td>
<td>41.2 (24.5)</td>
<td>9.17 (3.03)</td>
</tr>
</tbody>
</table>

Abbreviations: AUC_{24}, area under the curve to time infinity; t_{1/2}, terminal half-life; Vss, volume of distribution at steady state; CL, clearance.

aData are reported for patients treated at the MTD (3.6 mg/kg every 3 weeks; n = 15).

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overall survival; secondary endpoints include ORR, duration of response, patient-reported quality of life, and safety. MARIANNE (NCT01120184) is a randomized (1:1:1) 3-arm study comparing the efficacy and safety of single-agent T-DM1 plus placebo versus T-DM1 plus the HER2-targeted monoclonal antibody pertuzumab versus trastuzumab plus a taxane for the first-line treatment of HER2-positive, metastatic, or locally recurrent breast cancer (44, 45). The planned enrollment is 1,092 patients. The primary endpoint is PFS. Secondary endpoints include safety, ORR, overall survival, duration of response, and quality of life.

Ongoing Combination Studies of T-DM1

Additional studies are evaluating novel T-DM1 combinations in HER2-positive breast cancer. TDM4373g (NCT00875979) is investigating the safety and efficacy of T-DM1 combined with pertuzumab in recurrent \( n = 46 \) or newly diagnosed \( n = 21 \) HER2-positive MBC (46). Interim results showed ORRs of 34.8% \( n = 16 \); 95% CI, 22.2–50.0% \( n = 12 \); 95% CI, 34.0–78.2% \( n = 21 \); 95% CI, 30.9–60.2% \( n = 13 \); 95% CI, 39.8–80.3%) were reported, respectively. Most AEs were grade 2 or lower, with reports of mild neuropathy and infrequent cardiotoxicity. Only 1 patient was discontinued because of a decrease in LVEF. The efficacy and safety of T-DM1 plus pertuzumab is also being assessed in the second part of the phase II QTc study TDM4688g, following disease progression on single-agent T-DM1 (NCT00943670; ref. 47).

TDM4652g is a phase Ib, multicenter, open-label, 3+3 design, dose-escalation study evaluating T-DM1 (both weekly and every 3 weeks) plus paclitaxel and pertuzumab in patients with HER2-positive MBC who previously...
received HER2-directed therapy (48). Preliminary results from 24 patients showed an ORR of 33.3% (8 confirmed and 5 unconfirmed PRs) and manageable safety and tolerability, with no unexpected safety signals or pharmacokinetic interactions. The combination of T-DM1 and docetaxel for HER2-positive advanced breast cancer is being studied in the open-label, multistage phase II study BP22572 (49). The target enrollment is 50 patients. GDC4627g (NCT00928330), a phase Ib, open-label, dose-escalation study, is the first trial to combine a HER2-targeted agent with a PI3K inhibitor (GDC-0941; refs. 50, 51). The target enrollment is 65 patients.

In addition to the trials evaluating T-DM1 for HER2-positive MBC, the 3.6-mg/kg every 3 weeks regimen is being investigated in a multicenter, international phase II study of patients with early-stage HER2-positive breast cancer, following anthracycline-based adjuvant or neoadjuvant chemotherapy (TDM4874g; NCT01196052; ref. 34). The target enrollment is 135 patients.

Distinguishing T-DM1 from Trastuzumab

T-DM1 has several clinically relevant features that distinguish it from trastuzumab as a unique molecular entity. The first is that following internalization by HER2-overexpressing tumor cells, T-DM1 delivers the potent cytotoxic agent DM1 directly to the tumor cell cytoplasm, disrupting microtubule assembly/disassembly dynamics and inducing apoptosis after G2/M cell cycle arrest (18, 52–55). In vitro cell viability studies have shown that DM1 is 25- to 4,000-fold more potent than currently clinically used chemotherapy agents [e.g., 25- to 500-fold more potent than paclitaxel (20, 56) and 100- to 4,000-fold more potent than doxorubicin (20)]. The second feature is that T-DM1 differs from trastuzumab in terms of dosing (3.6 mg/kg every 3 weeks for T-DM1 vs. 6 mg/kg every 3 weeks for trastuzumab) and half-life (~4 days for conjugated T-DM1 vs. ~3-4 weeks for trastuzumab; refs. 37, 39, 57, 58). Furthermore, unlike trastuzumab, T-DM1 undergoes deconjugation, proteolytic degradation, and cytochrome P450-mediated metabolism of DM1-containing catabolites, which likely explains the faster clearance of T-DM1 compared with trastuzumab, an idea that is supported by data from an exploratory model (59). The third feature is that T-DM1 was active in trastuzumab- and lapatinib-insensitive disease in preclinical studies (18, 20), consistent with clinical results (24–26). Finally, T-DM1, specifically the DM1 component, also efficiently inhibits growth in tumor models with constitutively active PI3K signaling, a postulated mechanism of trastuzumab insensitivity (60). As a result of these differences, direct comparisons should not be made between T-DM1 and trastuzumab outside of a randomized study.

