**Breast Cancer Drug Trastuzumab Induces Cardiac Toxicity: Evaluation of HER2 as a Potential Diagnostic and Prognostic Marker**

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Canadian Journal of Physiology and Pharmacology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>cjp-2018-0005.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Review</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>08-Feb-2018</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Johnson, Taylor; University of Central Florida Burnett School of Biomedical Sciences, Division of Metabolic and Cardiovascular Sciences Singla, Dinender; University of Central Florida,</td>
</tr>
<tr>
<td>Is the invited manuscript for consideration in a Special Issue:</td>
<td>IACS Orlando</td>
</tr>
<tr>
<td>Keyword:</td>
<td>HER2; Breast Cancer; Trastuzumab; Trastuzumab Emtansine; Cardiac Toxicity</td>
</tr>
</tbody>
</table>
Breast Cancer Drug Trastuzumab Induces Cardiac Toxicity: Evaluation of HER2 as a Potential Diagnostic and Prognostic Marker

Taylor A. Johnson and Dinender K. Singla

Division of Metabolic and Cardiovascular Sciences, Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL, 32816

Running Title : HER2 Therapy and Cardiovascular Complications

Address for Correspondance :
Dinender K. Singla, PhD, FAHA, FIACS
Division of Metabolic and Cardiovascular Sciences
Burnett School of Biomedical Sciences
College of Medicine
University of Central Florida
4110 Libra Drive
Orlando, FL 32816, USA

Email: Dinender.Singla@ucf.edu
Phone: 407-823-0953
Fax: 407-823-0956
Abstract

Breast cancer is one of the most prevalent forms of cancer in the United States and worldwide. Cancer occurs through the uncontrolled development of new abnormal cell growth. Clinicians and researchers strive to improve diagnostics and treatments in pursuit of remedying breast cancer, while limiting or removing any potential side effects that may arise. Unfortunately, traditional treatments, such as anthracyclines (i.e. Doxorubicin) can damage the cardiovascular system. Recent strategies have utilized antibody-based compounds as singular treatments, or in conjunction with other treatments, with the aim to minimize side effects. The Human Epidermal Growth Factor Receptor 2 (HER2) protein has been the target of numerous antibody-based breast cancer therapies, such as Trastuzumab (TZM) and Trastuzumab Emtansine (T-DM1). This review will discuss the HER2 receptor as a diagnostic marker in targeting breast cancer using the therapeutic agents TZM and T-DM1, as well as discuss the induced cardiac toxicity following TZM and T-DM1 treatments.
Keywords:

HER2; Breast Cancer; Trastuzumab; Trastuzumab Emtansine; Cardiac Toxicity
Abbreviations

ASCO (American Society of Clinical Oncology)
ALT (Alanine Transaminase)
AST (Aspartate Transaminase)
CAP (College of American Pathologists)
CE (Cardiac Event)
Doxo (Doxorubicin)
EGFR (Epidermal Growth Factor Receptor)
FDA (Food and Drug Administration)
HER (Human Epidermal Growth Factor Receptor)
HF (Heart Failure)
IHC (Immunohistochemistry)
ISH (In Situ Hybridization)
LVEF (Left Ventricular Ejection Fraction)
PI3K (Phosphoinositide 3-Kinase)
RT-PCR (Real-time Polymerase Chain Reaction)
SMCC (N-Succinimidyl 4-(Maleimidomethyl) Cyclohexane-1-Carboxylate)
T-DM1 (Trastuzumab Emtansine)
TK (Tyrosine Kinase)
TZM (Trastuzumab)
VEGF (Vascular Endothelial Growth Factor)
Introduction

Normal tissue function requires that body cells maintain homeostatic conditions through normal cell division, mediated by apoptosis (Seitz et al., 2000). Unfortunately, in certain circumstances, such as in genetic disorders, cells can divide in an uncontrolled manner, leading to new abnormal cell growth (Rakoff-Nahoum, 2006). This group of new and atypical cells form a mass of tissue called a malignant tumor, more commonly known as cancer (Rakoff-Nahoum, 2006). Many different types of cancers can be developed in the body (American Cancer Society & Atlanta, 2017). The most prevalent types of cancers, according to incidence and mortality statistics reported by the American Cancer Society, are lung cancer, colon-rectal cancer, pancreatic cancer, prostate cancer, and breast cancer (specifically in females) (American Cancer Society & Atlanta, 2017).

