Alvarez · Cortés · Falzon · Gandy · Gianni
Harbeck · Piccart

Handbook of HER2-Targeted Agents in Breast Cancer

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Javier Cortés, MD, PhD, received his degree in Medicine and Surgery from the Universidad Autònoma de Madrid in 1996. He continued his studies at the University of Navarra, specialising in Medical Oncology. Since 2003, Dr Cortés has worked in the Department of Medical Oncology at the Hospital Vall d'Hebron, Barcelona, where he is head of the Breast Cancer Unit and Melanoma Unit. He is also the coordinator of the Medical Oncology Training Programme for oncology residents. Dr Cortés is the author of more than 90 publications, focusing on breast tumors and new drugs and has given more than 150 communications at medical conferences. He is an active member of the Spanish, European, and American Societies of Medical Oncology (SEOM, ESMO, ASCO), and member of the Scientific Committee of the European Society of Medical Oncology.

Leticia De Mattos-Arruda, MD, is a medical oncologist and translational/clinical investigator at the Medical Oncology Department and Translational Research Program of Vall d'Hebron Institute of Oncology, Vall d'Hebron

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Mary Falzon, MD, is a consultant histocytopathologist at University College Hospital, London and an honorary Senior Lecturer at University College London. She has been a consultant since 1990 and has extensive experience in breast and lung pathology, for which she is the lead clinician at her institution. Dr Falzon has been involved in HER2 testing since its inception and has been instrumental in the setting up of UCL Advanced Diagnostics (UCLAD) as a centre for HER2 testing and interprets tests for various hospitals in the UK and abroad.

Michael Gandy, BSc, MSc, FIBMS graduated with honors from the University of Northumbria in Biomedical Sciences in 2000. Michael took up the post of trainee Biomedical Scientist (BMS) within the Histopathology Department at St James's University Hospital Leeds, UK were he obtained his state registration as a Clinical Biomedical Scientist (BMS). Whilst practicing, he then went on to to complete his Master's degree in Pathological Science at Sheffield Hallam University. In 2004, Michael transferred to Diagnostic Oncology where he focused on biomarker testing, with particular focus the HER2 IHC and ISH testing service and on the technical aspects of breast prognostic and predictive biomarkers. In 2005, Michael took the post of product development supervisor at Leica Biosystems Newcastle where he was involved in the development of a range of antibodies/assays and companion diagnostics systems development for multiple region (CE IVD/FDA) release. In 2010, Michael joined University College London (UCL) as the Lead BMS & Clinical Services Manager at UCL-Advanced Diagnostics (and latterly as Head of Clinical & Research Services for UCL-AD and UCL/Sarah Cannon Labs), the diagnostic arm of the UCL Cancer Institute and the diagnostic service laboratory for UCLH NHS Trust, leading a team of scientists in the largest

clinical IHC and ISH referral centre in the UK. Currently based within the Research Department of Tissue and Energy at UCL, Michael is now helping to develop an Integrated Molecular and Cell Science Laboratory (IMCS), focusing on prognostic and predictive biomarker discovery. He is a UKNEQAS IHC, ISH, and CPT assessor and is a keen contributor to teaching workshops for all levels, lecturing for the University of Westminster Biomedical Science and UCL Cancer Institute MSc Cancer degree courses, and is one of the co-authors of the updated UK HER2 ISH testing guidelines published in 2010.

Luca Gianni, MD is Director of the Department of Medical Oncology and Head of the Strategic Project of New Drug Development in solid tumors at the Ospedale San Raffele in Milan, Italy. He obtained his MD with honors from the State University of Milan, Italy in 1976. He is board-certified in internal medicine and was trained in clinical pharmacology and experimental therapeutics at the clinical pharmacology branch of the National Cancer Institute, Bethesda, Maryland, USA from 1980 to 1984. From 1984 to 1998, Professor Gianni has worked at the Istituto Nazionale dei Tumori of Milan, Italy, as a close collaborator of Gianni Bonadonna. In that period, he started the Strategic Program of New Drugs and Phase I studies and the Laboratory of Clinical Pharmacology, and became Associate Director of the Division of Medical Oncology. From 1999 to 2011, he took the responsibilities of Director of the Division. For many years, Professor Gianni has worked on new drug development in the field of oncology, and on the pharmacological characterization and clinical application of innovative drug therapies in medical oncology, with special emphasis on breast cancer. Since 1996, he has been coordinator of a European Cooperative Group conducting trials in women with operable breast cancer, and led several trials in the field of neoadjuvant treatment with chemotherapy and with targeted therapies. Professor Gianni has been, and is, recipient of research grants from public and private funds to conduct clinical and translational studies. He has authored more than 193 articles in peer-reviewed journals. He sits on the editorial boards of many international oncology and pharmacology journals, and is a member of several scientific and medical societies. He is also Advisor and Expert for Research Applications to the Italian Ministry of University and Research, to the Institut