Conclusions

T-DM1, a unique ADC that is currently in clinical development for HER2-positive breast cancer, appears to be well tolerated as a single agent given at 3.6 mg/kg every 3 weeks. T-DM1 has also shown clinical activity in multiple phase I and phase II studies in previously treated patients, with ORRs ranging from 25.0% (24) to 37.5% (25), and CBRs ranging from 46.4% (26) to 73% (24). It has been suggested that CBR is not an appropriate endpoint for oncology clinical trials and is not consistently defined (61). However, it appears that a standard definition of CBR (i.e., CR + PR + stable disease for ≥6 months) has been emerging over the last several years. If the definition of CBR is provided to the reader, this endpoint can be quite useful in clinical trials. This is especially true for heavily pretreated patients, who currently have very few effective options. Historically, in women who have been treated with cytotoxic therapy for MBC, it has been observed that the greater the number of prior treatments, the less likely is the chance of an objective response. Given this, stable disease for a minimum of 6 months is noteworthy and indicative of real benefit to the patient.

Although enthusiasm for T-DM1 should be tempered by the knowledge that its development is still in relatively early stages, the currently available data are encouraging. Oncologists treating women with HER2-positive breast cancer have few options to offer their patients, as multiple therapies have proved ineffective at stemming disease progression. For example, the women who enrolled in earlier clinical trials of T-DM1 had HER2-positive breast cancer that had already progressed on several drugs prior to study entry, including trastuzumab, lapatinib, capecitabine, taxanes, and anthracyclines, at minimum. Additional agents, such as pertuzumab (62) and neratinib (63), are also in development for HER2-positive breast cancer. Together with T-DM1, these new agents have the potential to dramatically increase the treatment options for women with HER2-positive MBC.

In 2010, the FDA refused to file a biologics license application for T-DM1 under the Accelerated Approval Regulations (21CFR 601.40), which require single-arm trials to show that patients are unresponsive to all available therapies. If the early clinical data are replicated in larger randomized trials, TDM-1 could set a new standard for anticancer therapy as a drug that manifests minimal toxicity while offering clinical benefit in pretreated patients with HER2-positive disease, for whom there is currently no effective therapy. PFS and overall survival data from the ongoing randomized phase III (EMILIA) study in HER2-positive MBC patients are eagerly awaited, and data from this study may form the basis of a future FDA submission.

Questions remain regarding the relative contribution of the components of T-DM1 to its antitumor activity. Preclinical data have shown that T-DM1 maintains known mechanisms of action of trastuzumab (20) and that the integrity of the antibody and its therapeutic properties are maintained after conjugation. As outlined in the previous section, there are also several lines of evidence supporting the activity of the DM1 component of T-DM1 (i.e., differentiating it from trastuzumab; refs. 18, 20, 52–55, 57). Preclinical data have shown that T-DM1 is more potent
than trastuzumab in trastuzumab-sensitive settings (18, 55) and that T-DM1 is highly active in trastuzumab-refractory settings (18, 20). It is likely that in trastuzumab-sensitive settings, both the trastuzumab and DM1 components contribute to the activity of T-DM1, and in trastuzumab-refractory settings, DM1’s mechanisms of action are responsible for the antitumor activity. The relative contributions of trastuzumab and DM1 to the clinical activity of T-DM1 are less clear; however, it has been shown that T-DM1 is active in patients with disease that has progressed on lapatinib and trastuzumab (25, 26), suggesting that DM1 plays a role.

Also remaining unclear at this point is the ultimate potential of T-DM1. Can T-DM1 as a single agent replace the standard of care? Will T-DM1 have a role in the treatment of early-stage breast cancer or other HER2-positive cancers? Data from ongoing phase II (TDM4450g) and phase III (MARIANNE) studies of T-DM1 in the first-line setting will help dictate the path forward. As we await the data from these trials, T-DM1 remains a new molecular entity with a novel mechanism of action and the potential to improve clinical outcomes for patients with HER2-positive breast cancer.

Disclosure of Potential Conflicts of Interest

P. LoRusso received honoraria from and is a consultant for Genentech Inc. M. Sliwkowski is employed by and has an ownership interest in Genentech Inc. The other authors disclosed no potential conflicts of interest.

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Trastuzumab Emtansine: A Unique Antibody-Drug Conjugate in Development for Human Epidermal Growth Factor Receptor 2–Positive Cancer

Patricia M. LoRusso, Denise Weiss, Ellie Guardino, et al.

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