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Retrospective Assessment Of Treatment With T-Dm1 After Pertuzumab In Her2-Positive Metastatic Breast Cancer

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Retrospective Assessment of Treatment with T-DM1 after Pertuzumab in HER2+ Metastatic Breast Cancer

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Abstract

RETROSPECTIVE ASSESSMENT OF TREATMENT WITH T-DM1 AFTER PERTUZUMAB IN HER2+ METASTATIC BREAST CANCER

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T-DM1/ ado-trastuzumab emtansine, the most recent addition to the HER2-targeted therapies approved to treat HER2-positive metastatic breast cancer (MBC), is an antibody-drug conjugate with a favorable side effect profile. T-DM1 is currently approved for patients with HER2-positive MBC who previously received trastuzumab and a taxane. Since the trial resulting in T-DM1 approval was conducted, the standard first-line therapy for metastatic HER2-positive breast cancer has changed from trastuzumab and a taxane to a three-drug combination of trastuzumab and a taxane plus pertuzumab. Due to the timing of these approvals, there is no clinical trial or observational data on the activity of T-DM1 in patients who have received prior therapy that included pertuzumab. The goal of this study was to assess the efficacy of T-DM1 in routine clinical practice in a contemporary patient population that received both prior trastuzumab and pertuzumab. To address this goal, a retrospective chart review was performed for all patients with HER2-positive MBC who received T-DM1 after pertuzumab between March 1, 2013 and July 15, 2015 at three institutions (Smilow Cancer Hospital at Yale-New Haven, MD Anderson Cancer Center, and The James Cancer Hospital at the Ohio State University). We manually reviewed the medical records of each case to confirm treatment sequencing and outcome. Eighty-two patients were identified who had

received single agent T-DM1 and had received pertuzumab at any time previously. Demographic characteristics and prior therapy were reported for these patients. Seventy-eight patients were available for analysis of response. The rate of prolonged duration on therapy (PDT), defined as duration on therapy $\geq$ 6 months, was 30.8% (95% CI, 20.6-41.1%) and the tumor response rate was 17.9% (95% CI, 9.4-26.4%). The rate of any benefit (AB), defined as PDT and/or TR, was 37.2% (95% CI, 26.5-47.9%). The median duration on therapy was 4.0 months (95% CI, 2.7-5.1, range 0-22.5). The reason for discontinuation of T-DM1 was progression of disease in 84% of patients. Only 7 patients (10%) discontinued T-DM1 due to toxicity or poor tolerance. Overall, this retrospective analysis provides the first data demonstrating the efficacy of T-DM1 in patients who have received pertuzumab previously. Response rates were lower than prior reports in trastuzumab-resistant HER2-positive MBC, but one third of patients received therapy with T-DM1 for at least 6 months, which suggests tumor control and clinically relevant benefit to T-DM1 in patients who received prior trastuzumab and pertuzumab.

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Introduction

HER2 and Trastuzumab in Breast Cancer

The human epidermal growth factor receptor 2 (*HER2, ERBB2*) gene encodes a receptor tyrosine kinase that is a member of the epidermal growth factor receptor family of receptors, which mediate cell proliferation, migration, differentiation, and survival (1). The HER2 protein is overexpressed in approximately 20 percent of invasive breast cancers, and its overexpression is associated with more aggressive tumor biology and unfavorable prognosis (2-4).

The development of **trastuzumab (Herceptin®)**, a recombinant, humanized monoclonal antibody targeting HER2, led to significant improvements in survival for patients with HER2-positive breast cancers and established HER2 as a useful therapeutic target. Trastuzumab binds to the extracellular domain of HER2, suppressing its signaling activity, promoting receptor degradation, and inducing antibody-dependent-cell-mediated cytotoxicity (ADCC) (5). Ligand-induced dimerization of HER2 with other epidermal growth factor receptor family members activates multiple signaling pathways, but trastuzumab's best-known effect on HER2 signaling is inhibition of the MAPK and PI3K/Akt pathways, which results in cell cycle arrest and suppression of cell growth and proliferation (5). By ADCC, trastuzumab also attracts immune cells to HER2-overexpressing cells, resulting in immune-mediated cell death (6).

The clinical activity of trastuzumab was first studied in HER2-positive metastatic breast cancer (MBC). The single agent activity of trastuzumab was modest (around 20% objective tumor response) but the addition of trastuzumab to chemotherapy significantly improved progression-free and overall survival in MBC. (7, 8). In the pivotal phase III trial that led to the approval of trastuzumab in the US by the Food and Drug Administration (FDA), the addition of trastuzumab to anthracycline plus cyclophosphamide or paclitaxel compared to chemotherapy alone was associated with increased time to disease progression (median, 7.4 vs. 4.6 months; P<0.001), higher objective response rate (ORR) (50% vs. 32%; P<0.001), longer duration of response (median, 9.1 vs. 6.1 months; P<0.001), lower rate of death at 1 year (22% vs. 33%; P=0.008), and longer overall survival (median, 25.1 vs. 20.3 months; P=0.046) (7). Because of its success in the metastatic setting, trastuzumab was also studied in the adjuvant setting in early-stage HER2-positive breast cancer, in which it resulted in decreased disease recurrence and improved overall survival when given in combination with or after chemotherapy (9-12). Based on these favorable results, concurrent chemotherapy and trastuzumab followed by continued trastuzumab for a total of 12 moths is now standard of care adjuvant therapy for stage I-III, HER2-positive breast cancer (13).

Treatment of HER2-Positive Metastatic Breast Cancer

Despite the improvements in survival in both metastatic and early stage HER2-positive breast cancer, trastuzumab resistance remains a problem. Most patients with metastatic cancer will eventually progress on trastuzumab therapy (7,

14) and some early stage HER2-positive breast cancers relapse despite trastuzumab containing adjuvant therapy (9, 10). These clinical observations have motivated the development of several other HER2-directed therapies, three of which are currently approved by the FDA including lapatinib, pertuzumab, and ado-trastuzumab emtansine (T-DM1).