Breast cancer is considered to be a heterogeneous disease in which characterization depends on the cellular involvement, diversity between tumors, appearance of certain markers, and development of metastasis (Redig & McAllister, 2013). Therefore, the complexity of breast cancer development can influence the decision of which treatment options are best suitable for an individual patient. The most common treatment options include surgery (removal of the cancerous tissue), radiation (killing cancerous cells using x-ray), and chemotherapy (chemical treatment to kill cancer cells) (Baskar et al., 2014). Unfortunately, these treatments significantly affect the normal cell populations that reside adjacent to the cancer cells as well as in different tissues, such as the heart (Baskar et al., 2014). Therefore, recent research efforts have aimed to identify breast cancer cell specific markers. In this review, we will discuss the
breast cancer cell marker, human epidermal growth factor receptor 2 (HER2), in addition to methods of breast cancer diagnosis, treatment options, the mechanism of anti-breast cancer antibody treatment, and cardiotoxicity that results from anti-breast cancer treatment.

**Human Epidermal Growth Factor Receptor 2**

Human Epidermal Growth Factor Receptor 2 (HER2/erbB2), a 185-kD transmembrane protein in the epidermal growth factor receptor (EGFR) family of tyrosine kinases (TKs), is a receptor that helps regulate normal cell growth, division, and repair when needed (Dai et al., 2016). However, when the HER2 gene is not working correctly and expresses aberrant levels of the HER2 protein, it promotes breast cancer due to uncontrolled cell growth and division (Dai et al., 2016).

HER2 has also been shown to play a role in cardiovascular development and regulation. Studies have documented HER2 in the development of the fetal cardiovascular system, dilated cardiomyopathy prevention, ventricular muscle and valve development, cardiomyocyte survival, and sympathovagal coordination (Okoshi et al., 2004; Camenisch et al., 2002; Crone et al., 2002; Ozcelik et al., 2002; Zhao et al., 1998). Overexpression of HER2 has been shown to lower reactive oxygen species in cardiomyocytes and H9C2 cardiomyoblast cells, and has been suggested to play a role in antioxidant defenses (Belmonte et al., 2015). HER-activated cell survival networks normally protect the heart from agents that would cause cardiovascular damage (De Keulenaer et al., 2010). In fact, HER2-deficient mice demonstrated left ventricular dysfunction, chamber dilation, decreased contractility, and increased susceptibility to drug toxicity (Crone et al., 2002). In addition to cardiac cells, gastric cells (Abrahao-
Machado & Scapulatempo-Neto, 2016) and esophageal cells (Huang et al., 2013) were also observed to have the HER2 receptor.

HER2 oncogene expression is amplified in 25-30% of human primary breast cancers (Slamon et al., 1989) and expresses nearly 1-2 million copies of the receptor per breast cancer tumor cell (Lewis Phillips et al., 2008). HER2 is commonly linked to cell survival pathways such as the phosphoinositide-3-kinase (PI3K) pathway (Yuan & Cantley, 2008). Predominance of the HER2 receptor on cancer cells makes it a strong candidate to be used as a common diagnostic marker in breast cancer detection assays (Weigel & Dowsett, 2010). Furthermore, scientists have developed monoclonal antibodies against the HER2 receptor in order to restrict breast cancer cell growth.