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Nadia Harbeck, MD, PhD, is the Head of the Breast Center, Oncological Therapy and Breast Clinical Trials Unit, and holds the Chair for Conservative Oncology at the Department of Obstetrics and Gynecology (Hospitals Grosshadern and Maistrasse-Innenstadt), University of Munich (Ludwig-Maximilians-Universität München [LMU]), Germany. From 2009–2011, she was head of the Breast Center at the University of Cologne, Germany. Until 2009, she was Associate Professor and Head of Conservative Senology at the Technical University of Munich, where she obtained her OB&GYN specialist degree in 1998. In 1989, she received her medical degree from the University of Munich. Professor Harbeck is a member of the expert panel issuing the yearly updated evidence-based Working Group for Gynecologic Oncology (AGO) Guidelines for breast cancer therapy in Germany (www.ago-online.de). She is also a faculty and panel member of the International Conference on Breast Cancer in Young Women (BCY 2014) and of the International Consensus on Advanced Breast Cancer (ABC 2011, 2013, and 2015), as well as panel member of the 2015 St. Gallen Consensus meeting. Moreover, she served as an executive board member of the European Organization for Research and Treatment of Cancer (EORTC) between 2009–2015. She is Scientific Director of the West German Study Group (WSG) which focuses on early breast cancer trials with a strong translational research program (www.wsg-online.com) and co-chair of the TransALTTO committee. Professor Harbeck has authored more than 300 papers in peer-reviewed

journals and is coordinating editor-in-chief of Breast Care. For her clinical translational research, she has received numerous awards, including the 2012 Claudia von Schilling Award, the 2002 AGO Schmidt-Matthiesen Award, a 2001 American Association for Cancer Research (AACR) Research Award, and the 2001 American Society of Clinical Oncology (ASCO) Fellowship Merit Award.

Martine Piccart, MD, PhD, is Professor of Oncology at the Université Libre de Bruxelles (ULB) and Director of Medicine at the Jules Bordet Institute, in Brussels, Belgium. Earning her medical degrees at the ULB and oncology qualifications in New York and London, she is also member of the Belgian Royal Academy of Medicine. With a primary interest in breast cancer and drug development, Dr. Piccart is a leader in international research collaboration and is the principal or co-principal investigator of many clinical trials, including HERA, MINDACT and ALTTO. She is co-founder and chair of the Breast International Group (BIG), uniting 49 academic research groups from around the world and running over 30 trials under its umbrella. BIG's research programs include the European Commission supported TRANSBIG consortium of 28 institutions in 11 countries (running with the EORTC the MINDACT trial) and NeoBIG, an innovative biomarker and drug development program focused on neo-adjuvant trials. Dr. Piccart is active in numerous professional organizations. Since January 2012, she has been President of ESMO. She is immediate past-president of the EORTC, president-elect of ECCO and served on the ASCO Board. (Co) author of more than 420 publications in peer-reviewed journals, she has received numerous prestigious awards for her research contributions including the Jill Rose Award (New York), the William L. McGuire Award (San Antonio), and the Umberto Veronesi Award for the Future Fight against Cancer (Cancun). She has received the prestigious David A. Karnofsky Memorial Award at the 2013 American Society of Clinical Oncology (ASCO) meeting in Chicago.

Chapter 1

Introduction and background biology

Angelica Fasolo and Luca Gianni

Introduction

The successful targeting of growth factor receptors is one of the most fruitful areas of new drug discovery and development in recent years. A key moment in this chapter of modern pharmacology is the outstanding results obtained from targeting the receptor tyrosine kinase (RTK) coded by the *ERBB2* gene (also known as human epidermal growth factor receptor 2 [HER2]) with the humanized monoclonal antibody trastuzumab in women with HER2 overexpressing/amplified breast cancer. The basis for developing one of the emblematic therapeutic strategies of modern oncology stands on the original observation that amplification of HER2 was linked to a poorer outcome than recorded in non-amplified cases of breast cancer. It took almost two decades from that seminal article to the establishment of trastuzumab as standard of therapy for HER2-positive breast cancer, and the accompanying demonstration that there are a sizeable number of breast carcinomas that are 'addicted' to HER2 signaling and therefore are unable to survive a block or modulation of the signaling pathway(s) downstream of the receptor. In the following chapter, we will cover some key aspects of the biology and pathology of HER2 in breast cancer.

Epidemiology

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in women worldwide, accounting for 25% (1.67 million) of the total new cancer cases and 15% (522,000) of the total cancer deaths in 2012 [1]. More than half of all cases and 60% of the deaths are estimated to occur

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in economically developing countries [1]. The mortality for breast cancer has been decreasing over the past 25 years, largely as a result of early detection through mammography and improved treatment [2–5]. In fact, in Europe and USA, most breast cancers are diagnosed when the tumor is still confined to the breast and can be treated with curative intent. However, breast cancer remains a major cause of death in women aged between 35 and 59 years of age.

About 15–25% of breast cancers overexpress HER2 [6,7], which belongs to a family of transmembrane RTKs that mediate cell growth, differentiation, and survival [8,9]. HER2 overexpression is associated with aggressive tumor behavior [6] and until the advent of HER2-targeted therapies, patients affected by HER2-positive early breast cancer faced a poorer prognosis than patients with HER2-negative disease, including reduced relapse-free and overall survival, with a peak of recurrence at 2–3 years from diagnosis [10,11]. In addition, approximately 50% of ductal carcinomas in situ (DCIS) display HER2 amplification. The concept that a portion of DCIS eventually evolve into HER2 overexpressing infiltrating carcinomas is still the focus of discussion [12,13].

HER2 overexpression has a well defined association with prognosis. In addition, since the advent of trastuzumab and other HER2-targeting drugs, preclinical and clinical studies have shown that in women with advanced breast cancer the clinical benefit of HER2-targeting therapies are limited to those breast cancers that display the highest levels of overexpression [14], which is almost fully concordant with gene amplification. This clinical evidence has led to the routine testing of breast cancer for HER2 as a predictive factor to guide the therapeutic decision process [15].

