Evaluating Trastuzumab in the Treatment of HER2 Positive Breast Cancer

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Abbreviations: Angiotensin-converting-enzyme (ACE), Antibody-dependent cellular cytotoxicity (ADCC), Confidence interval (CI), Cyclin-dependent kinase (CDK), Epidermal growth factor receptor (ErbB), Focal adhesion kinase (Fak), Heart rate recovery (HRR), Human epidermal growth factor receptor (HER), Immunoglobulin G1 (IgG1), Insulin-like growth factor 1 receptor (IGF-IR), Left ventricular ejection fraction (LVEF), Maximum rate of oxygen consumption (VO_{2max}), Messenger RNA (mRNA), Mitogen-activated protein kinase (MAPK), Mucin 4 (MUC4), Neuregulin 1 (NRG1), Phosphatase and tensin homolog (PTEN), Phosphoinositide 3-kinases (PI3K), Protein kinase B (Akt), Proto-oncogene tyrosine-protein kinase Src (Src), Reactive oxygen species (ROS), Signal transducer and activator of transcription (STAT), The National Institute for Health and Care Excellence (NICE), Transforming growth factor beta (TGF-β), Trastuzumab Emtansine (T-DM1), Tumour necrosis factor alpha (TNF-α).

Reply to Editors Comments

We thank you very much for the comments and suggestions as these have been very valuable and helpful for revising and improving our manuscript. We have made revisions according to the referees' comments and suggestions, as described in the authors' response. Each point from the Editor/Reviewer has been answered below.

Point 1: The work described appears complete and clear, however considering the speculation about the cardio toxic effects of trastuzumab I would expand the part related to the exercise prescription. I would suggest adding targeted controls in the pre and post exercise periods such as the maximum aerobic capacity (VO2max); the magnitude of heart rate recovery (HRR) and the time constant of recovery based on different time intervals post-exercise. Those assessments should be proposed and discussed. They both correlate most closely with CVD risk factors and are therefore interesting outcome to be considered in the next generation therapies for patients with HER2-positive breast cancer.

Reply: Thank you for this suggestion. Information has been added that gives more explanation of VO2max and how this relates to CVD risk, as well as CVD risk in those with cancer. The suggestion of putting heart rate recovery (HRR) and the time constant of recovery based on different time intervals post-exercise has also been implemented and discussed in the text, making note of how these should be used alongside current and future practices in clinical trials as measurements of exercise tolerance, CVD risks and all-cause mortality.

Alongside this, exercise intensity was also explored as this is an area that gets little attention. This analysed different approaches on how to measure intensity, which were backed up by clinical trials and were discussed appropriately. Adherence to exercise was also examined further by reviewing current research to suggest recommendations of how best to implement behavioural strategies. The changes made are highlighted blue in the text below.

Point 2: Digital images. Black and white figures must be at gray scale. Line art files must have a 500dpi resolution, while other images must have a 300dpi resolution.

Reply: The images have been reduced to size and the resolution has been increase. Figures are to be uploaded in PowerPoint format as this retains the resolution of the images best.

Point 3: When more than one article is included in the same parentheses, they should be ordered by the year (the oldest the first), and those from the same year, alphabetically.

Reply: These have been changed and ordered by the year (the oldest first), and those from the same year, alphabetically (As highlighted in text).

Point 4: Please, carefully check that all the articles cited in the text are in the Reference list, and vice versa.

Reply: These have been checked and all references in the text and tables are now cited in the reference list.

Point 5: Please, check that the name of the authors is correctly spelled and that the place of work is correct.

Reply: The names of the authors and the place of work is spelled correctly.

Point 6: The Reference list must be re-written according to the Instructions of the Journal. You can get them in our web site. Please, follow them carefully.

Reply: The reference list has been changed to match the instructions of the journal, following the template as describe on the website.

Point 7: The following author(s) has(have) not answered to the Questionnaire: Sam Xu. All the authors must answer before publication of the article. We have recently sent a new e-mail with a link to answer to the questionnaire.

Reply: The author has now responded to the questionnaire in question.

Summary

The transmembrane oncoprotein HER2 is encoded by *ERBB2* gene and overexpressed in around 20% of invasive breast cancers. It can be specifically targeted by Trastuzumab (Herceptin®), a humanised IgG1 antibody. Trastuzumab has been regarded as one of the most effective therapeutic drugs targeted to HER2 positive cancers. However, there are drawbacks, notably cardiotoxicity and resistance, which have raised awareness in clinical use. Therefore, understanding the mechanism of action is vital to establish improved therapeutic strategies. Here we evaluate Trastuzumab application in the treatment of HER2 positive breast cancer, focusing on its mechanistic actions and clinical effectiveness. Alternative therapies targeting the HER2 receptor and its downstream anomalies will also be discussed, as these could highlight further targets that could be key to improving clinical outcomes.

Introduction

Breast cancer is the most common cancer amongst the female population with over 55,000 cases diagnosed in 2018 in the UK (Naseem, 2018). Of these, around 13 - 20% patients have tumour cells overexpressing with human epidermal growth factor receptor 2 (HER2) protein (Rakha et al., 2015). This transmembrane receptor with intracellular tyrosine kinase domains plays vital roles in cell growth and repair mechanisms through downstream signalling pathways (Slamon et al., 1987). HER2 positive cancer is associated with aggressive clinical progression and poorer prognostics outcomes (Mitri et al., 2012). As a result, a disproportionate number of the 11,500 sufferers that pass away each year will be HER2 positive (Slamon et al., 2005; Naseem, 2018).

Immunotherapy with monoclonal antibodies gives new hope to patients with HER2 positive breast cancer. Several therapeutic monoclonal antibodies have been developed to target different epitopes of HER2 receptor. Trastuzumab is one of the humanised monoclonal antibodies used for both early stage and metastatic chemotherapy regimens (Slamon et al., 2001; Mayor, 2006). Since its debut, the response rates in patients have significantly increased and higher survival times have been reported in several Phase III clinical trials (Bang et al., 2010; Slamon et al., 2011; Perez et al., 2014; Cameron et al., 2017), but questions involving resistance, efficacy and cardiotoxic side effects still need to be assessed. Only by answering these questions we will be able to understand the behaviour of HER2 overexpressed cells in its response to Trastuzumab. This brief review will critically discuss and assimilate current insights of immunotherapy and educate medical professionals about the cutting-edge research of HER2 positive breast cancer therapy.

HER2 Positive Breast Cancer

The HER2 receptor

HER2 is one member of epidermal growth factor receptors (ErbB) family. The human ErbB family consists of 4 members, i.e., ErbB1 (EGFR, HER1), ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4). These receptors comprise of an extracellular ligand binding domain and a cytoplasmic region with tyrosine kinase enzymatic activity. Activation relies on the binding of specific ligands to the extracellular domain inducing dimerisation of the receptor with similar or different ErB family members (Wieduwilt et al., 2008). Interestingly, no ligand has been found to directly activate HER2, but it has been proposed to be in either a constitutively activated state and/or activated upon heterodimerisation (Iqbal and Iqbal, 2014). During dimerisation, the two receptors cross-phosphorylate one another, which relay signalling complexes towards the mitogen-activated protein kinase (MAPK) and Phosphoinositide 3-kinase (PI3K) pathways (**Figure 1**) (Moasser, 2007). Downstream effects of these pathways can induce proliferation, Signal transducer and activator of transcription (STAT) signalling, migration/adhesion, survival/metabolism and nuclear receptor signalling (Wee et al., 2017).