**HER2 as a Diagnostic Marker**

There have been challenges with proper diagnosis of breast cancer. In 2002, the National Surgical Adjuvant Breast and Bowel Project (NSABP) generated the B-31 HER2 protocol to screen patients for study eligibility. 18% of the assays used were inconclusive using HercepTest™ immunohistochemistry (IHC) or fluorescent in situ hybridization (ISH) (Paik et al., 2002). In addition, smaller laboratories were shown to produce over-reaching positive results, resulting in inconsistent data (Paik et al., 2002). To address these inconsistencies in diagnosis, a joint panel, coordinated by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP), constructed guidelines in 2007 that included improved instructions on performing interpretation of IHC and ISH assays. The guidelines and protocols were revised in 2013 by ASCO and CAP due to the emergence of new testing methodology, such as bright-field in situ hybridization and real-time polymerase chain reaction (RT-
PCR), which warranted an updated review of the diagnostic procedures (Wolff et al., 2013). While the committee deemed these new DNA and mRNA techniques beneficial for clinical utility, they required validation with a Food and Drug Administration (FDA) approved assay as a confirmatory means of diagnosis (Wolff et al., 2013). HER2 testing is a critical component necessary to guide clinicians in the decision to prescribe therapeutics in a more personalized way, by targeting HER2 either specifically or non-specifically. The first method outlined by ASCO and CAP in the selection criterion of histological diagnostic principles makes use of an immunohistochemistry protocol; a procedure in which an antibody specific for the HER2 protein is used to identify expression as shown in Figure 1 (Wolff et al., 2013). In this procedure, tissue isolated from a patient’s primary tumor or metastatic site is fixed for preservation, and subsequently stained for HER2 expression. The staining intensity allows for classification of the patient’s specimen into one of four histological grades (IHC 0, 1+, 2+, 3+) that can be used to establish whether the patient is considered positive or negative for HER2 expression, and if additional testing is necessary. The process for this classification system is outlined in Figure 1. In this method, the ASCO and CAP guidelines define the patient as positive when the biopsy demonstrates intense circumferential cell staining of HER2 on greater than 10% of the biopsied tumor cells (Figure 1) (Wolff et al., 2013). Alternatively, when there is no or incomplete circumferential staining with faint intensity, the patient is considered HER2 negative (Wolff et al., 2013). This classification system is clinically significant because it provides a method of analysis that is standardized across all clinical providers; therefore, decreasing the likelihood of false-positives and administration of non- HER2 specific
and/or potentially harmful therapies. In addition to IHC, ASCO and CAP have defined criterion for a second diagnostic test using in situ hybridization. The procedure for this additional diagnostic differential method is shown in Figure 2. This test can be used either singularly or in combination with IHC to support a diagnosis. ISH utilizes a DNA probe that facilitates quantification of the number of HER2 copies present within the cell nucleus, termed as a single probe for HER2, shown in the bottom left section of Figure 2. The single probe ISH method quantifies the number of cells positive for the HER2 signal in analysis of twenty non-overlapping cells within the patient specimen. If the biopsy results in an average of ≥6.0 signals/cell among the quantified cells, the patient is reported as positive and therapies most effective against HER2 cancers are significantly considered (Figure 2). However, when the average number of signals is <4, the patient is reported as negative and non-HER2 specific therapies may prove more effective (Figure 2). The guidelines also outline the use of a dual probe for patient specimens that fall within the intermediate range of a ≥ 4.0 and <6.0 signal average (Figure 2). This classification is reported as equivocal, meaning that it requires additional testing, such as use of a dual probe ISH test. The dual probe targets HER2 in addition to a centromeric probe for chromosome 17 (CEP17), a HER2 genotypic abnormality that results in polysomy of chromosome 17 in cancer cells (Page et al., 2017). The ratio derived from the HER2 probe signal to CEP17 can subsequently classify a patient sample as HER2 positive and further clarify treatment options (Wolff et al., 2013).
HER2 Treatment with Trastuzumab (TZM)

Trastuzumab (Herceptin) (Genentech, Inc.), a monoclonal antibody used to treat HER2 receptor positive patients, was approved by the United States FDA for cancer therapy in 1998 (Slamon et al., 2001; Smith et al., 2007). Structurally, TZM contains two antigen-specific sites that bind to the HER2 extracellular domain (Albanell et al., 1996). Patients prescribed TZM every 3 weeks for one year following chemotherapy demonstrated an increase in disease-free survival (Piccart-Gebhart et al., 2005). In addition, tumor regression of up to 60% was exhibited in patients given weekly Trastuzumab doses after 3 weeks of single agent treatment (Mohsin et al., 2005).

Mechanism of TZM treatment in Cancer Cells

Recent in vitro and in vivo studies support TZM treatment of cancer cells, resulting in down modulation of HER2, dimerization prevention, reverse cytokine resistance, tumor growth inhibition, and impairment of vascular endothelial growth factor (VEGF) expression (Izumi et al., 2002; Ritter et al., 2007; Sliwkowski et al., 1999; Valabrega et al., 2007). Moreover, TZM has also been shown to exhibit multifactorial effects resulting in cancer growth inhibition such as inhibition of ligand-independent HER2 signaling, activation of antibody dependent cell-mediated cytotoxicity (ADCC), inhibition of extracellular domain (ECD) (Figure 3) (Clynes et al., 2000; Cooley et al., 1999), co-expression of EGFR and/or EGFR ligands (Ritter et al., 2007; Dua et al., 2010; El-Sahwi et al., 2010; Juntila et al., 2009; Tse et al., 2012), and modification of angiogenesis and properties of vasculature (Izumi et al., 2002; Petit et al., 1997; Viloria-Petit et al., 2001).
Mechanisms of TZM Treatment in Cardiac Cells