HER2 primary structure

HER2 is a 185 KD glycoprotein encoded by a gene localized on the long arm of chromosome 17 (17q12-21) and is normally expressed in the epithelia of various organs such as lung, bladder, pancreas, breast and prostate [16,17]. It belongs to the ErbB family of transmembrane RTKs, which are a subclass of cell-surface growth-factor receptors with an intrinsic, ligand-controlled tyrosine kinase activity. RTKs have a crucial role in the signaling pathways that govern key cellular processes, such as proliferation, migration, metabolism, differentiation, and survival, and signaling that regulates intercellular communication during development. RTK activity in normal cells is tightly controlled. Mutations or

structural alterations, however, cause abnormal activation of RTKs, which become potent oncoproteins involved in the development and progression of many human cancers.

The ErbB family of receptors, to which HER2 belongs, includes four members: EGFR (epidermal growth factor receptor, also known as ErbB1), HER2, HER3, and HER4 (human EGFR-related-2, -3, and -4, named for their high level of homology to human EGFR; also named ErbB2, ErbB3, and ErbB4). These receptors are characterized by a similar molecular structure, composed of an extracellular ligand-binding domain (ECD), a hydrophobic transmembrane region and an intracellular tyrosine kinase portion. The latter domain comprises an extended C-terminal tail that includes the adenosine triphosphate (ATP)-linking position for receptor autophosphorylation and phosphorylation of respective substrates [18,19].

The ligands of the ErbB RTKs are the epidermal growth factor (EGF) for EGFR and primarily neuregulins (NRG1–4) for HER3 and HER4. HER2 has no ligand binding site and it is still unknown if a ligand for HER2 exists; HER3 does not possess kinase activity and the receptor can initiate signal transduction only when dimerized with another HER2 family member (Figure 1.1) [20].

Signal transduction through receptor tyrosine kinases

The ErbB proteins stand on the surface of the plasma membrane in an inactivated state and are activated by ligand binding. The ligand-bound RTKs undergo a conformational change of the extracellular domain that induces the dimerization of the receptors in homodimers and heterodimers, if the dimerization involves the same receptors or two different receptors, respectively. Dimerization of the receptor(s) causes autophosphorylation of the tyrosine residues of the catalytic kinase domains, which results in the activation of intracellular tyrosine-kinase cascades responsible for the downstream signal transduction [21].

Unlike other ErbB family members, HER2 lacks a ligand binding site, is constitutively active, and can undergo ligand-independent dimerization. Importantly, HER2 is the preferred partner for the other ErbB proteins, and heterodimers containing HER2 are more potent in signal transduction than homodimers of HER2 or homodimers of other ErbB proteins. In particular, the combination of HER2 and HER3 is very potent in the activation of survival and proliferation networks

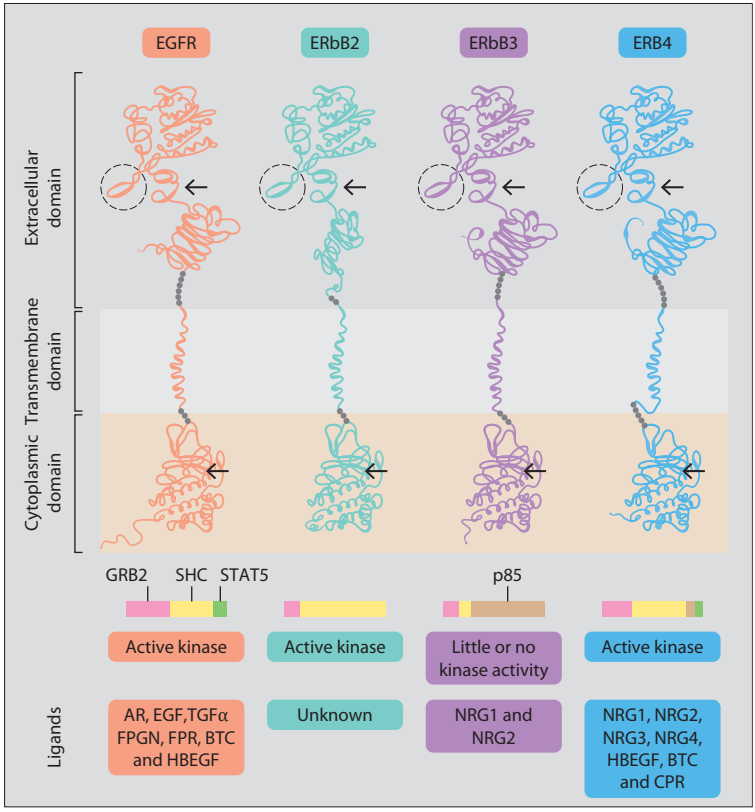

Figure 1.1 The four members of the epidermal growth factor receptor family. These are represented by their corresponding and highly homologous crystal structures. They have three major domains: the ligand-binding domain, the transmembrane domain and the kinase domain. The ligand-binding clefts are marked by upper arrows and the dimerization loops by dashed circles. ErbB2 has no ligand-binding cleft. Bottom arrows mark the ATP-binding sites. AR, amphiregulin; BTC, β-cellulin; EGF, epidermal growth factor; EPGN, epigen; EPR, epiregulin; HBEGF, heparin-binding EGF-like growth factor; NRG, neuregulin; STAT5, signal transducer and activator of transcription 5; TGFα, transforming growth factor-α. Reproduced with permission from Yarden and Pines [20] ©Nature Publishing Group.

because the cytoplasmic tail of HER3 contains binding sites for phosphatidylinositide 3-kinases (PI3K), which strongly activates the PI3K-Akt-mTOR (mammalian target of rapamycin) pathway, whereas HER2 powerfully signals through the mitogen-activated protein kinase (MAPK) pathway. The subsequent deregulation of PI3K and MAPK signalling strongly enhances cell proliferation and evasion of apoptosis [22–25]. Therefore, the overexpression of HER2 that occurs in HER2-positive breast

cancer can directly result in excess dimerization of ErbB proteins and subsequent increase of cellular signaling, resulting in a poor clinical outcome in breast cancer and resistance to various cancer therapies.