HER2 overexpression in breast cancer

The HER2 gene (*ErbB2*) is located on chromosome 17q12. In approximately 20% of invasive breast tumours, this gene is amplified and leading to an overexpression of HER2 protein across the plasma membrane (Lamy et al., 2011). This enhances intracellular signalling responses via heterodimerisation and promotes cell survival and tumorigenesis. Therefore, HER2 positive breast cancer tends to grow faster and are more likely to spread, leading to poorer prognoses and overall survival times (Mitri et al., 2012). Additionally, the Protein Phosphatase 1 Regulatory Inhibitor Subunit 1B (PPP1R1B) gene may also be amplified. This encodes dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP-32) and a truncated isoform, truncated isoform of dopamine- and cAMP-regulated phosphoprotein (t-Darpp), which act as signal transduction molecules and have been shown to play an important role in cancer metastasis (Gu et al., 2009; Denny et al., 2015; Lenz et al., 2018). Further amplification occurs in the C35 (C17ORF37) oncogene region(I) (Katz et al., 2010) where its function in invasive breast cancer is unknown, although studies in prostate cancer suggests it plays a role in migration and invasion via Akt phosphorylation pathways (Dasgupta et al., 2009). Genes involved in proliferation, such as topoisomerase II, are also frequently co-amplified and overexpressed, where its amplification with HER2 has shown to be associated with high growth and advancing tumour subtypes (Romero et al., 2011). Overall, these oncogenic variants induce potent disturbances in survival and proliferation mechanisms which highlight the aggressive nature of this specific cancer.

Diagnosis of HER2 positive cancer

The strong link between HER2 overexpression and poor clinical outcomes indicates that evaluation of HER-status is of vital importance. Positivity can be measured at either gene or protein expression level. In most countries, two different methods are used: Immunohistochemistry (IHC) and Fluorescence *In Situ* Hybridization (FISH), which identify any HER2 presence and messenger RNA (mRNA) expression in tumour cells. Other alternatives to FISH include real-time polymerase chain reaction (RT-PCR) or chromogenic *in situ* hybridisation (CISH). In addition to breast cancer, HER2 can be overexpressed in a range of tumours types including ovarian, bladder, pancreatic, and non-small cell lung cancer, where Trastuzumab can also be used as part of the chemotherapy regimen (Scholl et al., 2001).

Clinical use of Trastuzumab

Mechanism of action

Trastuzumab, or brand name called Herceptin, is a humanized monoclonal antibody (Immunoglobulin G1, IgG1). The IgG1 binds to the domain IV extracellular region of HER2 and causes G1 cell arrest by upregulating Cdk inhibitor p27 and blocking the Akt and MAPK pathways (Le et al., 2005);

subsequent loss of HER2 receptors thereby suppresses cells survival and growth mechanisms. The interaction of HER2 with Trastuzumab prevents tyrosine kinase signalling through a number of methods (**Figure 2A**). It can block HER2 forming dimers with other HER receptors and block cleavage of the extracellular domain. It can also induce passive endocytosis, which then subjects the targeted receptor to lysosomal degradation (Austin et al., 2004). Alternatively, the Fc region of Trastuzumab binds to the Fc gamma receptor III of effector immune cells, such as Natural Killer cells, which kill tumour cells via antibody-dependent cell mediated cytotoxicity (ADCC) (Collins et al., 2012). Due to the high specificity of the target, cytotoxic side effects are reduced compared to traditional chemotherapy agents, therefore maintaining a higher quality of life (Osoba et al., 2002). Trastuzumab also prevents the formation of HER2 heterodimers, thus downregulating intracellular PI3K-Akt pathways, and further suppressing cellular survival mechanisms via the Proto-oncogene tyrosine-protein kinase Src/Focal adhesion kinase (Src/Fak) pathway (Xu et al., 2009).

Interestingly, Trastuzumab has shown to have anti-angiogenic properties, decreasing vascular endothelial growth factor (VEGF) expression and possibly increasing blood vessel permeability (Petit et al., 1997; Sorace et al., 2016). This is proposed to boost drug delivery to the tumour but it is simultaneously linked to vascular side-effects.

Clinical use

Trastuzumab (Herceptin®) is available as an (150mg) intravenous infusion or (600mg) subcutaneous preparation. Both are currently produced by the Swiss drugmaker Roche but recently three intravenous biosimilars (Herzuma® produced by Celltrion, Ontruzant® produced by Samsung Bioepis, and Kanjinti® produced by Amgen and Allergan) have been launched and approved for use in the UK. These biosimilar's are highly similar to Trastuzumab in terms of their structure, and work in the same manner when targeting the HER2 receptor. More significantly, these have no clinically meaningful differences to Trastuzumab, and could therefore be used as a cheaper replacement, bringing down the cost of treatment.

Due to the embryo-fetal toxicity of these products, exposure during pregnancy can results in oligohydramnios, skeletal abnormalities and possible neonatal death. Therefore, Trastuzumab and its biosimilars are contraindicated in those who are pregnant (Durrani et al., 2018). As these drugs can remains present in the circulation for up to 7 months, women should require the use of contraception during, and at least 7 months after their treatment has finished (Lambertini et al., 2019). Breast feeding is also not advised due to the risk of IgG antibodies being passed on.

The National Institute for Health and Care Excellence (NICE) has set guidelines for Trastuzumab use in patients with early or metastatic breast cancer whose tumours have been validated to have either HER2 overexpression or HER2 gene amplification (Mayor, 2006). In early stage cancer, Trastuzumab is combined with docetaxel and carboplatin for 1 year following surgery, radiotherapy and/or chemotherapy. For patients with metastatic breast cancer who have undergone at least two prior

chemotherapy regimens, Trastuzumab monotherapy is instructed. In cases of no previous treatment, Trastuzumab is administered in combination with either docetaxel or paclitaxel, depending on the suitability of the patient. These guidelines advocate the continued usage of Trastuzumab until disease progression or until side effects develop, although this is still not universally accepted.

Further questions also remain on the optimal duration for Trastuzumab treatment. Currently, NICE have used the HERA trial as supportive evidence for its guidelines for early stage cancer, which found that 12 months of adjuvant chemotherapy tends to be optimal (Gianni et al., 2011). However, the more recent PHARE trial found that taking Trastuzumab over a 6-month period is just as effective as 12 months in early breast cancer patients, with better outcomes reported in cardiotoxicity (Pivot et al., 2013). Contrary to this, another recent trial (Finher) found that 9 weeks of Trastuzumab inclusive chemotherapy with 9 weeks of Trastuzumab failed to show non-inferiority of shorter administration, although the number of cardiac events were lower (Joensuu et al., 2018).

Clinical efficacy

The use of trastuzumab alongside chemotherapy has significantly improved survival rates amongst all patient subgroups (Marty et al., 2005; Buzdar et al., 2007; Gasparini et al., 2007; Rastogi et al., 2007; Robert et al., 2007; Kaufman et al., 2009; Joensuu et al., 2009; Perez et al., 2009; Spielmann et al., 2009; von Minckwitz et al., 2009; Blackwell et al., 2010; Gianni et al., 2010; Gianni et al., 2011; Huober et al., 2012; Moja et al., 2012; Balduzzi et al., 2014). An overview of the completed clinical trials using Trastuzumab are shown in **Table 1**.