TZM treatment significantly induces cardiac toxicity as a major side effect. It has been reported that HER2 receptor is necessary for cardiac development and cardiomyocyte cell survival, as we mentioned in this paper previously (Crone et al., 2002). Therefore, treatment with TZM inhibits HER2 receptor function to attenuate cancer cell growth, but unfortunately also inhibits HER2 receptor presence on healthy cardiomyocytes in the heart which subsequently induces mitochondrial dysfunction, oxidative stress and apoptosis (Figure 3) (Cardinale et al., 2010; Gordon et al., 2009). Development of oxidative stress and apoptosis has been well documented in various heart diseases in the progression of left ventricular dysfunction, ultimately leading to heart failure (Hansel et al., 2010; Chien, 2006; Levine, 2005; Singal & Iliskovic, 1998). Therefore, presence of increased oxidative stress and cardiomyocyte apoptosis with TZM treatment also leads to heart dysfunction in these cancer patients as a side effect (Figure 3), as reported elsewhere (ElZarrad et al., 2013; Hahn et al., 2014). Moreover, the exact mechanisms of cardiotoxicity induced by TZM is complex and not very well established; however, various other mechanisms leading to cell death has also been reported such as damage to myofibers and reduction of myofiber thickness (ElZarrad et al., 2013), hindering neuregulin function (Sandoo et al., 2015), inhibiting autophagy (Mohan et al., 2016), stimulating oxidative stress (Sandoo et al., 2015; Dirican et al., 2014; Mohan et al., 2016), inhibiting the PI3K/AKT pathway (Junttila et al., 2009; Mohsin et al., 2005) and several cytoskeletal proteins (i.e. ALC1, MLCA2a, Connectin) and transcription factors (i.e. ATF3, DBP) (ElZarrad et al., 2013). TZM treatment develops into acute and chronic heart dysfunction. Therefore, development of strategies for early...
detection of cardiac dysfunction following TZM treatment would reduce hospitalizations, and avoid development of chronic heart dysfunction.

**Acute Cardiac Dysfunction and Early Detection to Reduce Disease Burden**

Cardiotoxicity has been reported to occur in 3-36% of patients that receive TZM-based chemotherapy (Srikanthan *et al.*, 2017). TZM treatment hinders heart contractility by interfering with the QT interval and ventricular polarization (Hansel *et al.*, 2010). A recent study by Dirican *et al.* (2014) observed that patients, post-TZM administration, had a slower QT interval (.398 seconds pre-TZM vs. .430 seconds post-TZM), which indicates ventricular depolarization and repolarization dysfunction; however, the QRS complex duration was unchanged (Dirican *et al.*, 2014). LVEF also decreased from 65% to 60% upon TZM administration (Dirican *et al.*, 2014). In a separate study, ElZarrad (2013) revealed that TZM administration in C57BL/6 mice decreased left ventricular posterior wall thickness, LVEF, and fractional shortening, yet little changes occurred in the left ventricular diastolic and systolic diameter (ElZarrad *et al.*, 2013). A third study revealed 28% of TZM administered patients developed cardiotoxicity and registered significant reductions in LVEF, representative of decreased cardiac function (Baron *et al.*, 2014). Specifically, patients that registered between a LVEF baseline of 60.8 ±6.9 % declined over one year to 57.68 ±8.4 % (Baron *et al.*, 2014). Further, TZM administration has a long serum half-life (Leyland-Jones *et al.*, 2003), which may contribute to the higher prevalence of cardiotoxicity (2.5 fold) in TZM patients compared to non-TZM patients (Viani *et al.*, 2007). African-Americans had a higher risk of developing cardiotoxicity, however more studies are necessary to evaluate susceptibility in races and genders (Baron *et al.*, 2014). Collectively, it can be inferred that TZM
treatment promotes the development of cardiac contractility events with high incidence and reduces LVEF function; however, early detection of TZM induced cardiac dysfunction can decrease the risk of developing chronic heart dysfunction leading to heart failure.