Lapatinib (Tykerb®/Tyverb®)

Lapatinib is an orally active small molecule inhibitor of the HER2 and HER1/EGFR1 tyrosine kinases, which disrupts signaling pathways downstream of these receptors (15). On March 13, 2007, Lapatinib was approved by the FDA for the treatment of patients with metastatic HER2-positive breast cancer who have received prior therapy including an anthracycline, a taxane, and trastuzumab. A large randomized clinical trial demonstrated improved time to progression (TTP) with the addition of lapatinib to capecitabine compared to capecitabine alone with a hazard ratio (HR) of 0.57 (95% confidence interval [CI], 0.43-0.77; P<0.001) that corresponds to a 43% reduction in the risk of progression in the combination treatment arm (14, 16). Subsequently, lapatininb was also approved in combination with the aromatase inhibitor letrozole for the treatment of postmenopausal women with estrogen receptor (ER) positive MBC that overexpresses HER2. In a double-blind, placebo-controlled study, patients treated with lapatinib and letrozole experienced a 5.2 month increase in median progression-free survival (PFS) compared to women treated with letrozole alone (17) .

Pertuzumab (Perjeta®)

Pertuzumab is a humanized monoclonal antibody that binds to the extracellular dimerization domain of HER2 (a different epitope from where trastuzumab binds) and prevents HER2 from dimerizing with itself or other HER family members inhibiting subsequent intracellular signaling (18). Like trastuzumab, pertuzumab also stimulates ADCC (19). On June 8, 2012, pertuzumab was approved in combination with trastuzumab and docetaxel for the treatment of patients with HER2-positive MBC who have not received prior anti-HER2 therapy or chemotherapy for metastatic disease (20). The CLEOPATRA trial included 808 patients with metastatic HER2-positive breast cancer and compared trastuzumab and docetaxel with the same drugs plus pertuzumab in patients who have not received prior therapy for metastatic disease (but most of the patients included in the trial received trastuzumab and chemotherapy as adjuvant treatment) (21). The addition of pertuzumab to trastuzumab plus docetaxel resulted in increased ORR (69.3% vs. 80.2% [95% CI, 4.2 to 17.5; P=0.001]), increased PFS (12.4 vs. 18.5 months, HR 0.62; 95% CI, 0.51 to 0.75; P<0.001) (21) and improved overall survival (40.8 vs. 56.5 months, HR: 0.68; 95% CI, 0.56 to 0.84; P<0.001) (22). Another randomized trial that compared pertuzumab monotherapy to the combination of pertuzumab and trastuzumab in HER2-positive metastatic breast cancer that progressed on trastuzumab also demonstrated improved PFS with the combination of pertuzumab and trastuzumab (17.4 weeks; 80% CI, 6 to 29 weeks) compared to pertuzumab alone (7.1 weeks; 80% CI, 6 to 10 weeks) (23).

In September 2013, pertuzumab also received accelerated approval for neoadjuvant (i.e. preoperative) treatment of early stage HER2-positive breast cancer as part of a multidrug treatment regimen. In a randomized study, patients were assigned to receive one of four different preoperative treatment regimens: trastuzumab plus docetaxel, trastuzumab plus docetaxel plus pertuzumab, pertuzumab plus trastuzumab, and pertuzumab plus docetaxel. The highest pathologic complete response rate (i.e. no residual invasive cancer in the breast or lymph nodes) of 45.8% was achieved by the pertuzumab plus trastuzumab and docetaxel combination (24). As a result of these trials, most patients with HER2-positve breast cancer today receive pertuzumab either in the neoadjuvant treatment setting or as the initial treatment for metastatic disease when their cancer recurs.

Ado-trastuzumab emtansine / T-DM1 (Kadcyla®)

The most recent addition to HER2-targeted therapies is T-DM1/Ado-trastuzumab emtansine, an antibody-drug conjugate composed of the cytotoxic agent DM1 attached to trastuzumab via a stable thioether linker (25). DM1, a derivative of maytansine, is a potent microtubule polymerization inhibitor that possesses *in vitro* cytotoxicity 10 to 200 times greater than that of taxanes and vinca alkaloids (26). Conjugation of DM1 to trastuzumab via a non-cleavable linker minimizes the amount of free DM1 in circulation and results in less systemic toxicity; it also facilitates intracellular delivery of the drug through HER2-receptor mediated internalization and intracellular release by lysosomes (25).

Ado-trastuzumab emtansine was approved by the FDA on February 23, 2013 for patients with metastatic HER2-positive breast cancer who previously received trastuzumab and a taxane (27). The approval was based on the results of the EMILIA trial, which included 991 patients with HER2-positive metastatic breast cancer who previously received treatment for metastatic breast cancer with trastuzumab and a taxane. Patients were randomized to T-DM1 or lapatinib plus capecitabine (28). T-DM1 demonstrated increased progression-free survival and overall survival compared to capecitabine and lapatinib. The median PFS was 9.6 versus 6.4 months (HR 0.65; 95% CI, 0.55 to 0.77; P<0.001) and the median overall survival was 30.9 versus 25.1 months (HR 0.68; 95% CI, 0.55 to 0.85; P<0.001). The objective response rate was also higher in the group receiving T-DM1 (43.6% vs. 30.8%; P<0.001) (28). The TH3RESA study was another randomized, open label, phase III trial to test the activity of T-DM1 in patients who have progressed on multiple prior lines of HER2-targeted therapies (29, 30). Six hundred and two patients were randomly assigned to T-DM1 or treatment of physician's choice (83% of patients received trastuzumab or lapatinib together with chemotherapy in this arm) (31). T-DM1 demonstrated significantly improved progression-free survival (median PFS 6.2 months [95% CI, 5.59-6.87]) versus 3.3 months [95% CI, 2.89-4.14] (31). A small, randomized phase II trial TDM4450g (n= 137 patients) compared the activity of T-DM1 to trastuzumab plus docetaxel in patients who received no prior HER2-targeted therapy for metastatic breast cancer (i.e. first line therapy) (32). Treatment with T-DM1 resulted in improved median progression-free survival (9.2 vs. 14.2 months; HR, 0.59; 95% CI, 0.36 to 0.97) (32). To confirm these results, a large, 3-