Moja *et al.* systematically reviewed 11,991 early stage breast cancer patients over eight studies, Buzdar (Buzdar et al., 2007); B31 (Rastogi et al., 2007); BCIRG006 (Robert et al., 2007); FinHer (Joensuu et al., 2009); N9831 (Perez et al., 2009); PACS-04 (Spielmann et al., 2009); NOAH (Gianni et al., 2010); HERA (Gianni et al., 2011) found that overall survival and disease-free survival following a median 36 month period had a Hazard ratio of 0.66 (0.57-0.77 95% Confidence intervals) and 0.60 (0.50-0.71 95% CI) respectively. Cardiac problems such as congestive heart failure had as risk ratio of 5.11 (3.00-8.72 90% Cl) and left ventricular ejection function had a risk ratio of 1.83 (1.36-2.47 90% Cl). Three trials (Buzdar et al., 2007; Rastogi et al., 2007; Perez et al., 2009) were stopped early because of an observed benefit, strongly supporting the efficacy of the drug. This led to three other trials (Robert et al., 2007; Joensuu et al., 2009; Gianni et al., 2010) offering patients in the control arm to switch to Trastuzumab. Five trials (Rastogi et al., 2007; Joensuu et al., 2009; Perez et al., 2009; Spielmann et al., 2009; Gianni et al., 2011) also reported that the risk of brain metastases was significantly high in the Trastuzumab group (RR 1.75; 1.29-2.38 90% Cl), but due to statistical anomalies the evidence is of low quality and further research is necessary in order to increase confidence in this estimate.

Balduzzi *et al.* reviewed of the use of Trastuzumab inclusive chemotherapy for metastatic breast cancer looking at seven trials (Slamon et al., 2001; Marty et al., 2005; Gasparini et al., 2007; Kaufman et al., 2009; von Minckwitz et al., 2009; Blackwell et al., 2010; Huober et al., 2012)

involving 1,497 patients (Balduzzi et al., 2014). These found that overall survival and disease-free survival following a median 2-year period had a hazard ratio of 0.82 (0.71-0.94 95% Cl) and 0.61 (0.54-0.70 95% Cl). Congestive heart failure had a risk ratio of 3.49 (1.88-6.47 90% Cl) in the Trastuzumab inclusive arm, although no measurements of LVEF changes were taken. The quality of these studies shares some weaknesses. Firstly, all the studies were not blinded so bias may have affected the outcome measurements, with two studies (Gaspirini et al., 2007; Huober et al., 2012) choosing not to publish their mortality results. Furthermore, in three trials half the control group were permitted to switch to the Trastuzumab arm at disease progression, thereby making it difficult to estimate the benefit of Trastuzumab on mortality. One study administered Trastuzumab in addition to Lapatinib. Also, two trials (von Minckwitz et al., 2009; Huober et al., 2012) were stopped early due to slow recruitment, with another (Gaspirini et al., 2007) closing prematurely due to data suggesting Trastuzumab benefits only patients with strong HER2 expression. It is also important to note that these trials were given to women who had not previously been administered Trastuzumab. Therefore, the effectiveness for women relapsing after adjuvant Trastuzumab therapy needs to be explored.

Studies of first-line treatment have demonstrated improvements in both overall and disease-free survival for Trastuzumab when used in combination with either an anthracycline or paclitaxel regimen, compared with chemotherapy alone (Slamon et al., 2001; Marty et al., 2005; Untch et al., 2010). Unexpectedly however, the combination of Trastuzumab with an anthracycline-containing regimen has been strongly linked to significant increases in cases of cardiac dysfunction, so it is not advisable to use these two drugs in an adjuvant setting. It is also necessary to not rule out the implications of blinding bias on the results of these studies due to all of them being open label.

Resistance

Despite its clinical effectiveness, a significant number of patients may eventually develop resistance to Trastuzumab (Cobleigh et al., 1999; Vogel et al., 2002). Intensive studies have proposed mechanistic insights of how drug resistance arises, and key aspects are summarised in (**Figure 2B**).

The activated signalling pathways in HER2 overexpressed cell lines are compensated during Trastuzumab treatment by increased signalling from other receptors. Both Insulin-like growth factor 1 (IGF-1) and tyrosine kinase C-Met receptors have been shown to be upregulated in response to Trastuzumab which prevent apoptosis through their signalling pathways (Shattuck et al., 2008; Luque-Cabal et al., 2016). The HER3 (ErbB3) receptor has also been shown to counterbalance HER2 inhibition through transcriptional and posttranslational upregulation. Additionally, the PPP1R1B gene is often coamplified producing DARPP-32 and t-Darpp, with the latter recently been found to activate

IGR-IR/HER2 heterodimerisation, increasing cellular glycolytic capacity and Akt signalling, thereby promoting resistance (Lenz et al., 2018).

Variants of the HER2 receptor have shown to impart some, if not all, resistance to Trastuzumab. An excellent example is p95HER2, where 2 main isoforms (95-100 kDa and 110-115 kDa) exist through proteolytic cleavage or immature translation (Arribas et al., 2011). Due to the lack of extracellular domain, it is resistant to Trastuzumab and so is constitutively active. Likewise, another variant HER2Δ16 lacks part of the extracellular domain, although it is not wholly resistant to Trastuzumab. However, it does possess some potent qualities, such as constitutively forming homodimers that powerfully couples downstream signalling pathways involved with tumorigenesis (Mitra et al., 2009).

Resistance can also arise from epitope masking from membrane glycoproteins found on the cell surface. Notably, glycoproteins such as Mucin 4 (MUC4) and CD44/hyaluronan have shown to have anti-adhesive properties which masks the extracellular IV domain that Trastuzumab attaches to, and thereby offers protection for the tumour cell (Pohlmann et al., 2009). Current evidence proposes that transforming growth factor-alpha (TNF-α) upregulates MUC4 expression, where it can be used as a predictive biomarker due to its poor disease-free survival prognosis (Mercogliano et al., 2017). Naturally, TNF-α is an inflammatory mediator generated by tumour cells and plays a key role in the progression of cancer by inducing the protein complex nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), the main transcription factor, which can enhance cell survival through proliferative mechanisms (Coussens et al., 2013).

In terms of molecular networking, tumours deficient in phosphatase and tensin homolog (PTEN) have been observed to have remarkably lower overall response rates to Trastuzumab. Ubiquitously expressed, PTEN is a tumour suppressor that can reduce PI3K and MAPK signalling through its lipid and protein phosphatase activity respectively. A report (Depowski et al., 2001) found 36% of HER2 positive specimens from metastatic patients were Trastuzumab resistant and had worse outcomes. The same report also found that two mutations in PI3KCA (E545K and H1047R) had noticeably shorter progression free survival times in 25% of cases. These two disturbances act on the phosphatidylinositol 3-kinase/serine-threonine kinase (PI3K/Akt) pathway contributing to resistance. Patients with Src activation were also found to be resistant to Trastuzumab. Analysis found that Src is a central mediator in most resistant pathways and can prevent Akt phosphorylation and downstream signalling (Vu and Claret, 2012). This highlights a potential target for future therapies, especially in those with poor responses to Trastuzumab.

Cardiotoxic effects

Most side effects of Trastuzumab tend to be mild and mostly manageable (**See Box 1**), but serious events may arise (Sodergren et al., 2016). One major concern is the cardiotoxic effects it produces, where reductions in left ventricular ejection fraction (LVEF) and congestive heart failure have been

seen to be significantly higher in those treated with Trastuzumab, although changes in LVEF appear to be at least partially reversible once treatment has stopped (Ewer and Ewer, 2015).