Recent studies show that TZM induced left ventricular cardiac dysfunction leading to chronic heart dysfunction is actually reversible (Zeglinski et al., 2011; Nair & Gongora, 2016; Wadhwa et al., 2009) if proper management is provided to the patients. Standard methods to detect chemotherapy related cardiac dysfunction (CRCD) include the use of echocardiography and presence of clinical symptoms. The use of echocardiography determines structural damage which may not have been clearly developed in the early stage of the disease development, and therefore may be reversible once the TZM regimen is stopped (Onitilo et al., 2014; Jiji et al., 2012). However, this acute dysfunction may damage the cardiomyocytes in the myocardium which then start releasing specific proteins and lead to apoptosis. In this case, it is too late for reversal. Therefore, implementing the use of biomarker panels is becoming a newly established method for early detection of CRCD (Srikanthan et al., 2017). Moreover, after TZM treatment cardiac troponin I has been shown to increase in animal models and humans (ElZarrad et al., 2013; Zeglinski et al., 2011) which could be harnessed as a future ELISA target to detect cardiac dysfunction developing in the heart. Identification of the level of cardiac dysfunction following TZM treatment will allow for optimization of TZM administration to significantly reduce potential of long-term side-effects and severe consequences.
Transtuzumab Induced Chronic Heart Dysfunction

Several studies have documented the severity of cardiac events (CE) by assigning grades and reporting changes in LVEF that occur during treatment. Guarneri (2006) revealed 28% of patients in a metastatic breast cancer study reported experiencing a CE; 15.6% experienced a grade 2 CE (asymptomatic with 40-50% LVEF), and 10.4% experienced a grade 3 CE (congestive heart failure with 40-50% LVEF) (Guarneri et al., 2006). Furthermore, in a 2 year follow-up to the Herceptin Adjuvant (HERA) trial, eleven percent of patients prescribed TZM for one year experienced a grade 3 or 4 cardiac event as defined by the New York Heart Association as symptomatic congestive heart failure with LVEF below 50% and a 10% decrease from baseline (Smith et al., 2007). Eight years post-HERA, approximately 1/3 of the patients that were discontinued from the trial were due to a cardiac-related disorder, including congestive heart failure and decreased LVEF (de et al., 2014). In addition, 8 years post-HERA significant decreases in LVEF were noted in 4.1% and 7.2% of patients prescribed 1 and 2 years of TZM treatment, respectively. Interestingly, patients that previously demonstrated significant reductions of LVEF (less than 50%) showed recovery of LVEF over time, suggesting some reversibility (de et al., 2014).

Further studies were conducted to evaluate cardiac toxicity in elderly patients. In a study of 68,536 elderly breast cancer patients, 10% (6,829) had experienced cardiomyopathy or HF (Tsai et al., 2014). Importantly, use of TZM is associated with a two-fold increase in cardiac disorders in patients at 66 years of age and older, regardless of the stage of the disease in the patients’ diagnosis, history of hypertension, or prior drug treatment (i.e. anthracyclines, taxanes). This study alludes to the potency
of utilizing TZM in elderly patients. A separate study reports approximately 9% of TZM-treated patients 70 years of age and older developed symptomatic HF (Serrano et al., 2012). These sets of data suggest that TZM is an excellent cell specific anti-breast cancer drug, though it still can cause significant acute and chronic heart dysfunction.

**Cardiac Toxicity with Modified TZM Drug Trastuzumab Entansine (T-DM1)**

Although TZM is considered the standard therapy for HER2 breast cancer, nearly 40% of patients do not respond to the current regimens and patients develop cardiotoxicity (Marty et al., 2005). The exact reason for ineffectiveness of TZM and developed cardiotoxicity is not clearly understood. Recently, a new drug called Trastuzumab Emtansine (T-DM1) was developed through the combination of two components: (a) a monoclonal antibody against HER2 receptor and (b) a cytotoxic agent.