arm, phase III study, the MARIANNE trial was conducted. MARIANNE included 1,095 patients with previously untreated metastatic or locally-advanced HER2-positive breast cancer who were randomized to receive either T-DM1 plus pertuzumab, T-DM1 plus placebo, or trastuzumab plus a taxane. The preliminary results were reported at the Annual Meeting of the American Society of Clinical Oncology (ASCO) in 2015 with a median follow-up of more than 34 months (33). In contrast to the results from the TDM4450g trial, all 3 arms appeared to perform similarly and the T-DM1 plus pertuzumab combination was not superior to trastuzumab plus taxane in terms of progression-free survival. Median PFS was 15.2 months in the T-DM1 plus pertuzumab arm (HR 0.87; 95% CI, 0.69 to 1.08; p=0.14) and 14.1 months with T-DM1 alone (HR 0.91; 95% CI, 0.73 to 1.13; p=0.31) compared to 13.7 months with trastuzumab plus a taxane, and the objective response rates were 64.2%, 59.7%, and 67.9%, respectively (33). The median duration of response was 21.2 months (95% CI, 15.8 to 29.3) in the T-DM1 plus pertuzumab arm, 20.7 months (95% CI, 14.8 to 25.0) in the T-DM1 monotherapy arm, and 12.5 months (95% CI, 10.5 to 16.6) in the trastuzumab plus taxane arm (33). Due to the lack of superior results with T-DM1 as first-line therapy in the MARIANNE trial, trastuzumab and pertuzumab plus a taxane remains the first-line standard of care treatment for metastatic HER2-positive breast cancer with T-DM1 remaining the second-line treatment option for those who progress.

T-DM1 Toxicity and Side Effect Profile

In EMILIA, MARIANNE, and TDM4450g, treatment with T-DM1 resulted in lower overall rates of grade ≥3 adverse events compared to the other treatment arms (EMILIA: 40.8% vs. 57.0%, MARIANNE: 45.4% vs. 54.1%, and TDM4450: 46.4% vs. 90.9%, respectively) (28, 32, 33). In the EMILIA trial, T-DM1 also resulted in improved patient-reported outcomes, measured as a delay in clinically significant symptom worsening (7.1 months vs. 4.6 months; HR=0.796; 95% CI, 0.667-0.951; P=0.0121) (28, 34). In TDM4450g, T-DM1 also showed fewer AEs leading to treatment discontinuations (7.2% vs. 40.9%) and fewer serious AEs (20.3% vs. 25.8%) (32). In a pooled analysis of six studies (n=884 patients) with HER2-positive metastatic breast cancer who received the standard T-DM1 dose of 3.6 mg/kg every 3 weeks, the most commonly reported adverse events were fatigue (46.4%), nausea (43.0%), thrombocytopenia (32.2%), headache (29.4%), and constipation (26.5%) (35). The most common grade 3-4 AEs were laboratory abnormalities: thrombocytopenia (11.9%) and increased AST serum concentrations (4.3%) (35).

Thrombocytopenia was the most common grade ≥3 adverse event observed in patients treated with single-agent T-DM1, although the majority of thrombocytopenia events were grades 1-2 (35). In the pooled analysis, 128 patients experienced grade 3-4 thrombocytopenia, while 56 (43.8%) experienced grade 1 bleeding (primarily epistaxis), five (3.9%) experienced grade 2 bleeding (primarily epistaxis), and six (4.7%) experienced grade 3-4 bleeding, but only two had grade 3-4 thrombocytopenia at the same time (35). Similarly, in EMILIA grade ≥3 thrombocytopenia was more common in the T-DM1 arm than the capecitabine plus

lapatinib arm (12.9% vs. 0.2%), and the overall incidence of bleeding events was higher in the T-DM1 arm (29.8% vs. 15.8%), but the rates of grade ≥3 bleeding events were still low in both groups (1.4% vs. 0.8%) (28).

In clinical trials, T-DM1 also demonstrated a potential to induce infrequent but moderately severe hepatotoxicity. Hepatic aminotransferase elevations were the second most common grade ≥3 adverse event in pooled analysis, but the rates were low (AST 4.1% and ALT 2.8%), and elevations were generally transient, allowing patients to remain on therapy (35). In addition to elevations in aminotransferases, there have been cases of biopsy-confirmed nodular regenerative hyperplasia (NRH) (3 cases in the pooled safety analysis) in patients receiving T-DM1 (35). NRH, a rare liver condition that can lead to non-cirrhotic portal hypertension, has no suggestive lab value and can only be diagnosed by liver biopsy (36). If portal hypertension develops while on T-DM1, NRH should be investigated as a possible cause, and if NRH is diagnosed T-DM1 should be discontinued permanently (36).

Sequencing of HER2-targeted Therapies in Metastatic Breast Cancer

The current NCCN (National Comprehensive Cancer Network) guidelines recommend the following sequencing of the above drugs during the management of HER2-positive metastatic breast cancer (37). The standard first-line therapy for metastatic HER2-positive breast cancer is the combination of pertuzumab, trastuzumab, and a taxane. The recommended second-line therapy for those who have progressed on the above therapy is T-DM1. There is no preferred third-line

treatment option after progression on pertuzumab and T-DM1 containing therapies but options include: capecitabine plus lapatinib or combinations of trastuzumab and vinorelbine or eribulin and other chemotherapy drugs (38, 39). A diminishing number of patients who have not previously received pertuzumab or T-DM1 as first or second line therapy, may also receive these drugs as third- and fourth-line therapy.

Rationale of Current Study

The simultaneous clinical development and almost simultaneous approval of pertuzumab (June 2012 for MBC and September 2013 as neoadjuvant therapy) and T-DM1 (February 2013) in the US to treat metastatic HER2-positive breast cancer resulted in the unusual circumstance that the patient population that was enrolled in the pivotal trial which resulted in the approval of T-DM1 no longer exists. The phase 3 EMILIA trial which established T-DM1 as the preferred second-line treatment for metastatic HER2-positive breast cancer did not include any patients who had received pertuzumab as neoadjuvant/adjuvant therapy or as first-line treatment for MBC (28). Since the EMILIA trial was conducted, the standard first-line therapy for metastatic HER2-positive breast cancer has changed from trastuzumab and a taxane to the three-drug combination of trastuzumab and a taxane plus pertuzumab. Unfortunately, there is no clinical trial or observational data on the activity of T-DM1 in patients who have received prior therapy that included pertuzumab. The goal of this study is to assess the efficacy of T-DM1 in

routine clinical practice in a contemporary patient population that includes patients who have received prior therapy with pertuzumab.