The primary cause of cardiotoxicity is the downregulation of Neureglin-1 (NRG1), a ligand for HER receptors that activate the MAPK and PI3K/Akt pathways in cardiomyocytes by dimerising with HER2 (Rupert and Coulombe, 2015). Trastuzumab effectively reduces cardiac NRG1 release in the myocardial and endocardial endothelial cells and consequently prevents cell growth and alters protein regulation. This provokes the onset of heart failure as suppression of the cell survival and growth mechanisms neglect cardiac myofibril structure and function (Bersell et al., 2009). Furthermore, as cardiac HER2 receptors have a high affinity for Trastuzumab, interactions between the drug and receptor interrupts the NRG/HER2 cardioprotective signalling pathways. Therefore, Trastuzumab is contraindicated in patients that have a history of coronary heart or vascular disease, myocardial infarctions, congestive heart failure and/or high blood pressure.

The inhibition of NRG1/HER2 pathway by Trastuzumab increases production of reactive oxygen species (ROS) from arising abnormalities in the mitochondria of cardiomyocytes (Mohan et al., 2017) (**Figure 1**). The subsequent intracellular response increases the upregulation of angiotensin II, platelet-derived growth factor, TNF-α and thrombin, which together stimulate vascular smooth muscle cells. This activity further increases the formation of ROS in a positive feedback loop, which damages the endothelium and contributes to cardiac dysfunction (Cai and Harrison, 2000).

Other pathways are also involved in promoting cardiotoxicity. HER2 degradation suppresses cell survival mechanisms via the Src/Fak pathway and supress protein hypertrophy by reducing MAPK activity (Xu et al., 2009). This in turn promotes cytostasis via the transcription factor C/EBPβ (Arnal-Estape et al., 2010). Modulation of ventricular remodelling is also seen through transforming growth factor-beta (TGF-β) signalling, which activates Smad2/3 and Smad4 signalling complexes (Padua and Massague, 2009). Downstream effects cause apoptotic and anti-hypertrophic processes that can progress to heart failure. TGF-β signalling also regulates the production of matrix metalloproteinases (MMPs), which subsequently activate TGF-β in the extracellular matrix and enhance tumour progression (Krstic and Santibanez, 2014).

Potential cardioprotective interventions

Although there is evidence that Trastuzumab can impair cardiac function, the clinical complications that may arise are far outweighed by the benefits (Moja et al., 2012; Balduzzi et al., 2014). Regardless, it should still be acknowledged that these clinical obstacles have damaging effects and often stand in the way to better health outcomes. Therefore, it necessary to investigate interventions that could help prevent these complications from arising.

One promising area of intervention is exercise, which may attenuate Trastuzumab induced cardiotoxicity as research has highlighted improvements in cardiopulmonary fitness from changes in ErbB/NRG-1 signalling. In cases of heart failure, it has been found that patients undertaking aerobic exercises prevented ventricular remodelling and protein degradation due to the increase in Akt in cardiomyocytes and inhibition of the TGF-β signalling pathway (Scott et al., 2013).

Evidence from rodent models is also encouraging. Drug-enhanced NRG1/HER2 signalling showed improvements in cardiac performance and cellular proliferation in those with left ventricular failure (Liu et al., 2006). This was further reflected during exercise, which caused the mechanical strain of the heart to encourage NRG1 synthesis and release and has suggested to evoke suppression of neurohormonal factors and prevent the onset of myocardial injury (Scott et al., 2013). It has also been proposed that aerobic exercise can prevent Trastuzumab-induced cardio remodelling by inhibiting the TGF-β and C/EBPβ pathways (Bostrom et al., 2010). Previous research has shown that exercise reduces expression of TGF-β and C/ERPβ which leads to increases in cell division and cardiomyocyte size, resulting in hypertrophy. Together, these studies show improvements in lifespan and reduction of cardiac dysfunctionality in those with dilated cardiomyopathy, and myocardial ischemia, further advocating the use exercise as a potential cardioprotective therapy in scenarios where HER2 treatment may induce cardiac stress. However, this has not been investigated thoroughly in patients treated with Trastuzumab. Of the limited data that exists, a single group study (Haykowsky et al., 2009) of 17 patients underwent aerobic training during the first 4 month of Trastuzumab treatment. Despite exercise training, it failed to prevent left ventricular decreases and cavity dilation, though limitations regarding the lack of a control group, small sample size, short duration and lack of adherence may have impacted on the obtained results.

Table 2 summarizes the most recent ongoing clinical trials, which study the effects of exercise training during Trastuzumab inclusive chemotherapy. To assess these changes, various parameters are used to determine cardiovascular changes. The fitness capacity, i.e. the maximum rate of oxygen consumption (VO_2max), can be applied as a predictor of Trastuzumab-induced left ventricular abnormalities and cardiovascular disease, due to being determined by cardiac output (Jones et al., 2011). VO_2max, along with global cardiovascular reserve, is a powerful predictor of cardiovascular events and all-cause mortality (Kavanagh et al., 2003; Jones et al., 2010) with recent evidence showing that VO_2max can be predictive of all-cause mortality in cancer patients (Jones et al., 2010; Kelsey et al., 2014; Lakoski et al., 2015). However, to the best of our knowledge, there have been no longitudinal studies of VO_2max during and following Trastuzumab treatment. Other parameters measured include ventricular modelling, as well as changes in the left ventricular ejection fraction (LVEF), which are regularly monitored when receiving Trastuzumab-inclusive treatment (Visser et al., 2016).

For current and future clinical trials, targeted controls should be implemented in exercise prescription, and measured pre- and post-exercise. The magnitude of heart rate recovery (HRR) and the time

constant of recovery based on different time intervals post-exercise should also be advocated, since emerging evidence has shown that these parameters are closely correlated to cardiovascular risks (Qiu et al., 2017). Research also suggests that recovery periods within the first 60 seconds are strong predictors of cardiovascular risks (Morshedi-Meibodi et al., 2002), with changes in heart rate over 10 seconds post-exercise being a stronger predictor for all-cause mortality (van de Vegte et al., 2018). These parameters may therefore be looked at as a new approach to how cardiovascular risks are measured by implementing their use alongside VO_2max and LVEF.

An area of practical concern refers to the difficulties of measuring exercise intensity in cancer patients. As Trastuzumab cardiotoxicity may alter oxygen delivery and affect other physiological systems, it is unlikely that patients undergoing chemotherapy will be able to carry out vigorous exercise. Therefore, empirical methods of how to accurately estimate exercise intensity in cancer patients need to be identified. The current guidance suggests 105 minutes of moderate or 75 minutes of aerobic exercise for cancer survivors (Schmitz et al., 2010), although this is yet to be extended to those undergoing treatment. Relevant recommendations may come from a recent trial looking at different levels of exercise intensity in healthy adults and breast cancer survivors (Scharhag-Rosenberger et al., 2015). A significant difference between these two groups was observed when measuring HRR and VO_2max, and for his reason concluded that intensity may have to be individualised to the patient. This is further supported by another clinical trial showing the accuracy of HRR as not achieving the equivalent exercise intensity within the three subgroups tested: breast cancer patients, survivors, and healthy controls (Kirkham et al., 2013). However, it was the most accurate of the methods that were tested in the cancer patients and survivors' subgroups, which suggests it could be effective in tailoring exercise intensity in those categories. The HRR method also benefits from requiring less equipment compared to using gas exchange measurements, and thus is the most feasible one for clinical use.