Receptor tyrosine kinases: site of therapeutic intervention

The observation that deregulation of the RTK-signaling network is crucial for tumor growth and survival constitutes the rationale for the development of targeted anticancer therapies. Neutralizing antibodies, which block the bioactivity of RTK ligands, RTK-targeted antibodies, which target overexpressed receptors, and small-molecule inhibitors of RTK kinase activity have been developed to interfere with RTK signal transduction.

An overview of the HER2-signaling network and the therapeutic strategies directed against HER2 are summarized in Figure 1.2 and Figure 1.3.

Signaling through HER-receptor family dimers leads to the activation of downstream cascades

The signaling and metabolic networks involving HER2 are characterized by great plasticity that confers robustness to the system through amplification and redundancy of signals, but also fragility through feedback loops that elicit resistance to anti-HER2 agents [26]. Two main mechanisms of resistance have been identified so far: HER2 can evade the targeted drug, or the driving role of HER2 in cancer 'addiction' is taken over by another pathway [27,28]. HER2 may elude trastuzumab (but not lapatinib or other HER 2-targeted tyrosine kinase inhibitors) by alternative splicing or proteolytic cleavage, which generates an intracellular constitutively active fragment called p95 [29], or changes in the structure of the ECD, which prevent the antibody-receptor interaction and the consequent immune response (antibody-dependent cell-mediated cytotoxicity [ADCC]) [30,31]. In addition, compensatory pathways such as upregulation of ErbB3 or insulin-like growth factor 1 receptor (IGF1R) or activation of the PI3K-Akt pathway through loss of *PTEN* or PI3K mutation have been described in trastuzumab-resistant model systems [32–36]. Similarly, resistance of breast cancer cells to lapatinib may involve overexpression of other RTKs or de-repression of the estrogen receptor (ER) pathway [37,38]. A more recent analysis showed that mutations in exon 21 of the *HER2* gene are also involved

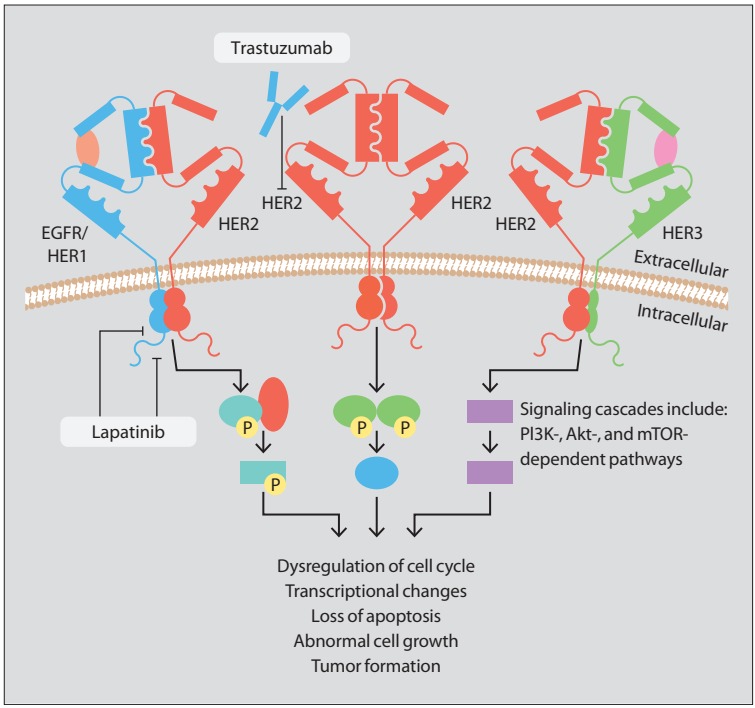

Figure 1.2 Signaling through HER-receptors family dimers leads to the activation of downstream cascades. Downstream signaling pathways include PI3K-, Akt-, mTOR- and MAPK-pathways, which control cell cycle, cell growth and survival, apoptosis, metabolism and angiogenesis. Signaling through HER2 homodimers is inhibited by the monoclonal antibody trastuzumab. Lapatinib is a small molecule that inhibits HER1 and HER2 tyrosine kinase activities. Akt, protein kinase B; EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; mTOR, mammalian target of rapamycin. Adapted from Ahn and Vogel [24].

in resistance to trastuzumab, without directly affecting trastuzumab binding to the receptor because exon 21 codes for the intracellular tyrosine kinase domain and mutations in this region do not change the structure of HER2 ECD [39].