In clinical trials, objective response rate and progression-free survival are measured using the RECIST (Response Evaluation Criteria in Solid Tumours) criteria, which include strict definitions for measurement of response and assignment of response categories (complete response, partial response, stable disease, and progressive disease) (40). In routine clinical practice, the same terminology is often used to describe the outcome of therapy but it rarely reflects the same rigorous tumor evaluation as called for by RECIST. Therefore, we use an alternative terminology for assessing clinical benefit in routine practice outside the structure of a prospective trial to avoid confusion with RECIST terms. In routine care, medical oncologists continue treatment with a given drug until disease progression or until intolerable side effects develop and therefore, the **duration on therapy** is a practical combined measure of clinical benefit and tolerability. Disease progression is usually indicated by new or enlarging lesions on tumor imaging or by symptomatic deterioration. Tumor response usually signifies a radiological report that states tumor response (with or without actual tumor measurements) and/or symptomatic improvement. Based on these outcomes, we created four categories as efficacy measures for our chart review study: (i) **tumor response (TR)** indicates physician reported clinical or imaging response, (ii) **prolonged duration on therapy (PDT)** indicates T-DM1 therapy ≥ 6 months, (iii) **minimal benefit (MB)** indicates physician reported stable disease as the initial response to T-DM1 but discontinuation of T-DM1 before 6 months, and (iv) **progressive disease (PD)**

indicates symptomatic deterioration or progression on routine radiologic assessment.

Specific Aims

The specific aims of this study are:

1. To assess the duration of T-DM1 therapy in patients who have received pertuzumab previously.
2. To assess the rates of tumor response, prolonged duration on therapy, and any benefit with T-DM1 in patients who have received pertuzumab previously.

Methods

Patient Population

This study is a retrospective chart review of medical records of patients with HER2-positive metastatic breast cancer who received T-DM1 after pertuzumab therapy at three institutions, including Smilow Cancer Hospital at Yale-New Haven (Yale), MD Anderson Cancer Center (MDACC), and the James Cancer Hospital at the Ohio State University (OSU). Patients received treatment with T-DM1 and were followed during treatment as per routine practice in the respective institutions. This study was designed and led by Hannah Dzimitrowicz (H.E.D.), under the supervision of Dr. Lajos Pusztai (L.P.), Chief of the Breast Medical Oncology section, and was conducted under the Yale Human Investigations Committee (HIC) approved protocol titled "*A retrospective assessment of treatment with T-DM1 after pertuzumab in HER2-positive metastatic breast cancer*" (HIC #1505015954) (protocol written by H.E.D. with input from L.P.). Each collaborating site's institutional review board also separately reviewed and approved the study (protocol written by H.E.D.), and each site had a designated scientific collaborator who served as the local principal investigator (PI) (MDACC: Dr. Rashmi Murthy; OSU: Dr. Michael Berger).

The respective hospital pharmacy information systems (at Yale and OSU) and a prospectively maintained departmental database (at MDACC) were queried for the names and medical record numbers of all patients with metastatic breast cancer

who received T-DM1 between March 1, 2013 (T-DM1 was approved by the FDA on February 23, 2013) and July 15, 2015. This list of patients was then cross-referenced against the list of patients who also received pertuzumab any time since its first approval in June 2012 to identify patients who have received both drugs and represent our study population (identification of eligible patients performed by pharmacy collaborators at Yale and outside collaborators at their respective institutions).

Data Collection

The electronic medical records of patients who received both T-DM1 and pertuzumab were reviewed manually by the local study PIs who were asked to complete a data acquisition form provided by the lead investigator (H.E.D.) at Yale. H.E.D. designed the data acquisition form (with input from L.P.) and manually reviewed the records of all patients from Yale. The following data items were extracted from the medical records and stored in de-identified form in an Excel data sheet on password protected computers:

(i) *Patient demographics:* date of birth and race

(ii) *Tumor characteristics*: date of diagnosis of primary breast cancer, estrogen and progesterone receptor status of the primary tumor (or the metastatic lesion if receptor status was reassessed), HER2 status (including IHC result and FISH result if both were available), date of metastatic recurrence, sites of metastases at the time of starting T-

DM1 therapy (bone, visceral, brain or soft tissue [i.e. skin, lymph node or breast]).

(iii) *Treatment history:* Prior neoadjuvant or adjuvant chemotherapy (yes vs. no and type of regimen), adjuvant endocrine therapy (yes vs. no, and names of agents), number of treatment lines for metastatic breast cancer including name of each drug during each line of therapy, dates of first and last courses of pertuzumab, and dates of first and last courses of T-DM1 therapy.

(iv) *Response to T-DM1:* Best tumor response during T-DM1 therapy by the assessment of the treating physician (i.e. complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) based on routine radiology reports and clinical assessment during T-DM1 therapy as documented in the medical records, deferring to the treating physician's assessment in the event that it conflicted with radiology reports), reason for discontinuation of T-DM1 (i.e. progression, toxicities that prompted discontinuation, other), date of death if applicable.

The ER and HER2 status of tumors was determined by routine clinical pathology using immunohistochemistry and/or fluorescence in situ hybridization (FISH) as per institutional standards of care. Tumors were classified as HER2-positive if the protein was overexpressed on immunohistochemistry (score of 3+) or if the *HER2* gene was amplified on FISH (HER2/CEP17 ratio ≥ 2 with an average

HER2 copy number ≥ 6.0 signals/cell) (41). A tumor was considered to be positive for ER or progesterone receptor (PR) expression if the respective immunostaining demonstrated expression in 1% or more of tumor cells and all controls were adequate (42).

H.E.D. created a final combined database by merging data from all three sites for analysis. The final study population included patients who received trastuzumab and pertuzumab any time before starting T-DM1.

Statistical Analysis

The primary endpoints of this study are: duration of therapy with T-DM1, tumor response rate, rate of prolonged duration on therapy (PDT), and rate of any benefit (AB). Duration on T-DM1 therapy was measured from day zero (date of a patient's first T-DM1 treatment) to the date of the patient's last T-DM1 treatment. **Tumor response (TR)** is defined as: physician reported partial or complete response based on imaging and clinical assessment. **Prolonged duration on therapy (PDT)** is defined as: duration on T-DM1 therapy for ≥ 6 months regardless of best response. **Minimal benefit (MB)** is defined as: an initial response of stable disease by physician assessment but T-DM1 treatment duration less than six months. **Progressive disease (PD)** is defined as: symptomatic deterioration or progression on routine radiologic imaging determined by physician assessment. **Any benefit (AB)** includes patients with tumor response (regardless of treatment duration) and/or PDT. Descriptive statistics are reported with point estimates and 95% confidence intervals for duration on therapy, tumor response rates, PDT rates,

and AB rates. Rates of TR, PDT, and AB were calculated for all patients as well as for the following pre-defined patient subsets: ER- and/or PR-positive tumors, ER- and PR-negative tumors, T-DM1 as ≤ second-line therapy for metastatic disease, T-DM1 as greater than second-line therapy for metastatic disease, de novo metastatic disease, metastatic recurrence less than 1 year after initial diagnosis, metastatic recurrence greater than 1 year after initial diagnosis, and prior lapatinib. All analysis and representation of data were performed by H.E.D.