T-DM1 is constructed using the antibody, TZM, and a maytansinoid, DM1. T-DM1 is a larger, hydrophilic molecule, that requires cells to undergo endocytosis for drug activity (Dieras & Bachelot, 2014). T-DM1 requires N-Succinimidyl 4-(Maleimidomethyl) Cyclohexane-1-Carboxylate (SMCC), a cross-linking agent that creates a thioester bond (Lewis Phillips et al., 2008; Burris, III et al., 2011) allowing the antibody to release the drug upon tumor site arrival (Xie & Blattler, 2006). TZM acts as the delivery vehicle for DM1, which acts independently of HER2 signaling to have a cytotoxic effect (Dieras & Bachelot, 2014). DM1 is a derivative of maytansine, which has been previously recognized as an anticancer drug that inhibits microtubule construction, induces cell cycle arrest, and stimulates apoptosis (Dieras & Bachelot, 2014; Cassady et al., 2004; Chari, 2008). DM1 has been shown to be 25-270 times more potent than
common chemotherapy agents, such as Paclitaxel, and 180-4000 times stronger than Doxorubicin (Doxo) (Junttila et al., 2011). In addition, T-DM1 has shown tumor growth inhibition and regression in the in vivo model 30 days post treatment in mouse mammary tumor virus (MMTV)-HER2 carrying mice (Lewis Phillips et al., 2008). These initial results suggest T-DM1 could be a more potent and targeted treatment option in comparison to TZM alone.

Due to the recent approval of T-DM1 (2013, compared to TZM approval in 1998), little is known about its adverse effects. However, thus far data shows modified HER2 treatment has reduced cardiac toxicity (Lewis Phillips et al., 2008). Currently, most studies evaluate T-DM1 in replacement of TZM or in combination with another drug. Since T-DM1 contains Trastuzumab, the FDA requires patients treated with T-DM1 have baseline and regular assessments during treatment. Current studies have shown decline in LVEF due to T-DM1 treatment (Callahan, 2014; Dieras et al., 2014). Additionally, T-DM1 administration has shown to stimulate other complications in patients. T-DM1 was administered in a clinical trial to 148 early stage breast cancer (EBC) patients, comprising of many ages, races, and geographical locations (Krop et al., 2015). Patients were administered T-DM1 approximately 1 year after anthracycline-based chemotherapy. 31.8% of patients reported epistaxis and 21.6% reported thrombocytopenia. Hypertension was also reported in 5.4% of patients. Grade 3 events documented in patients included thrombocytopenia (8.1%), neutropenia (5.4%), and increases in aspartate and alanine transaminases (AST and ALT) (both at 7.4%) (Krop et al., 2015).
Telangiectasia has been documented in multiple clinical studies. One case study evaluated a 43-year-old woman who developed telangiectasia accompanied with gingival bleeding, nasal mucosal bleeding, and rectal mucosal bleeding (Kwon et al., 2016). Echocardiography revealed normal left ventricular function, but right ventricular dilation and flattening of the interventricular septum. Although catheterization further confirmed severe pulmonary hypertension, multiple procedures ruled out other causes of the hypertension. Upon exiting the T-DM1 trial, her telangiectasia significantly improved; however, she continued to have breathing problems (Kwon et al., 2016). A separate study documented 5 different female patients, ranging from 43 to 66 in age, which developed telangiectasia post-initial treatment with T-DM1 (Sibaud et al., 2014).

Additionally, multiple groups prescribed singular treatments or mixed therapies that included TZM or T-DM1. The combined therapy of TZM, and cyclophosphamide (an anthracycline) revealed cardiotoxicity in 27% of patients in addition to anemia (35%) and leukopenia (52%). In contrast, the group that received TZM and Paclitaxel had the lowest cardiovascular complications (13%, 14%, and 24%, respectively) (Slamon et al., 2001). A separate study revealed that cardiovascular complications were high in patients prescribed either Docetaxel or a combined TZM-Docetaxel treatment (Marty et al., 2005). The percentage of patients that experienced epistaxis was higher by 15% in patients prescribed TZM and Docetaxel compared to Docetaxel alone. In addition, more TZM-Docetaxel patients experienced Grade 3 and 4 leukopenia or neutropenia, as well as reductions in LVEF (Marty et al., 2005).
These results support the potency and superiority of therapies containing multiple treatments in reducing HER2 breast cancer; however, the combination of therapies could result in developing cardiovascular and other complications.

**Future Perspectives**

In conclusion, Trastuzumab and Trastuzumab Emtansine should be considered a treatment option for patients with HER2 breast cancer; however, each treatment results in complications. At this time, TZM has been well documented to hinder contractility of the heart, in particular reduction LVEF. T-DM1, on the other hand, is relatively new and requires more clinical and non-clinical studies to further understand its potential side effects. Although both treatments have been documented to promote hematological pathologies, such as neutropenia, T-DM1 treatment is strongly correlated with thrombocytopenia and elevated transaminases, AST and ALT. Further testing is necessary to evaluate how these antibody-based treatments influence cardiovascular function, induce these pathologies, and determine whether damage can be minimized or reversed over time.