Other molecules currently being investigated in clinical trials of HER2-resistant breast cancer include Heat Shock Protein 90 (HSP90) inhibitors and telomerase inhibitors. Indeed, HSP90 acts as a chaperone protein which promotes the stabilization of many other proteins, including HER2, and prevents their rapid degradation. Telomerase expression is crucial for cellular proliferation and telomerase overexpression has been linked to tumorigenesis, whereas the inhibition of telomerase results in apoptosis or cell senescence. In trastuzumab-resistant cell lines, the inhibition of HSP90 or the inhibition of

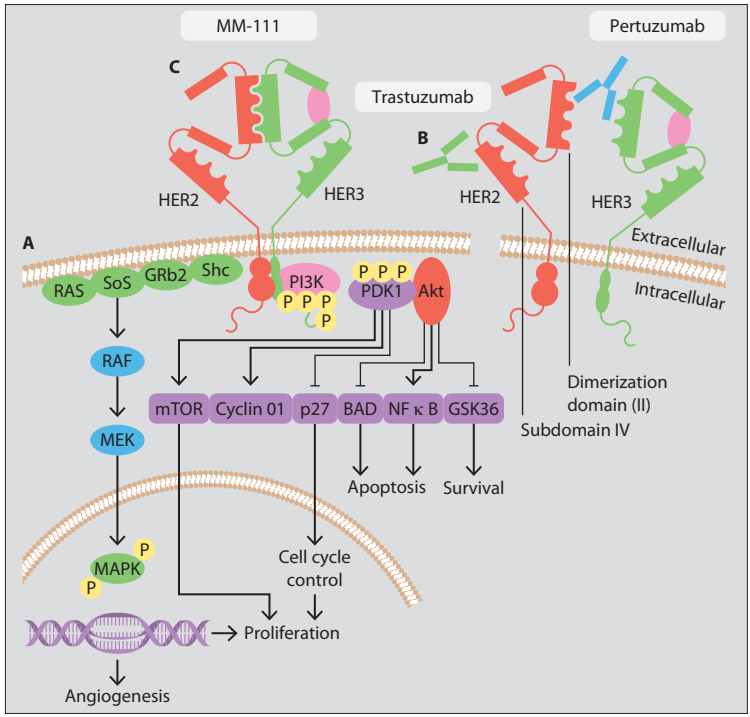

Figure 1.3 The HER2:HER3 heterodimer. (A) The HER2:HER3 heterodimer is a potent trigger of downstream signaling cascades, especially the PI3K/Akt cascade and the MAPK cascade. **(B)** HER2:HER3 signaling can be inhibited by the monoclonal antibody pertuzumab, preventing HER2:HER3 dimer formation by blocking the HER2 dimerization domain (subdomain II), which is distinct from the site of trastuzumab binding (subdomain IV). (**C**) A bispecific antibody for both HER2 and HER3 (MM-111) is being evaluated in combination with trastuzumab. Akt, protein kinase B; HER, human epidermal growth factor receptor; GRb2, growth factor receptor-bound protein 2; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog. Adapted from Ahn and Vogel [24].

telomerase was able to restore trastuzumab sensitivity [40,41]. Furthermore, several lines of evidence recently showed that the crosstalk between the ER and HER2 pathways plays a role in resistance to HER2-directed agents [42,43]. In fact, signaling from EGFR can downregulate ER and, conversely, the inhibition of HER2 with either trastuzumab or lapatinib, results in upregulation of ER and increased transcription of ER-regulated genes, which act as an 'escape' mechanism that contributes to resistance to HER2-directed agents [44]. This observation suggests that the combined inhibition of ER and HER2 may be critical to prevent the development of resistance to HER2-targeted therapies and

that the identification of mechanisms of resistance to trastuzumab has important implications for the rational selection of subsequent targeted therapies against other pathways and molecules implicated in HER2 resistance (Figure 1.4) [45].

The great plasticity of the HER2-regulated network and the many functions played by the different intracellular and extracellular domains of the HER2 receptors have led to the concept that dual targeting of HER2 may lead to enhanced therapeutic results in HER2-overexpressing tumors. Preclinical evidence in the KPL4 model of trastuzumab-resistant breast cancer have clearly shown that the combined use of trastuzumab with the monoclonal antibody pertuzumab, which blocks receptor dimerization, was more active than either monoclonal antibody alone. In addition, evidence was provided that tumor regrowth after initial response to trastuzumab could be reversed upon introduction of pertuzumab as second antibody [46]. The clinical evidence collected over the years is in line with the evidence obtained from animal models. Introduction of pertuzumab while continuing trastuzumab led to a high rate of objective responses and long-lasting stable disease in women with HER2-positive breast

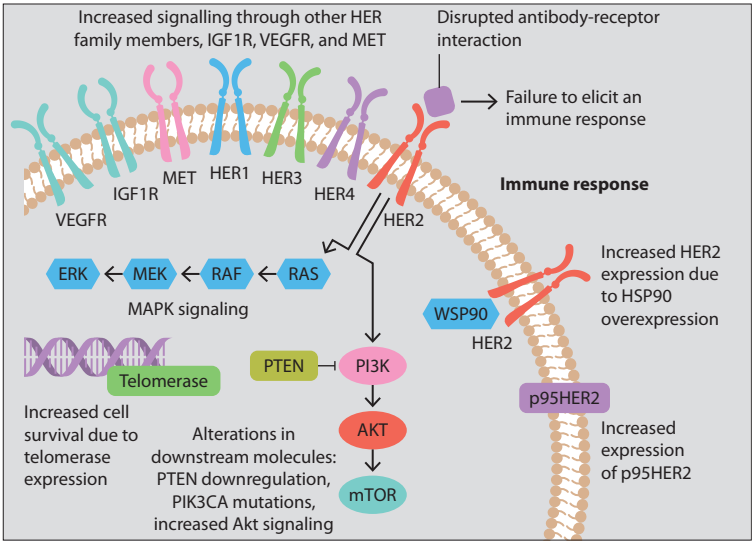

Figure 1.4 Proposed mechanisms of HER2 resistance. Akt, protein kinase B; HER, human epidermal growth factor receptor; HSP90, heat shock protein 90; IGF1R, insulin-like growth factor receptor 1; MAPK, mitogen-activated protein kinase; MET, mesenchymal epithelial transition factor; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; VEGFR, vascular endothelial growth factor receptor. Reproduced with permission from Mohd Sharial et al [45] ©Oxford University Press.