Results

Efficacy Results

The database search identified a total of 82 patients across the three participating institutions who have received single agent T-DM1 between March 1, 2013 and July 15, 2015 as treatment for metastatic HER2-positive breast cancer and received pertuzumab any time before T-DM1 treatment began. Table 1 lists the patient characteristics. Ninety-six percent of patients received trastuzumab and pertuzumab, 88% received a taxane, and 23% also received lapatinib as treatment for metastatic disease before receiving T-DM1 (Table 2). Three patients received pertuzumab as neoadjuvant or adjuvant therapy (3.7%) before metastatic recurrence, two of which subsequently had no therapy for metastatic disease prior to T-DM1. Thirty-two percent of patients received T-DM1 as first or second line

therapy (i.e. after one prior line of treatment for metastatic cancer) and 48% received it as fourth or greater line treatment.

Seventy-eight patients were available for outcome analysis. The tumor response rate was 17.9% (95% CI, 9.4-26.4%), the rate of prolonged duration on therapy (PDT) (at least 6 months on T-DM1) was 30.8% (95% CI, 20.6-41.1%), and the rate of any benefit (AB) was 37.2% (95% CI, 26.5 to 47.9%)(Table 3). Nine patients with PDT also had a tumor response (Figure 1). Ten patients (12.8%) demonstrated minimal benefit from T-DM1 (best response of stable disease but T-DM1 < 6 months), and 39 patients (50%) were demonstrated to have only progressive disease (Figure 1).

The median duration on therapy was 4.0 months (95% CI, 2.7-5.1) with a range of 0 to 22.5 months (Figure 1). The median duration on therapy for patients receiving T-DM1 as ≤ second-line therapy (N=26) was also 4.0 months (95% CI, 2.4-6.8). Twenty-four patients were on therapy for 6 months or longer and 6 patients were on treatment for one year or longer. Eight patients continued to be on T-DM1 at the time of data collection.

The reason for discontinuation of T-DM1 in 84.3% of cases (59 of 70 patients) was progression of disease. One patient discontinued therapy after continued fatigue and falls resulting in transition to hospice care. One patient refused further therapy due to disbelief in her diagnosis despite a partial response to T-DM1. Another patient elected to discontinue medical care despite a complete response to T-DM1. One patient discontinued T-DM1 after continued stable disease. Only 7 patients (10%) discontinued T-DM1 due to toxicity or poor tolerance. Two patients discontinued T-

DM1 due to thrombocytopenia. Each of the remaining five patients discontinued T-DM1 due to a different toxicity. These included: 1) arthralgia, fatigue and anorexia, 2) a serum sickness-like presentation including fever and joint pain, 3) neuropathic foot pain, 4) reduced cardiac ejection fraction, and 5) elevated hepatic aminotransferases.

T-DM1 Side Effects

In previous trials, T-DM1 has been shown to have a favorable side effect profile and low rates of discontinuation due to toxicity. In EMILIA, MARIANNE, and TDM4450g, treatment with T-DM1 resulted in lower overall rates of grade ≥3 adverse events compared to the other treatment arms (EMILIA: 40.8% vs. 57.0%, MARIANNE: 45.4% vs. 54.1%, and TDM4450: 46.4% vs. 90.9%, respectively) (28, 31, 32). In our study, only seven patients (10%) discontinued T-DM1 due to toxicity or poor tolerance. In a pooled analysis of six studies of T-DM1 at the typical dose of 3.6 mg/kg every 3 weeks including 884 patients, 62 patients (7.0%) discontinued treatment due to an adverse event, the most common of which involved laboratory abnormalities, primarily thrombocytopenia (1.5%) and increased hepatic aminotransferases (0.8% for increased AST, 0.5% for increased ALT) (35). In our study, two patients discontinued treatment due to thrombocytopenia, and there was one case of elevated hepatic aminotransferases resulting in treatment discontinuation. Although not a treatment-ending side effect in previous studies of T-DM1, fatigue was the most commonly reported AE (46.4%) and arthralgia of any grade was common (20.1%) in the pooled analysis of 884 patients (35). Our study captured serious adverse events that resulted in treatment discontinuation, but it

did not capture less severe AEs or AEs successfully mitigated with dose reductions. In previous trials of T-DM1, AEs were predominantly asymptomatic laboratory abnormalities that were manageable with dose reductions, which were not captured in this study. Collectively, our data suggests that the particularly good safety profile of T-DM1 seen in previous studies extends to patients previously treated with pertuzumab.

Discussion

In this retrospective analysis of patients with HER2-positive metastatic breast cancer who received T-DM1 after trastuzumab and pertuzumab, T-DM1 exhibited clinical activity and a safety profile comparable to those observed in clinical trials (Table 4). To the best of our knowledge, this is the first clinical data demonstrating the efficacy of T-DM1 in a contemporary patient population that has received pertuzumab.

Comparison of Our Results to Clinical Trial Results with T-DM1

Clinical trials of T-DM1 have taken place in patient populations with differing degrees of pre-treatment and resulted in varied responses that are summarized in Table 4. The highest antitumor activity was reported in the phase II TDM4450g trial (ORR 64.2%, PFS: 14.2 months) and the phase III MARIANNE trial (ORR: 59.7%, PFS: 14.1 months) that were conducted to assess the efficacy of T-DM1 as first line

therapy (i.e. no prior therapy for MBC) (33). In the EMILIA trial, which included 61% of patients who received no or only 1 prior regimen for metastatic disease, the ORR was 43.6% and PFS was 9.6 months (28). The TH3RESA trial that accrued more heavily pre-treated patients reported an ORR of 31% with median PFS of 6.2 months (31). In the Phase II M4374g trial, which also accrued heavily pre-treated patients, the clinical benefit rate (CBR), defined as the rate of CR, PR, or SD ≥ 6 months, was 48.2%, ORR was 34.5%, and median PFS was 6.9 months (30).