Overall, clinical trials in this area are rather limited. This is possibly due to higher survival rates compared to other more aggressive types of cancers, as well as the partial reversibility of Trastuzumab induced cardiotoxicity. Exercise intervention may also cause setbacks in terms of adherence. For example, patients who are older, female or have psychiatric issues are shown to have poorer adherence to exercise therapy, whereas lifestyles factors such as financial difficulties and socioeconomic class have also been highlighted to be strong predictors (Conraads et al., 2012). This is also evident from a recent study which investigated exercise adherence of 68 cancer patients undergoing treatment, all of whom were prescribed aerobic and resistance exercise therapy three times a week (Kirkham et al., 2018). As chemotherapy progressed, attendance decreased significantly for both aerobic and resistance training. The two most common reasons for poor adherence were that patients felt the exercise was too difficult and their treatment symptoms prevented them attending. This highlights the need for behavioural strategies to be explored in order to improve adherence. One idea put forward is offering two different intensity levels of aerobic exercise at 40%

or 60% of maximal heart rate (Galanti et al., 2013). This has been modelled as a flexible design option for exercise tolerance, allowing for greater attraction of participants. In the long term, this may further identify possible changes in cancer reoccurrence, as well as improvements in the quality of life for patients, particularly those who may have a longer life expectancy.

Beyond exercise therapy, the use of Beta-blockers and ACE inhibitors to reduce mortality in non-cancer patients with heart failure have also been proposed for possibly use in mitigating Trastuzumab cardiotoxicity. As angiotensin is a potent down-regulator of the NRG1/ErbB pathway, ACE inhibitors may reverse this suppression and could be effective in reducing cardiac dysfunction. β-blockers have been shown to increase pro-survival signalling by transactivating the EGFR pathway via recruitment of β-arrestin (Kim et al., 2008).

Recently, the first randomised control trial, MANTICORE 101 (Pituskin et al., 2017), looked at the prevention of Trastuzumab-induced cardiotoxicity using this pharmacological route. The results showed that the ACE inhibitor, Perindopril, and the Beta-blocker, Bisoprolol, each attenuated LVEF declines often induced by Trastuzumab, but failed to prevent left ventricular remodelling. This is further reflected by another study using lisinopril (ACE inhibitor) and carvedilol (β-blocker) (Guglin et al., 2017). A further trial is also underway investigating the protective capacity of the β-Blocker, Bisoprolol and the ACE inhibitor, Ramipril, for HER2 positive cancer patients treated with Trastuzumab (NCT02236806, www.clinicaltrials.gov).

As the synthesis of cardiac NRG-1 is influenced by the regulation of blood pressure, it has been suggested that other blood pressure reducing drugs, such as angiotensin receptor blockers (ARB's), may help improving cardiotoxic outcomes. However, a trial looking at the effects of Candesartan (ARB), showed no significant changes in protecting the decrease in left ventricular fraction during or shortly after trastuzumab treatment in early breast cancer patients (Boekhout et al., 2016).

Although recent studies have shown only mild improvements in clinical outcomes, further work is needed to directly evaluate the effect of β-blockers and ACE inhibitors on cardiac energetics, specifically focusing on longer term studies and what beneficial effects these interventions have on cardiac dysfunction, heart failure risk and overall survival rates.

Trastuzumab conjugates

Trastuzumab has several limitations. Firstly, it is not effective in every patient that is HER2 positive, and even in those that are sensitive, resistance often arises in a significant number of patients over the course of treatment. Secondly, it possesses cardiotoxic properties, increasing the risk of cardiac problems such as early heart failure. Thirdly, Trastuzumab only targets one member of the receptor dimer, thereby preventing the signalling blockade from being complete (Wang et al., 2016). For these reasons, it is important to look at agents that could target other areas of the HER2 receptor which could be used alongside Trastuzumab.

A new modified form of Trastuzumab, Trastuzumab Emtansine (T-DM1) has currently undergone several Phase III clinical trials (Chen et al., 2017; Dieras et al., 2017; Perez et al., 2017; Hurvitz et al., 2018). This modified version not only shares the biological action of Trastuzumab, but after the HER-2-T-DM1 complex undergoes endocytosis and degradation, the Emtansine breaks away to inhibit the polymerisation of tubulin (**Figure 3**). Thus, T-DM1 has a double anti-tumour effect, through the activity of Trastuzumab and the selective antimitotic agent in the cytoplasm (Fabi et al., 2016). In terms of treatment, NICE permits its use as a monotherapy in HER2 positive locally advanced or metastatic breast cancer patients, who have received prior treatment with Trastuzumab and a taxane in combination or separately. Patients given Trastuzumab who have relapse within 6 months of completing adjuvant therapy are also qualified to received T-DM1.

A new generation of therapeutic drugs are being looked at which have shown to be clinically effective in targeting the HER2 receptor in several clinical trials (Goss et al., 2013; Marin et al., 2013; Pivot et al., 2015; Awada et al., 2016; Urruticoechea et al., 2017) (**Table 3**); the most promising of these are Lapatinib, Pertuzumab and Neratinib. Further studies are also underway (**Table 4**) which will evaluate safety and side effects of combined therapy in extending remission and disease-free survival rates.

Pertuzumab (Perjeta®) is a recombinant humanised monoclonal antibody that blocks the dimerisation of HER2 with other ErbB receptors by binding to the extracellular domain II (Harbeck et al., 2013). This is useful due to HER2/HER3 dimers having been shown to drive proliferation and tumour progression, where Trastuzumab has little effect (Wang et al., 2016)[82)]. Pertuzumabs mechanism is similar to Trastuzumab, where it blocks intracellular signalling cascades as well as activate the ADCC pathway (**Figure 3**).

Lapatinib (Tykerb®) works by preventing the activation of downstream signalling pathways, but targets EGFR in addition to HER2 (Oakman et al., 2010). This works by competing for ATP in the ATP-binding pocket at the intracellular domain of the tyrosine kinases, which subsequently, blocks the downstream MAPK and PI3K pathways (**Figure 3**) (Dai et al., 2008), resulting in growth arrest and apoptosis. A significant benefit of Lapatinib is its ability to inhibit $p95^{HER-2}$ where Trastuzumab is unable to do due to the absence of the extracellular domain (Arribas et al., 2011).

Another drug, Neratinib (Nerlynx®), blocks signal transduction events resulting in G1 arrest and suppression of cellular proliferation (Schwab et al., 2015). Evidence suggests that this irreversibly binds to the EGFR and HER2 receptor via covalent interaction with cysteine residues C805 on HER2 and C773 on EGFR (Zhang et al., 2016). Downregulation of HER2 transcription follows due to HSP90 release from HER2, causing subsequent ubiquitin-mediated endocytic degradation in presence of lysosomes (**Figure 3**).

Targets not associated with HER2 are also being examined. One example is the integrin αvβ6, which promotes invasive and survival mechanisms of cancer cells, although this is not fully understood

(Yang et al., 2015). Cells containing high levels of mRNA or protein for β6 integrin have been associated with increase metastasis and poor prognosis, which is worsened by co-expression of HER2 (Moore et al., 2014). A human monoclonal antibody 264RAD targeting this integrin has been shown to reduce tumour growth and metastasis through inhibition (Eberlein et al., 2013). It should therefore be of interest to see clinical trials started on 264RAD, in addition to Trastuzumab or alongside drugs previously described here.

Better diagnosis procedures should also be given attention, as intracellular anomalies in the ErbB network, such as PI3KCA and PTEN are said to be independent predictors of prognosis[61]. Future therapies should target these anomalies that enhance the tumorigenic and resistance mechanisms and bring about a designed therapy regimen that is tailored towards the individual. PI3K, Mammalian target of rapamycin (mTOR), Src, and CDK inhibitors are currently being investigated for potential use in chemotherapy regiments, however clinical trials are still in the early stages.