Marker based trials have been previously proposed as a method to define patients that would benefit from dual-therapy treatments (Blank *et al.*, 2010; Bonastre *et al.*, 2012). To determine cardiotoxicity following TZM treatment, additional biomarkers specific for heart proteins such as cardiac troponin I (cTnI), cardiac myosin light chain 1 (cMLC1), myeloperoxidase (MPO), placental growth factor (PIGF), and growth differentiation factor 15 (GDF-15) need to be evaluated in order to understand their role in pathogenesis prior to the development of cardiac dysfunction and subsequent heart failure (ElZarrad *et al.*, 2013; Putt *et al.*, 2015). In fact, a recent study revealed 62% of
patients that developed TZM-induced cardiotoxicity had elevated Troponin I levels (Cardinale et al., 2010; Fallah-Rad et al., 2011). These biomarkers should be combined with physiological measurements in order to determine which parameter can first, or more accurately, predict drug-induced cardiotoxicity. In a recent editorial utilizing the echocardiography parameter, left atrium global longitudinal strain (LAGLS) was shown to decrease in both Doxo and TZM induced toxicity prior to a decrease in LVEF (Moreno et al., 2016).

Exosomes, small 30-100 nm cell-derived vesicles, have recently become the subject of many research efforts. These vesicles have been utilized as vehicles to deliver a variety of cargo, including numerous anti-cancer drugs (i.e. Doxo) (Batrakova & Kim, 2015; Johnsen et al., 2014; Tavakoli et al., 2017). The contents of exosomes are also of interest, as they contain a variety of miRNAs, siRNAs, and proteins, which could play a beneficial role in inhibition/reversal of disease progression (Batrakova & Kim, 2015; Johnsen et al., 2014). Collectively, the roles of exosomes provide an exciting area of further research that warrants investigation into their use as a delivery method to reduce cardiac toxicity or as a combination therapy approach with TZM or TZM-DM1.
Acknowledgements

The authors would like to thank Kaley Garner and Zahra Tavakoli Dargani for technical assistance in preparing the manuscript.
Disclosures

There are no conflicts of interest to report.
Reference List


American Cancer Society & Atlanta G. Cancer Facts & Figure 2017. 2017. Ref Type: Pamphlet


Figure Legends

Figure 1: Immunohistochemistry (IHC) based HER2 specific diagnostic procedure to identify breast cancer patients.

Tissue is initially isolated from a patient that has been clinically diagnosed with breast cancer using several different methods such as core needle biopsy, extraction of cytologic specimens, and resection. Upon isolation, the tissue is fixed for 6 to 72 hours in a 10% neutral buffered formalin solution within 1 hour of isolation. The tissue is then sectioned and prepared for immunohistochemistry staining using a primary antibody to the HER2 protein. When the circumferential staining pattern is not observed or is incomplete with ≤10% of tumor cells positive for HER2, the patient is considered to be negative and the histological score reported as IHC 0. Similarly, when there is faint or incomplete staining with HER2 expression in >10% of tumor cells, the patient is considered to be negative and reported as IHC 1+. Upon faint or moderate staining with >10% of cells positive for HER2 or complete circumferential staining with ≤10% of cells positive for HER2, the patient is considered to have equivocal expression which warrants confirmation by repeating IHC with a new sample or performing an additional test such as ISH. When circumferential staining is strong and >10% of cells are positive for HER2, the patient is considered HER2 positive and assigned a histological score of 3+. If the tissue sample has any apparent histopathologic discordance, either IHC or another HER2 test must be repeated on a new sample.
Figure 2: *In situ* Hybridization (ISH) based HER2 Assay to diagnose breast cancer patients.

In situ hybridization is used to determine the number of HER2 gene signals per cell nucleus using a DNA probe. Initially, a patient diagnosed clinically with breast cancer undergoes a procedure to isolate tissue from a primary tumor or metastatic site. Subsequent to tissue fixation, a single or double probe can be used to determine HER2 signal or HER2 signal in relation to CEP17, respectively. Using the single probe method, 20 non-overlapping cells are quantified for an average HER2 signal/cell. When this value is <4 the sample is reported as negative. A value equal to or greater than 6 is considered positive. When the average HER2 signal is between these values (4 or greater, but less than 6), it is considered equivocal and a dual probe or IHC test needs to be performed to confirm. The dual probe method analyzes the presence of HER2 in addition to CEP17. HER2/C17 ratios ≥2.0 are considered to be positive. HER2/C17 ratios <2 are further classified based on whether HER2 signal is <4. A HER2/C17 ratio <2 accompanied by a HER2 signal <4 is reported as negative. A HER2/C17 ratio <2 with a HER2 signal >4 and <6 is considered equivocal and requires ISH to be repeated or confirmed with an additional test.