In our study we examined T-DM1 activity as predominantly second or greater line of treatment in patients who have progressed on trastuzumab and pertuzumab and observed a rate of prolonged duration on therapy of 30.8% (95% CI, 20.6-41.1%), tumor response rate of 17.9% (95% CI, 9.4-26.4%), any benefit rate of 37.2% (95% CI, 26.5-47.9%) and median duration on therapy of 4.0 months (95% CI, 2.7-5.1) with a range of 0 to 22.5 months. These results are less favorable than the response rates and PFS observed in earlier trials of T-DM1 (Table 4). Of these trials, however, our results are most comparable to the results obtained in the TH3RESA and M4347g trials that enrolled heavily pretreated patients (Table 4)(30, 31). Overall, these results suggest that the anticancer efficacy of T-DM1 declines as the number of prior therapies, in particular the number of HER2-targeted therapies, increases. In our study, patients who received T-DM1 as ≤ second-line therapy after the first-line combination of two HER2-targeted agents (trastuzumab and pertuzumab) had response rates (TRR 23.1%) lower than rates observed in previous trials of T-DM1 as second-line therapy. Still, clinically important activity is observed in patients who have received prior pertuzumab. Importantly, 31% of

patients in our study remained on T-DM1 for 6 months or longer which demonstrates a meaningful tumor control rate and overall benefit in patients who have received prior pertuzumab (and multiple other lines of therapy).

Limitations in Methodology

Our study is a retrospective evaluation of patients treated in routine clinical practice in contrast to a prospective clinical trial, so there are limitations in methodology and comparison to trial results. In clinical trials, patient eligibility is strictly defined and accrual is often limited by the number of prior therapies and therefore the study population is more homogeneous than the patient cohorts included in retrospective chart reviews. Tumor response assessment is also codified and measurements occur at regular pre-specified intervals, which is quite different from the response assessment performed at the discretion of the treating physician in routine practice. In retrospective chart review studies some patients are assessed less frequently than others, adding variability to duration on therapy and response measures. In clinical trials, response and progression-free survival are measured using the RECIST (Response Evaluation Criteria in Solid Tumours) criteria, which include definitions of minimum size of measurable lesions, instructions on how many lesions to follow, and the use of unidirectional measures for overall evaluation of tumor burden and response (40). Under RECIST, there are strict criteria for responses, including: complete response being a disappearance of all target lesions, partial response being a 30% decrease in the sum of the longest diameter of target lesions, progressive disease being a 20% increase in the sum of

the longest diameter of target lesions, and stable disease corresponding to small changes not meeting these criteria (40). In routine practice, radiologists provide their overall best estimate of disease status often without specific measurements. Traditionally, trials measure time to progression (TTP) and progression-free survival (PFS) as the time from randomization until tumor progression. Because patients in our study were not enrolled in an organized trial and our data collection was retrospective, PFS was not a strictly recorded measurement, and we used duration on therapy as an approximation. While this is not a precise measurement it reflects the clinical utility of a drug because in routine clinical practice, medical oncologists continue treatment with a given drug until disease progression or until intolerable side effects develop. Therefore if a drug has few side effects, length of time on treatment is a reasonable estimate of PFS, which is why we used this metric as an efficacy endpoint in our study. The reason for discontinuation of T-DM1 was progression of disease in 84% of cases in our study supporting the use of duration on treatment as a surrogate for PFS. We do note that 16% of patients discontinued therapy before progression due to side effects, poor tolerability, or personal decisions; in these cases duration on therapy potentially under-estimated PFS (in clinical trials patients often have tumor measurements or are censored at the time of treatment discontinuation for toxicity)(43). Additionally, one patient remained on T-DM1 for several months beyond initial progression, which leads to over-estimation of PFS in our analysis. Toxicities are also assessed differently and more rigorously in clinical trials using the NCI toxicity grading compared to routine administration of a drug. All of these methodological differences indicate caution

when numerical outcome results from a chart review study such as ours are compared to historical benchmark results reported by prospective clinical trials.

We also noticed an unusual distribution of de novo stage IV disease in our study population. In large, population based studies, approximately, 5 to 10% of breast cancers present with de novo stage IV disease (44). Our study population included 44% of patients who presented with de novo metastatic disease, which was confirmed by review of their medical records. This high proportion of de novo stage IV disease was also observed in all three patient cohorts from the separate institutions. This may be due to peculiarities in patient populations referred to and treated at large academic centers or may reflect a genuine change in the HER2-positive metastatic patient population. Highly effective trastuzumab and pertuzumab containing adjuvant chemotherapies significantly reduced recurrence rates of stage I-III HER2-positve breast cancer which could lead to a shift in the proportion of recurrent versus de novo metastatic cases. Registry data does in fact suggest that in a community setting, de novo HER2-positive MBC approaches 50% of newly diagnosed cases (45). This unusual patient composition raises the possibility that our patient mix might have influenced our results and could also limit the extrapolation of our results to different patient populations. In one previous observational cohort study of patients with HER2-positive MBC (N=1,023), patients with de novo MBC had a 28% and 23% lower hazard of progression and death, respectively, compared with patients with recurrent MBC (46). However, in another retrospective analysis of 331 patients with HER-positive MBC, the response rates and PFS to first-line trastuzumab-based therapy did not differ significantly

between de novo and recurring stage IV disease (47). In our study, when we examined the rates of PDT in patients with de novo metastatic disease (PDT 30.3%) and patients with metastatic recurrence greater than 1 year after initial diagnosis (PDT 29.0%), we observed no significant difference. Overall, this suggests that the high percentage of de novo metastatic disease in our study population may not limit the generalizability of the results.

Conclusions and Future Directions

T-DM1 was approved by the FDA on February 23, 2013 as second-line therapy for patients with HER2-positive MBC who previously received trastuzumab and a taxane (27). As evidenced in this retrospective analysis, T-DM1, however, is broadly used after many previous lines of therapy for metastatic disease which is supported by results from several Phase II and Phase III trials. Additionally, the combination of trastuzumab and chemotherapy was recently replaced by pertuzumab plus the combination of trastuzumab and chemotherapy as the first line for metastatic HER2-positive breast cancer with pertuzumab's approval on June 8, 2012 (20). The order of these drug approvals combined with the current treatment recommendations result in patients now diagnosed with metastatic HER2-positive breast cancer receiving pertuzumab in combination with trastuzumab and a taxane as first-line therapy followed by T-DM1 when they progress. In our study, many patients were diagnosed with metastatic disease before the approval of either pertuzumab or T-DM1, resulting in a population that received multiple prior lines of therapy before receiving these newer HER2-targeted therapies. Despite the

multiple prior lines of therapy including pertuzumab, T-DM1 has shown clinical benefit in our patients comparable to results reported in clinical trials (with caveats outlined above).