Conclusion

Our improved understanding of the ErbB network and its unnatural oncotic disturbances has improved our approach in treating both advanced and early stage breast cancers, with the creation of specific targeting agents such as Trastuzumab. Undoubtedly, Trastuzumab has been a significant breakthrough in HER2 breast cancer therapy, in both prolonging and saving the lives of breast cancer patients. However, questions surrounding the optimal duration and resistance still need to be answered more precisely. The side effects regarding cardiac toxicity should also not go unnoticed, and prospective beneficial regimens should look at reducing this further. Customised exercise prescription tailored to the patients' needs is a promising tool for improving health outcomes in cancer patients. Moving forward, research into the downstream anomalies from the ErbB receptors could highlight more targets that are specific for future therapeutic agents. These should focus on suppressing the enhanced tumorigenic potential, which may improve and prolong life even further.

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Figures:

Figure 1 – Cellular response to HER/ErbB receptors dimerisation. HER/ErbB family receptors are activated upon homo-/hetero-dimerisation. With exception of HER2, activation is dependent on ligands binding to the extracellular domain, which subsequently causes phosphorylation of the intracellular kinase domain (except for the absent kinase domain for HER3) and initiates downstream cascades via PI3K and MAPK. The resulting effects of this cascade include proliferation, repair and resistant mechanisms that enhance survival. Trastuzumab binds to the HER2 receptor which either causes passive endocytosis leading to HER2 degradation, or the tumour cell is destroyed via antibody-dependent cell-mediated cytotoxicity (ADCC). Because of its inhibition, pro-survival signalling is reduced through decreased activity in downstream pathways. Cytotoxicity mediated through this inhibition is caused by generation of ROS and mitochondria dysfunctions, as well as increase of proapoptotic Bcl-xS. These cellular disruptions led swollen mitochondria and ATP depleted stores.

Figure 2 – Mechanisms of action and resistance for Trastuzumab. (A) Trastuzumab binds to the extracellular domain blocking dimerisation of HER2 with other Her family members, thus preventing the activation of tyrosine kinase signalling pathways. Upon attachment of HER2, passive endocytosis takes place which causes HER2 degradation. The Fc region of Trastuzumab can attach to the Fc receptor of effector immune cells such as Natural Killer cells which destroys the tumour cell via antibody-dependent cell-mediated cytotoxicity (ADCC). **(B)** In response to Trastuzumab, several biological alterations occur that help cells evade Trastuzumab treatment. Firstly, cells respond to treatment by rapidly upregulating C-Met and IGF-IR, where the former protects against Trastuzumab by abrogating p27 induction. Both activate downstream pathways normally sustained by HER2. IGF-IR signalling can also be activated through heterodimerisation with HER2 using t-Darpp which further increases resistance. Resistance can also arise from epitope masking from extracellular structures. Mucins, specifically MUC4 has been shown to play a role in the progression of cancer due to its anti-adhesive properties. In tumours, MUC4 can be overexpressed, presenting at the cell surface, where it is heavily glycosylated giving a highly extended and tough conformation. Its close association with HER2 helps mask Trastuzumab's target thereby offering protective resistance. Several isoforms of HER2 which are partly, if not all resistant to Trastuzumab exist; the most notable being HER2Δ16 and p95HER2. HER2Δ16 is formed by alternative splicing where it forms homodimers and constitutively actives downstream pathways. p95HER2 fragments can be form from either alternative initiation of translation (100-115kDa) or proteolytic cleavage of HER2 by metalloproteases (95-100kDa). These fragments are hyperactive due to their ability to form dimers, and in the case of 100-115 kDa is maintained by disulphide bonds. The result are operational intracellular kinase domains, resistant to Trastuzumab.

Figure 3 – Action sites of Trastuzumab Emtansine (T-DM1), Pertuzumab, Lapatinib and Neratinib. After attachment of Trastuzumab Emtansine to the extracellular domain IV of the HER2 receptor, the complex can either activate the antibody-dependent cellular cytotoxicity (ADCC) pathway (if an immune effector cell binds to the Fc region) or undergo endocytosis, whereby lysosomes degrade it. This releases DM1 metabolites which inhibits the assembly of microtubules by binding to tubulin and preventing cell division in tumour cells. Additionally, Trastuzumab binding to HER2 prevents both homodimerisation as well as HER2/HER3 heterodimerisation, which results in the inhibition of the PI3K and MAPK signalling cascades. This suppresses cellular proliferation and survival mechanisms that are fundamental to tumorigenesis. Similarly, Pertuzumab can also activate the ADCC pathway or cause endocytosis, although it attaches to extracellular domain II. Both small molecule inhibitors, Lapatinib and Neratinib, target the intracellular domain activity of the HER2 receptor. These work by blocking the downstream PI3K/Akt pathways, which regulates ErbB2 transcription. In the presence of either of these drugs, the drug-receptor complex undergoes passive endocytosis and lysosomal degradation. Neratinib can also trigger potent ubiquitination and vigorous degradation, whereas Lapatinib has stronger suppressive effects on ErbB2 transcription.

Tables:

Box 1 - Common and severe side effects of Trastuzumab (Sodergren et al., 2016)

Common	Severe
Fever	Reduced cardiac output
Nausea	Heart failure
Diarrhoea	Hypersensitive reaction
Vomiting	Pulmonary oedema
Increased risk of infection	Foetal death
Cough	Neutropenia
Skin Reactions	Anaemia
Muscle pain	

Table 1 - Completed Clinical Trials using Trastuzumab (Herceptin) in Early and Metastatic HER2 positive breast cancer