cancer progressing on trastuzumab either alone or in combination with chemotherapy [47]. This led to the initiation of the neoadjuvant study NEOSPHERE, which showed a significantly higher rate of pathologic eradication of operable breast cancer with the combination of pertuzumab and trastuzumab with docetaxel than with the conventional combination of trastuzumab and the taxane [48]. Conclusive evidence of the superiority of this approach to dual blockade of HER2 has been provided by the Phase III trial CLEOPATRA, which showed superior response, progression-free survival and overall survival with trastuzumab, pertuzumab and docetaxel compared with trastuzumab and docetaxel in HER2-positive metastatic breast cancer [49,50].

In a different approach to dual targeting, the tyrosine kinase inhibitor lapatinib was combined with trastuzumab. This approach leads to a more complete inhibition of receptor signaling while maintaining the immune mechanisms of activity afforded by the immunoglobulin (IgG) nature of trastuzumab. In addition, lapatinib should also block the signaling of truncated forms of HER2, for which trastuzumab is devoid of any activity. The preclinical evidence in favor of combining trastuzumab with a HER2 tyrosine kinase inhibitor found clear-cut correspondence with the clinical findings of NeoALLTO, a neoadjuvant study in which lapatinib and trastuzumab with paclitaxel were compared with trastuzumab and paclitaxel or lapatinib and paclitaxel [51]. The results showed a significant benefit for the dual HER2 targeting approach.

The success of treating HER2-positive breast cancer with HER2-targeted drugs with different mechanisms of action confirms that superior results can be expected by inhibiting the HER2-associated signaling pathway at different points. This is consistent with results from preclinical studies showing a potential benefit from concomitant use of trastuzumab and inhibitors of the PI3K [52], or inhibitors of mammalian target of rapamycin (mTOR) [53,54].

The immune system and response to HER2-targeted treatment

A growing body of preclinical and clinical evidence shows that the innate and adaptive immune system contributes substantially to the therapeutic effects of trastuzumab in vivo [55,56]. A correlation has been noted between a higher level of immune infiltration and a lower risk of relapse in patients not receiving

adjuvant treatment, irrespective of molecular subtype. The most consistent association between good prognosis and immune infiltration has been recorded in triple-negative and HER2-positive tumors [57–59]. Study findings in patients with breast cancer treated with neoadjuvant chemotherapy show that augmented expression of immune-associated genes and extensive lymphocyte infiltration in the tumor before treatment are associated with increased likelihood of a pathological complete response in HER2-positive tumors [60]. Furthermore, findings indicate that immune-related markers can provide useful predictive information and that increased clinical activity might follow activation of the immune system. Development of immunomodulatory drugs with remarkable activity in many solid tumors defines a scenario in which the combination of immune modulation with trastuzumab, or other HER2-directed drugs, will result in augmented response and clinical outcome [61–64].

Conclusions

The thorough characterization of HER2 biology and the involvement of this growth factor receptor in the pathogenesis and maintenance of about 25% of breast carcinomas has contributed to the development of one of the most successful therapeutic interventions in the era of targeted oncology drugs. In addition, it has contributed to the elucidation of mechanisms relevant to other therapeutic approaches and other neoplastic diseases. Importantly, it has clearly shown the power of the concomitant targeting of the HER2 pathway in HER2 'addicted' carcinomas, illustrating the benefit of a multipronged treatment approach with targeted agents in oncology. For all the above, the clarification of HER2 biology and pathology and the still ongoing development of multiple targeted approaches against HER2-driven tumors stands as a reference in the field of modern oncology.

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Chapter 2

HER2 testing

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Introduction

The human epidermal growth factor receptor 2 (HER2) *ERBB2* gene has been at the forefront of the therapeutic management of breast cancer since 1998, following the US Food and Drug Administration (FDA) approval of trastuzumab. This drug was initially introduced into clinical practice for patients with metastatic breast cancer, and then subsequently used in patients with early-stage primary disease [1]. Positive confirmation of HER2 status, in conjunction with the wider clinicopathologic characteristics of the patient and their disease, now determines eligibility for treatment with a range of HER2-targeted therapies.

The HER2 protein, a receptor tyrosine kinase molecule, spans the cell membrane with an N-terminus extracellular domain, transmembrane region, and nuclear carboxy-terminal fragments [2]. It is encoded for by the *HER2* gene located on the long arm of chromosome 17 at position 17q21 [3]. The normal function of HER2 is associated with cellular processes of differentiation, growth, development, and apoptosis via activation of tyrosine kinase activity through dimerization of HER2, with itself and other members of the epidermal growth factor receptor family [2]. Approximately 15% of patients with breast cancer overexpress the HER2 protein or show amplification of the gene [4]. It is this constituent link between gene amplification and protein overexpression, and the availability of the HER2 extracellular domain as a target for humanized monoclonal antibody-based therapies, which has driven the HER2 therapeutic and testing rationale.