Figure 3. Flow diagram shows proposed mechanisms of action using Trastuzumab in the induction of cardiotoxicity (left side) and anti-cancer effects on cancer cells (right side). (ADCC: Antibody-dependent cell-mediated cytotoxicity, EDC: Extracellular Domain)
Patient Clinically Diagnosed with Breast Cancer

Isolation of tissue from the primary tumor or metastatic site for HER2 protein expression
(Examples of tissue isolation: Core needle biopsy, cytologic specimens, resection specimens)

Tissue is fixed within 1 hour of isolation in 10% neutral buffered formalin for 6 to 72 hours

Immunohistochemistry Staining (IHC)
Use of antibody to analyze HER2 Protein Expression

No observed staining OR Incomplete circumferential staining; Faint intensity; ≤ 10% of tumor cells

Negative HER2 Expression (IHC 0)

Patient is HER2 Negative (Consideration of Treatment options non-specific for HER2)

Incomplete circumferential staining; Faint intensity; > 10% of tumor cells

Negative HER2 Expression (IHC 1+)

Patient is HER2 Negative (Consideration of Treatment options non-specific for HER2)

Incomplete circumferential staining; Faint/Moderate intensity; > 10% of tumor cells OR Complete circumferential staining; Strong intensity; ≤ 10% of tumor cells

Equivocal HER2 Expression (IHC 2+)

Consideration of performing additional HER2 tests or repeating IHC on a new sample

Complete circumferential staining; Strong intensity; > 10% of tumor cells

Positive HER2 Expression (IHC 3+)

Patient is HER2 Positive (Consideration of Treatment options specific for HER2)
Patient Clinically Diagnosed with Breast Cancer

Isolation of tissue from the primary tumor or metastatic site for HER2 gene expression
(Examples of Tissue isolation: Core needle biopsy, cytologic specimens, resection specimens)

Tissue is fixed within 1 hour of isolation in 10% neutral buffered formalin for 6 to 72 hours

In situ Hybridization (ISH)
Determines the number of HER2 copies per nucleus using a DNA probe
(Examples: Bright-Field ISH and FISH assay)

Single Probe for HER2 Signal
Quantification of 20 Non-overlapping cells

Average HER2 Signal/Cell: < 4.0
Report as Negative
Perform Dual Probe ISH or IHC to Confirm

Average HER2 Signal/Cell: ≥ 4.0 and < 6.0
Equivocal

Average HER2 Signal/Cell: ≥ 6.0
Report as Positive

Dual Probe for HER2 and CEP17
Quantification of 20 Non-overlapping cells

HER2/C17 Ratio: < 2.0
Equivocal

HER2 Signal/Cell: < 4.0
Repeat on new specimen or perform IHC

HER2/C17 Ratio: ≥ 2.0
Report as Negative

HER2/CEP17 Ratio: < 2.0
With HER2 Signal/Cell: ≥ 4.0 and < 6.0
Equivocal

HER2/CEP17 Ratio: ≥ 2.0
With HER2 Signal/Cell: ≥ 6.0
Report as Positive

Genetic Aneusomy of Chromosome 17 (CEP17)
Quantification of 20 Non-overlapping cells

Average HER2 Signal/Cell: ≥ 4.0 and < 6.0
Equivocal

Repeat on new specimen or perform IHC

Average HER2 Signal/Cell: ≥ 6.0
Report as Positive
Trastuzumab
(HER2 Receptor Antibody)

HER2 Receptor on Cardiomyocytes
- Impaired Myocardial Genes
- Impaired Mitochondrial Function
- Damages Myofibers
- Increased Oxidative Stress
- Apoptosis of Cardiomyocytes
- Heart Dysfunction / Cardiotoxicity

HER2 Receptor on Cancer Cells
- Inhibits Ligand-independent HER2 Signaling
- Activates ADCC
- Prevents HER2 ECD Shedding
- Apoptosis of Cancer Cells
- Anti-Cancer Effects

Figure 3. Johnson and Singla 2018