Currently, there is one ongoing study that will examine the efficacy of T-DM1 specifically in patients who have received prior pertuzumab (Clinicaltrials.gov identifier: NCT01835236). This study is recruiting patients with previously untreated metastatic HER2-positive breast cancer and randomizes patients to two arms: pertuzumab plus trastuzumab plus chemotherapy vs. pertuzumab plus trastuzumab, both followed by T-DM1 in the case of progression. This study provides the first clinical data on the efficacy of T-DM1 in a pertuzumab pre-treated population. A company-sponsored nationwide registry study (SystHERs Registry, NCT01615068) is also underway to collect information on treatment patterns and treatment sequencing for HER2-positive MBC. Otherwise, there are no other ongoing or planned trials specifically examining the efficacy of T-DM1 in a pertuzumab pre-treated population that represents the majority of future HER2-positive metastatic patients. Without trials examining the efficacy of T-DM1 in patients who previously have received pertuzumab, our current retrospective analysis provides valuable and otherwise unavailable data on T-DM1's currently utilized role in the treatment of metastatic HER2-positive breast cancer.

Table 1. Demographic and disease characteristics of study population (N=82)

Characteristic	No.	%
Age—years		
Median	54	
Range	29 – 97	
Treatment Location		
Yale	21	25.6
OSU	23	28.0
MD Anderson	38	46.3
Hormone-receptor status		
ER and/or PR-positive	51	62.2
ER and PR-negative	31	37.8
Neoadjuvant or Adjuvant Therapy		
Chemotherapy + Trastuzumab + Pertuzumab	2	2.4
Chemotherapy + Trastuzumab	23	28.0
Chemotherapy alone	7	8.5
Trastuzumab + Pertuzumab without Chemotherapy	1	1.2
Trastuzumab without Chemotherapy	1	1.2
Endocrine Therapy	15	18.3
Unknown	1	1.2
Adjuvant Trastuzumab for One Year		
Yes, Completed	22	26.8
Yes, Did not Complete	5	6.1
No	53	64.6
Unknown	2	2.4
Prior Lines of Therapy for Metastatic Disease Before T-DM1		
0	2	2.4
1	24	29.3
2	17	20.7
3	19	23.1
4	13	15.9
5 or more	7	8.5
Number of Distinct Sites of Metastases		
1	23	28.0
2	22	26.8
3	22	26.8
4	11	13.4
5	4	4.9
Site of Metastasis at Time of Starting T-DM1		
Bone	53	64.6
Lung	38	46.3
Liver	33	40.2
Soft Tissue	49	59.8
Brain	23	28.0
Disease Free Interval		
De Novo Stage IV Disease	36	43.9
Metastatic Recurrence ≤1 Year after Diagnosis	14	17.1
Metastatic Recurrence >1 Year after Diagnosis	32	39.0

Table 2. Prior therapies for metastatic disease before T-DM1

Prior drugs in the metastatic setting	**Number of patients**	**(%)**
Trastuzumab	79	96.3
Pertuzumab	79	96.3
Taxane	72	87.8
Hormonal Therapy	34	41.5
Lapatinib	19	23.2
Capecitabine	13	15.9
Vinorelbine	10	12.2
Carboplatin	9	11.0
Gemcitabine	5	6.1
Doxorubicin	4	4.9
Eribulin	3	3.7
Bevacizumab	3	3.7
Cyclophosphamide	2	2.4
Her2 Vaccine Trial	2	2.4
Ixabepilone	2	2.4
IGF-1R/IR Inhibitor	1	1.2
HDAC Inhibitor	1	1.2

Table 3. Investigator reported rate of tumor response (TR), rate of prolonged duration on therapy (PDT) and rate of any benefit (AB). TR is defined as physician reported clinical or imaging response. PDT is defined as duration of T-DM1 therapy ≥ 6 months regardless of best response. AB is defined as TR (for any duration) and/or PDT. Results with 95% confidence intervals (CI).

Characteristic	No. of Patients	%	95% CI
All Patients	78		
PDT	24	30.8	20.6 to 41.1
TR	14	17.9	9.4 to 26.4
AB	29	37.2	26.5 to 47.9
Hormone Receptor Status			
ER-positive and/or PR-positive	49		
PDT	16	32.7	19.6 to 45.8
TR	10	20.4	9.1 to 31.7
AB	21	42.9	29.0 to 56.8
ER-negative and PR-negative	29		
PDT	8	27.6	11.3 to 43.9
TR	4	13.8	1.3 to 26.4
AB	8	27.6	11.3 to 43.9
T-DM1 as ≤ 2nd Line Therapy	26		
PDT	9	34.6	16.3 to 52.9
TR	6	23.1	6.9 to 39.3
AB	11	42.3	23.3 to 61.3
T-DM1 as > 2nd Line Therapy	52		
PDT	15	28.8	16.5 to 41.1
TR	8	15.4	5.6 to 25.2
AB	18	34.6	21.7 to 47.5
Prior Lapatinib	18		
PDT	5	27.8	7.1 to 48.5
TR	2	11.1	0 to 25.6
AB	5	27.8	7.1 to 48.5
Recurrence Free Interval			
De Novo Metastatic Disease	33		
PDT	10	30.3	14.6 to 46.0
TR	7	21.2	7.3 to 35.2
AB	13	39.4	22.7 to 56.1
Met. Recurrence <1 year after diagnosis	14		
PDT	5	35.7	10.6 to 60.8
TR	3	21.4	0 to 42.9
AB	6	42.9	17.0 to 68.8
Met. Recurrence >1 year after diagnosis	31		
PDT	9	29.0	13.0 to 45.0
TR	4	12.9	1.1 to 24.7
AB	10	32.3	15.8 to 48.8

Table 4. Our Results in the Context of Phase II and III trials of T-DM1 to date.