Trial	Intervention arm(s)	Primary Outcomes	Main finding	Ref
B-31 (n=2043)	4 x AC → 4 x P q3w or 12 x P qw 4 x AC → 4 x P q3w or 12 x P qw + H (1 year)	I. DFS II. Cardiotoxicity	10 of 872 (1.3%) control patients had CEs (9 CHFs and 1 cardiac death) compared with 35 of 932 (3.9%) H-treated patients (35 CHFs and no cardiac deaths). Cumulative incidence difference at 5 years was 2.7%. Joint analysis of NSABP B-31 was done alongside N9831.	Rastogi et al., 2007
BCIRG006 (n=3222)	4 x AC → 4 x D q3w 4 x AC → 4 x D q3w + 12 x H qw → 13 x H q3w D + 6 x Carboplatin p3w + 18 x H qw → 11 x H q3w	I. DFS II. OS, Adverse effects, QoL	At median follow up at 36 months, the DFS and OS significantly improved in both H groups compared to control. Reduction risk of relapse 39% (p<0.0001) for H without Carboplatin and 33% (p=0.0003) with Carboplatin. Relative reduction in risk of death was 41% (p=0.0041) and 34% (p=0.017) respectively.	Robert et al., 2007
Buzdar (n=42)	4 x P q3w → 4 x FEC 4 x P q3w → 4 x FEC + 24 x H qw	I. pCR II. Clinical complete remission	Complete remission rate in chemotherapy group was 54.5% (32.2-75.6%; 95% CI). In the chemotherapy with H, remission rate was 60% (44.3-74.3%; 95% CI). No recurrences were seen in patients in the Herceptin group at 16.3 months follow up.	Buzdar et al., 2007
Finher (n=1010)	3 x D q3w or 8 x Vinorelbine qw → 3 x FEC q3w 3 x D q3w or 8 x Vinorelbine qw + 9 x H qw → 3 x FEC q3w	I. DDFS II. OS, Adverse effects, Recurrence	Cox model analysis showed the DDFS of patients in the Docetaxel, Herceptin and FEC was superior to those treat with vinorelbine, FEC and Herceptin (HR=0.31; 0.11-0.83 95% CI, p=0.020), and those treated with docetaxel and FEC (HR=0.32; 0.12-0.89 95% CI, p=0.029)	Joensuu et al., 2009
HERA (n=5102)	Observation 1 Year Herceptin 2 Years Herceptin	I. DFS II. Cardiotoxicity, OS, Recurrence	At 4 years DFS benefitted the 1-year Herceptin group compared to the observation (78.6% to 72.2%; HR 0.76, 0.66-0.87 95% CI, p<0.0001). 885 patients in observed group crossed over to Herceptin, where the cohort had fewer DFS events than those remaining in the observed group (HR 0.68; 0.51-0.90 95% CI, p=0.0077). Higher grade 3-4 incidences were reported in 1-year Herceptin group.	Giannini et al., 2011
N9831 (n=2766)	4 x AC → 12 X p qw 4 x AC → 12 X p qw → 1-year H qw 4 x AC → 12 X p qw + 1-year H qw	I. DFS II. OS, Adverse effects, Recurrence	Joint analysis was done alongside NSABP B-31. The addition of H sequentially to AC→T significantly improved DFS, univariately (HR=0.70; 57-86% 95% CI, p=0.0005). 5 yr DFS was increased from 72% with AC→T to 80% with AC→T→ H. Median follow up of 5.3 yrs for Arm B (AC→T→H) vs. Arm C (AC→T+H→H) showed Adjusted HR(Arm C/Arm B)=0.75 (0.60-0.94 95% CI). 5 yr DFS was increased from 80% with AC→T→H to 84% for AC→T+H→H.	Perez et al., 2009
NOAH (n=235)	3 X AP q3w → 4 x P q3w → 3 x CF + methotrexate q4w 3 x AP q3w + 3 x H q3w → 4 x P q3w + 4 x H q3w → 3 x CF + methotrexate q4w + 3 x H q4w	I. EFS II. pCR, OS, Cardiotoxicity	3-year EFS was 71% (61-78% 95% CI) with Herceptin vs 56% (46-65% 95% CI) without Herceptin, HR=0.59 (0.38-0.90 95% CI, p=0.013). Only 2 patients developed symptomatic cardiac failure.	Giannini et al., 2010
PACS-04 (n=3010)	6 x ED or 6 x FEC → Observation 6 x ED or 6 x FEC → 1-year H p3w	I. DFS II. OS, EFS, Adverse effects	Three-year DFS rates were 78% (72.3-82.5%, 95% CI) for observation, and 81% (75.3-85.4% 95% CI) in Herceptin arms. After median 47 month follow up, 1-year Herceptin was not associated with a significant decrease in relapse risk.	Spielmann et al., 2009
Slamon 2001 (n=469)	6 x A or E + C or P q3w 6 x A or E + C or P q3w + H qw	I. Time to disease progression II. OS, ORR, Clinical response	Herceptin with chemotherapy was associated with longer median time to progression (7.4 months vs 4.6 months, p<0.001) and lower rate of death at 1 year (22% vs.33%, p=0.008). Higher incidences of cardiac dysfunctions (27%) occurred in the group given an anthracycline, cyclophosphamide and Herceptin.	Slamon et al., 2001
Group 03-05 (n=156)	Capecitabine Monotherapy Capecitabine + Herceptin	I. Time to disease progression II. Clinical response, OS	Median time to progression was 5.6 months in the capecitabine group vs 8.2 months in Capecitabine + Herceptin (HR = 0.69; 0.48-0.97 95% CI, p=0.0338). OS was not significantly different. ORR were 27% without Herceptin and 48.1% with Herceptin (OR 2.50, p=0.115)	von Minckwitz et al., 2009
M77001 (n=188)	6 x D q3w alone 6 x D q3w + H qw	I. ORR II. OS, PFS, Clinical response	H + D was superior to D alone in ORR (61% to 34%, p=0.0002), OS (median 31.2 to 22.7 months; P =0.0325), and time to disease progression (median 11.7 to 6.1 months; P =0.0001). There was little difference in the number and severity of adverse events.	Marty et al., 2005
TAnDEM (n=208)	Anastrozole qd Anastrozole qd + H qw	I. PFS II. TTP	The H plus anastrozole arm experienced significant improvements in PFS compared to anastrozole alone (HR = 0.63; 0.47 to 0.84 95% CI) with median PFS at 4.8 vs. 2.4 months (P=0.0016). Incidence of grade 3-4 adverse events was higher in H arm.	Kaufman et al., 2009
eLEcTRA (n=57)	Letrozole qd Letrozole qd + H qw or q3w	I. PFS II. TTP, ORR, Clinical response, OS	Median time to progression in arm A was 3.3 months compared to 14.1 months in arm B (HR=0.67, p = 0.23). Clinical benefit rate was 39% for arm A compared to 65% in arm B (odds ratio 2.99, 1.01-8.84 95% CI).	Huober et al., 2012
Gasparini 2007 (n=123)	P qw P qw + H qw	I. ORR II. TTP, Safety, Clinical response	P + H was statistically superior for ORR (75% vs. 56.9%; P = 0.037). A statistically significant better median time to progression was seen in the subgroup with IHC 3+ (369 vs. 272 days; P = 0.030).	Gasparini et al., 2007
Blackwell 2010 (n=296)	Lapatinib qd Lapatinib qd + H qw	I. PFS II. ORR, OS, QoL	Lapatinib with Herceptin was superior to Lapatinib alone for PFS (HR=0.73; 0.57-0.93 95% CI, P =0.008) and Clinical benefit rate (24.7% vs. 12.4%; P=0 .01), but no difference in ORR (10.3% to 6.9%, P=0 .46).	Blackwell et al., 2010

Abbreviations: *n,* total number of enrolled patients; *D,* Docetaxel; *E,* Epidoxorubicin; *F,* 5-FU; *H,* Herceptin; *P,* Paclitaxel; *RT,* Radiotherapy; *q3w,* every 3 weeks; q4w, every 4 weeks; *qw,* every week; *qd,* every day; *DFS,* Disease-free survival; *OS,* Overall survival; *OR,* Odds ratio; *QoL,* Quality of life; pCR, Pathologic complete response; *EFS,* Event-free survival; *DDFS,* Distant disease-free survival; *TTP,* Time to tumour progression; *PFS,* Progression-free survival; *ORR,* Objective response rate; *CI,* confidence interval; *CE*, Cardiac events*; CHF,* Congestive heart failure.

Table 2 - Ongoing Clinical Trials examining the effects Exercise Intervention has on Breast Cancer Patients treated with Trastuzumab. (Up to 1 March 2019)

Study No.	Age (Years)	Summary	Study type	Cancer Stage	Primary Outcome Measurements	Clinicaltrail.gov ID
NCT02454777	>18	The feasibility of high-intensity interval training in improving cardiovascular fitness	Interventional	I-IIIC	I. Individual and Group compliance II. Maximum volume of oxygen uptake (VO2 peak), III. Left Ventricular function	Recruiting
NCT02433067	18-65	The effects of 3 months physical activity on myocardial function	Interventional	I-III	I. Left Ventricular Ejection Fraction (LVEF)	Recruiting
NCT03089502 *	>18	Effectiveness of a Cardio-Oncology Rehabilitation Exercise Program to Improve Cardiorespiratory Fitness	Interventional	I-III	I. Cardiopulmonary Exercise test following the Bruce protocol.	Terminated
NCT03176888 *	35-60	Efficacy of High-intensity Interval Training for Improving Health and Well-being	Interventional & Behavioural	I-III	I. Change in maximal aerobic capacity (VO_2 max)	Not yet recruiting
NCT03131024 *	>18	Caloric Restriction and Exercise for Protection from Anthracycline Toxic Effects	Interventional	I-IIIC	I. Left ventricular ejection fraction reserve	Recruiting
NCT03656731 *	>70	Effect of exercise intervention among older participants with breast cancer	Interventional	IV	I. 30-second chair stand test	Not yet recruiting
NCT01621659 *	>18	Effect of early interventions in people diagnosed with cancer, during and after treatment.	Interventional	I-IV	I. Echocardiography (% longitudinal strain) II. Serum biomarkers	Active, not recruiting