The assessment of HER2 in clinical practice commonly takes place within a histopathology laboratory, and both the histopathology processes

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and the specialist area of HER2 testing are governed by regional regulatory bodies (eg, US College of American Pathologists HER2 proficiency programs; UK National External Quality Assessment Scheme for HER2). In 2007, the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) defined the histologic phases which span the HER2 testing process as pre-analytic (sample handling [fixation and tissue processing]), analytic (testing), and post-analytic (interpretation) [1]. Of note, the ASCO/CAP recommendations for HER2 testing guidelines were updated in 2014 [5,6].

Pre-analytic phase: sample handling

HER2 assessment in breast cancer can be made using a variety of sample types, including primary core biopsies, resection specimens, and metastatic biopsies. On the rare occasion when no histological material is available, cytology cell blocks may be utilized. The HER2 protein in each of these sample types is subject to the stresses associated with histopathologic processing. The critical steps in the process include:

- *Cold ischemic time*: time taken from removal of the sample from the patient to fixing of the sample in an appropriate fixative (10% neutral buffered formalin) [1,7, 8]. Prolonged cold ischemic times can result in degradation of the HER2 protein and other cellular structures [9].
- *Fixation*: stabilization of tissue structures and genetic material in preparation for tissue processing [10]. A fixation time of 6–72 hours is recommended for breast cancer samples, depending on size and type of the sample [1,5].
- *Tissue processing*: complete dehydration, clearing, and paraffin embedding of the tissue sample in preparation for sectioning [1,8].
- *Slide preparation*: tissue sectioning at 3–4 μm and adherence on to charged microscope slides via incubation of the slide at 37°C overnight (or 60°C for 1 hour) in preparation for staining [8]. Prolonged storage of cut paraffin sections can result in antigen degradation [1].

Since the introduction of guidance in the pre-analytic phase for clinical practice, there has been a concerted development towards a more standardized approach to assessment of HER2 status across testing [1,6,11,12].

Analytic phase: HER2 testing methodologies

Pathologic assessment

Breast cancer is not one disease, but many different diseases. Even when tumors are classified together based on their morphology, they can act differently because of different genetic makeups. HER2-positive breast cancer is one form and is characterized by aggressive growth and a poor prognosis; it is caused by the overexpression or amplification of the HER2 gene in tumor cells [1]. In approximately 15% of women with breast cancer, there is a genetic alteration in the HER2 gene that produces an increased amount of the growth factor receptor protein on the tumor cell surface [4]. A recent study also found a 14.9% rate of HER2 overexpression in men with breast cancer [13]; however, due to the low incidence of male breast cancer and variability of HER2 testing clinically in this demographic, further and wider studies to determine HER2 positivity rates in this setting are required.

In the UK, routine testing for HER2 is recommended for all patients diagnosed with invasive breast cancer because the results may affect treatment recommendations and care plan decisions [6,11]. All types of epithelial-derived invasive primary breast cancers should be tested at diagnosis for HER2 status by immunohistochemistry (IHC) in the first instance [14]. HER2 testing is not currently performed routinely for ductal carcinoma in situ [6,11].

Whenever breast cancer recurs or metastasizes, the tumor should be retested for HER2 as well as for hormone receptor (ie, estrogen receptor [ER] and progesterone receptor [PR]) status, as these change from the original primary cancer in up to 20–30% of cases [15]. In patients with metastatic breast cancer, accurate determination of HER2 status is critical for guiding treatment decisions. The National Comprehensive Cancer Network (NCCN) guidelines recommend HER2 testing at relapse, particularly if HER2 expression was originally unknown or negative [14].

Clinical testing methodologies

In the UK, laboratories undertaking HER2 testing should have Clinical Pathology Accreditation, participate in the recognized national external quality assessment scheme, and carry out a formal annual audit of its testing services [6,11,12]. It is recommended that testing is restricted to laboratories undertaking

a minimum of 250 tests per annum for IHC and 100 tests per annum for in situ hybridization (ISH) techniques [6,11]. A similar level of accreditation and safety audits should be adhered to in all regions where possible.

Within the analytic phase, a number of testing methods have been developed. However, IHC and ISH remain the predominant methods used to assess HER2 status in the clinical setting [6,11]. These target the HER2 protein and gene, respectively, and are clinically validated, commercially available, and subject to regional regulatory clearance and control (eg, FDA) [1,16,17]. It has been shown via external quality assessment that the introduction of standardized and validated companion diagnostic assays have improved the quality of clinical HER2 testing. Examples of these include the Dako HercepTest (Dako Denmark A/S, Glostrup, Denmark) and Abbott PathVysion assays, co-approved on first approval of trastuzumab, and the widely used Ventana Pathway HER2 4B5 and DDISH assays (Ventana Medical Systems, Inc, Tucson, Arizona, USA), which have helped standardize HER2 testing in conjunction with fully automated laboratory instrumentation [1,2,16–18].

Within the laboratory, irrespective of which assay is used, testing is controlled by a mix of analyte control standards, which often come in the form of commercially available breast cancer cell lines or xenografts [1]. These are a constituent part of the diagnostic assays and, via locally prepared laboratory-specific tissue control slides, demonstrate each of the four HER2 pathologic scoring criteria (described below) on the individual laboratories' own prepared histologic material. Where possible, the use of a same-slide "control + test" is now seen as the gold standard in slide-based biomarker testing, controlling for individual slide positions on fully automated staining platforms. Internal structural controls with the test sample can also help guide assessment, with benign breast ducts showing little or no overexpression with IHC and expressing normal HER2:CEP17 gene ratios with ISH [1].