Trial	Study Design	Patients' Prior Therapies for Metastatic Disease	Treatment Arm(s)	T-DM1 Results
MARIANNE (32)	Randomized Phase III	-No prior therapy for MBC	3 arms: - T-DM1 + pertuzumab (n=363) - T-DM1 (n=367) -Trastuzumab + taxane (n=365)	ORR: -TDM1: 59.7% -TDM1 + P: 64.2% PFS: - TDM1: 14.1 mo. - TDM1 + P: 15.2 mo.
TDM4450g (31)	Randomized Phase II	-No prior therapy for MBC	- T-DM1 (n=67) - Trastuzumab + Docetaxel (n=70)	-ORR: 64.2% -PFS: 14.2 mo.
EMILIA (27)	Randomized Phase III	- Trastuzumab and a taxane. No prior lapatinib or capecitabine. -Previous regimens for MBC: - 0 or 1: 61% - >1: 39%	2 arms: - T-DM1 (n=495) - Lapatinib + Capecitabine (n=496)	-ORR: 43.6% - CR: 1.0%, PR: 42.6% -PFS: 9.6 mo.
TH3RESA (30)	Randomized Phase III	-2+ previous HER2-targeted agents (trastuzumab and lapatinib). -Previous regimens for MBC: - ≤3: 39% - 4-5: 33% - >5: 28%	2 arms: - T-DM1 (n=404) - Physician's choice (n=198)	- ORR: 31% - PFS: 6.2 mo.
TDM4258g (28)	Single-arm Phase II	-At least 1 prior HER2-targeted therapy and chemotherapy. -Median prior anticancer agents for MBC: 5.0	-T-DM1 (n= 112)	- ORR: 25.9% -CR: 0%, PR: 25.9% - PFS: 4.6 mo.
TDM4374g (29)	Single-arm Phase II	- Trastuzumab, lapatinib, an anthracycline, a taxane, and capecitabine - Median prior anticancer agents for MBC: 7.0	-T-DM1 (n=110)	- ORR: 34.5% -CR: 0%, PR: 34.5% - CBR: 48.2% - PFS: 6.9 mo.
Our Study	Single-arm, Retrospective	-At least prior trastuzumab and pertuzumab. -Prior lapatinib: 23% -Previous regimens for MBC: -0 or 1: 32% - >1: 68%	T-DM1 (n=82)	- TRR: 17.9% - PDT: 30.8% -median duration on therapy: 4.0 mo.

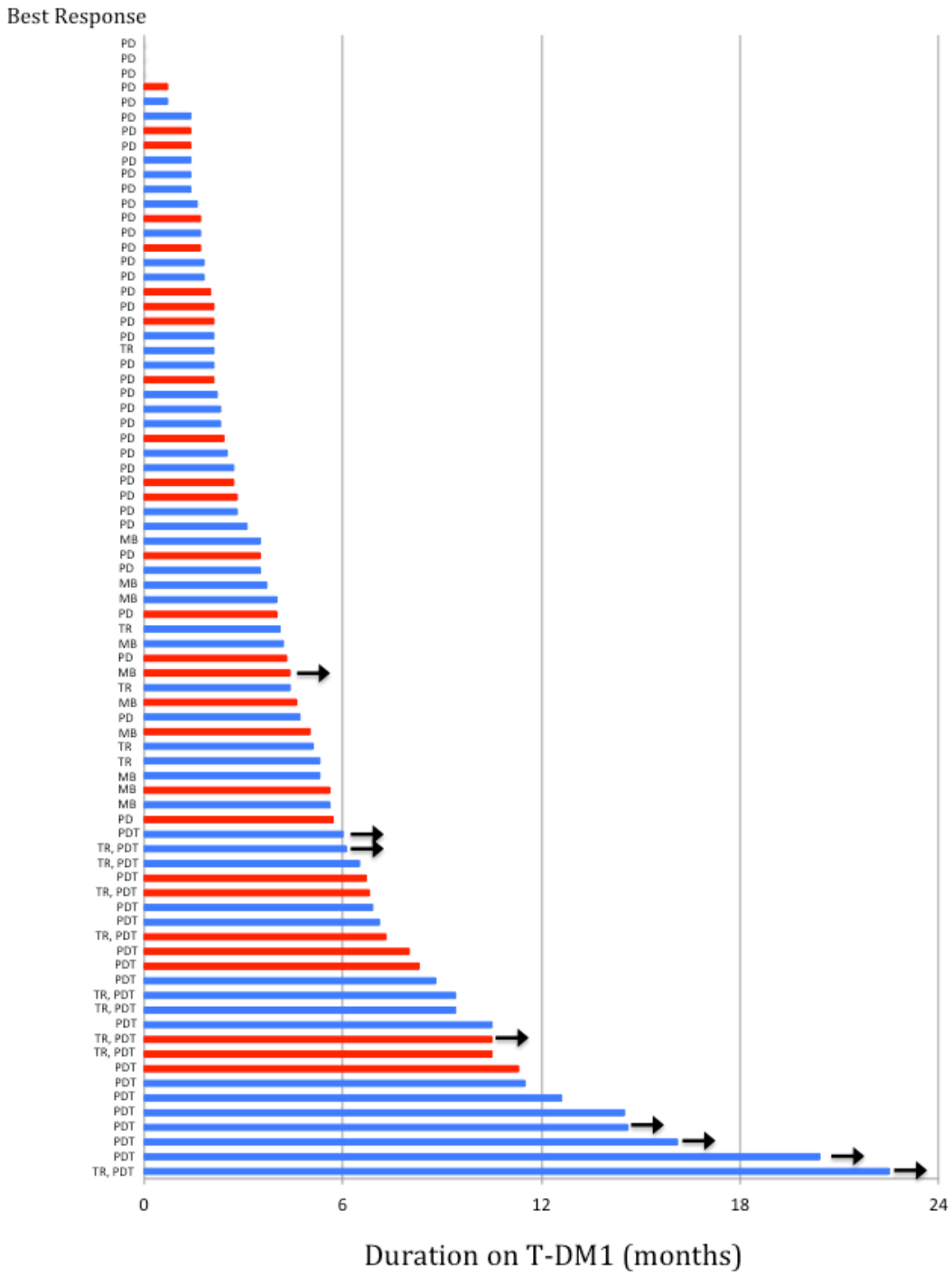

Figure 1. Duration of T-DM1 therapy (in months) and best response by investigator assessment. Each bar represents the duration of therapy for the corresponding patient, beginning at day zero (administration of first dose of T-DM1). Three patients received only one dose of T-DM1 (duration=0). Arrow indicates that patient was still receiving T-DM1 at time of data collection. Patients with ER and/or PR-positive disease are represented in blue; patients with ER and PR-negative disease are represented in red. Best response for each patient is indicated in the left column. PD, progressive disease; MB, minimal benefit; TR, tumor response; PDT, prolonged duration on therapy. Nine patients with PDT had a tumor response.

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