* = Trial includes some patients not receiving Trastuzumab treatment

Table 3 - Overview of completed clinical trials of HER2 inhibitors used in patients with HER2 positive breast cancer in the last 6 years. (Up to 1 March 2019)

Intervention	Comparator	Clinical phase	Hypothesis	Primary Outcomes	Main finding	Reference
T-DM1	Capecitabine + Lapatinib	III	Trastuzumab Emtansine increases survival rates compared to Lapatinib and capecitabine	I. PFS II. OS	OS was longer with T-DM1 (29.9 months) than control (25.9 months). Fewer AE's with T-DM1.	Chen et al., 2017
T-DM1 + Pertuzumab	Carboplatin + Docetaxel + Pertuzumab + Trastuzumab	III	Whether targeted therapy is better than tradition systemic chemotherapy	I. pCR	Tradition chemotherapy achieved better pCR. Less AE's occurred in T-DM1 + Pertuzumab group.	Wang et al., 2016
Trastuzumab + Capecitabine + Pertuzumab	Trastuzumab + Capecitabine	III	Addition of Pertuzumab to Trastuzumab and Capecitabine increases PFS and OS outcomes	I. PFS II. OS	Hierarchical testing showed OS increased by 9.1 months in Pertuzumab included group. AE's increased slightly.	Fabi et al., 2016
Neratinib	Lapatinib + Capecitabine	II	Neratinib increases OS and PFS compared to Lapatinib and capecitabine	I. PFS II. OS	Inconclusive since neither therapy demonstrated superiority.	Urruticoechea et al., 2017
Neratinib + Paclitaxel	Trastuzumab + Paclitaxel	II	Comparison of two regimens in their safety and effectiveness to shrink tumours and extend lives	I. PFS II. CBR	Neither treatment was superior in terms of PFS.	Martin et al., 2013
Lapatinib + Capecitabine	Trastuzumab + Capecitabine	III	Lapatinib effect on the incidences of brain metastases compared to Trastuzumab	I. CNS progression II. PFS	No differences in incidences. Better PFS and OS observed with Trastuzumab + Capecitabine.	Awada et al., 2016
Lapatinib	Placebo	III	Evaluate and compare the safety and efficacy of Lapatinib versus placebo	I. Reoccurrence II. OS	Little or no benefit with lapatinib in terms of DFS.	Pivot et al., 2015

Abbreviations: *T-DM1*, Trastuzumab Emtansine; *AE*, Adverse events; *PFS*, Progression-free survival; *pCR*, Pathologic complete response; *OS*, Overall S=survival; *CNS*, Central nervous system; *CBR* Clinical benefit rate; *DFS*, Disease-free survival.

Table 4 - Currently active/recruiting clinical trials of drugs that target HER2 positive breast cancer. (Up to 1 March 2019)

Experimental Drug(s) vs Placebo	Age	Clinical Phase	Summary/Overview	Primary Outcomes	Status	Clinicaltrail.gov ID
T-DM1	≥60	II	Efficacy of T-DM1 in older patients	I. IDFS	Active, not recruiting	NCT02414646
T-DM1 vs Lapatinib + Capecitabine	≥18	III	Efficacy of T-DM1 in patients with Locally Advanced or Metastatic cancer	I. PFS	Active, not recruiting	NCT03084939
T-DM1 vs Trastuzumab	≥18	III	Compare T-DM1 to Trastuzumab as Adjuvant Therapy	I. IDFS	Active, not recruiting	NCT01772472
T-DM1 vs Paclitaxel + Trastuzumab	≥18	II	Effectiveness of T-DM1 for Stage I breast cancer	I. DFS	Active, not recruiting	NCT01853748
T-DM1	≥60	II	Long-term benefits and side effects of T-DM1	I. 5-year IDFS	Recruiting	NCT03587740
T-DM1 + Pertuzumab	≥18	II	HER2 Heterogeneity on the Treatment of Early-stage Cancer	I. pCR	Active, not recruiting	NCT02326974
Pertuzumab + Trastuzumab + Chemotherapy	18-65	II	Effects of Pertuzumab in combination chemotherapy regimen	I. pCR	Active, not recruiting	NCT01855828
Pertuzumab + Trastuzumab + chemotherapy vs Trastuzumab + chemotherapy	≥18	II	Effect of Pertuzumab addition in patients previously treated with T-DM1	I. PFS	Active, not recruiting	NCT02229149
Pertuzumab + Trastuzumab + Docetaxel vs Trastuzumab + Doxataxel	≥18	III	Effects of Pertuzumab addition to chemotherapy in patients with early stage or locally advanced cancer	I. tpCR	Active, not recruiting	NCT02586025
Pertuzumab + Trastuzumab + Docetaxel vs Trastuzumab + Doxataxel	≥18	III	Effects of Pertuzumab addition to chemotherapy in untreated metastatic patients	I. PFS	Active, not recruiting	NCT02896855
Neratinib + Capecitabine vs Lapatinib + Capecitabine	≥18	III	Effectiveness of Lapatinib and Neratinib in metastatic patients with 2+ prior treatments	I. PFS II. OS	Active, not recruiting	NCT01808573
Lapatinib + Paclitaxel + Trastuzumab	≥18	II	Feasibility and Safety for the Lapatinib addition to chemotherapy	I. Safety	Active, not recruiting	NCT01827163
Lapatinib + Trastuzumab	≥18	II	Effectiveness of combined drugs on metastatic patients	I. ORR	Active, not recruiting	NCT00470704
Lapatinib + Trastuzumab	≥60	II	Effectiveness of combined drugs in treating older patients	I. Grade 3+ toxicities	Active, not recruiting	NCT01273610
Lapatinib + chemotherapy vs Trastuzumab + chemotherapy	≥18	III	Comparing taxane based chemotherapy in metastatic patients with two different drugs	I. PFS	Active, not recruiting	NCT00667251
Lapatinib + Chemotherapy	≥18	II	Effectiveness of Labatinib in addition to chemotherapy in newly diagnosed patients	I. pCR	Recruiting	NCT03273595
Paclitaxel + Trastuzumab ± Lapatinib	≥18	III	Effectiveness of drug combination on patients with operable tumours	I. pCR	Active, not recruiting	NCT00770809
Capecitabine + Neratinib	≥18	Ib/II	Assess the safety of Neratinib in combination with capecitabine	I. MTD	Recruiting	NCT03377387
Neratinib	≥60	II	Effectiveness of Neratinib in treating older patients with metastatic cancer	I. Grade 2+ toxicities	Recruiting	NCT02673398
Neratinib and T-DM1	≥18	Ib/II	Assess the highest dose of Neratinib given safely and its effects	I. Feasibility/Safety	Recruiting	NCT02236000

Abbreviations: *T-DM1*, Trastuzumab Emtansine; *IDFS*, Invasive disease-free Survival; *PFS*, Progression-free survival; *DFS*, Disease free Survival; *pCR*, Pathologic complete response; *tpCR*, Total pathologic complete response; *OS*, Overall survival; *ORR*, Objective response rate; *MTD*, Maximum tolerated dose