Post-analytic phase: screening and interpretation

Immunohistochemistry

The use of IHC in HER2 assessment requires input and governance by a specialist breast pathologist. The first stage of all HER2 assessments is to ensure that invasive breast carcinoma is being screened for, rather than carcinoma in situ. The sample is then tested for HER2. All regulatory-cleared IHC assays target the intercellular domain of the HER2 protein. Although extracellular or

external domain monoclonal antibodies exist and are readily available for use in IHC assays, none have been officially approved for use in clinical practice.

The scoring of HER2 IHC utilizes 4 distinct grades: 0, 1+, 2+ and 3+ (Figure 2.1). Screening is based on percentage of invasive tumor cell staining, the completeness of the circumferential staining, and the intensity of the stain [19]. It is recognized that each tumor when viewed microscopically is a single 'snapshot in time' of its activity and is representative of a much larger three-dimensional biologically active structure.

Immunohistochemistry interpretation determines if patients are suitable for HER2-targeted therapy (positive, 3+) or not (negative, 0–1+). There is also an equivocal category (2+) to identify patients who require further confirmatory testing and analysis (Table 2.1) [5,6,11]. This second-line testing component of the clinical HER2 testing algorithm utilizes ISH methods to examine the HER2 gene and classifies patients for treatment purposes. When samples are handled correctly in the pre-analytic phase and IHC testing is performed correctly, HER2 IHC staining can be localized to the cell membrane and can be accurately interpreted, as illustrated in Figure 2.1.

Reported variations in IHC screening results may be due to subtle differences between different assays (eg, epitope binding site, detection system sensitivity, sample heterogeneity, laboratory protocol, and pathologist/screener interobserver variation). Also, compromising the pre-analytic processes can often result in diffuse or granular IHC staining, which can be difficult to interpret. Despite all of these factors, IHC has proven to be an accurate, robust, and cost-effective method for assessing patients' HER2 status.

In recent times, difficult-to-interpret cases, or those that are suspected of having undergone suboptimal pre-analytic factors, are also referred for ISH testing. This may be to help clarify unique expression patterns as a result of heterogeneity or cases where tissue protein is damaged.

In situ hybridization screening

In situ hybridization screening methods (eg, fluorescence, chromogenic, or silver-based techniques) are all variations based on the same core ISH technology [1]. Utilizing either fluorophore- or hapten-labeled probes that are complementary to regions of the HER2 gene and chromosome 17, ISH screening tests microscopically visualize HER2 and chromosome 17 [6,11]. Standard

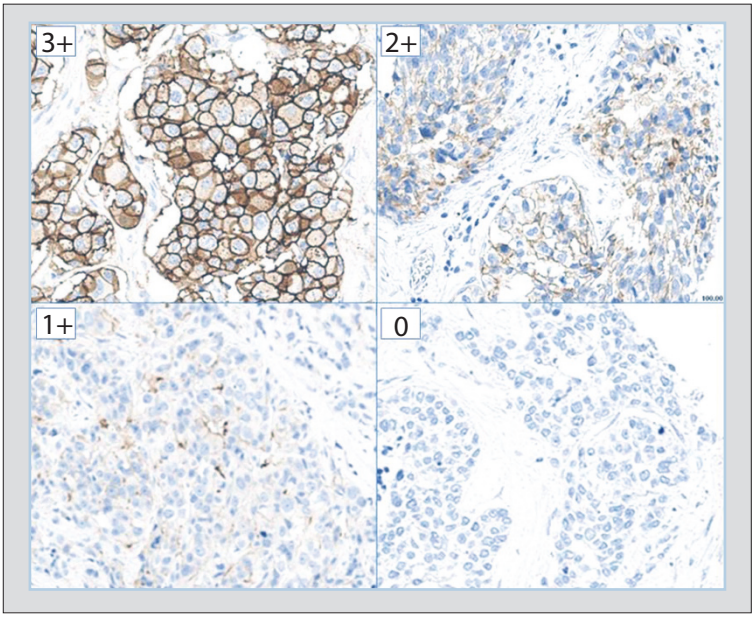

Figure 2.1 Immunohistochemical staining for HER2-positive breast cancer. This figure illustrates the 4 distinct immunohistochemical grades: 0, 1+, 2+, 3+.

Score to report	HER2 protein overexpression in assessment	Staining pattern
0	Negative	No staining observed or membrane staining that is incomplete and is faint/barely perceptible and within 10% of the invasive tumor cells
1+	Negative	Incomplete membrane staining that is faint/barely perceptible and within >10% of the invasive tumor cells
2+	Weakly positive* (equivocal)	Circumferential membrane staining that is incomplete and/or weak/moderate and within >10% of the invasive tumor cells or complete and circumferential membrane staining that is intense and within ≤10% of the invasive tumor cells
3+	Strongly positive**	Circumferential membrane staining that is complete, intense and within >10% of tumor cells

Table 2.1 Recommended immunohistochemical scoring method. *Weakly positive cases (2+): may be considered equivocal and reflexed to ISH testing. **Strongly positive cases (3+): based on recent testing guidelines, a 10% cut-off is recommended. FDA-approved scoring guidelines recommend a 10% cut-off for reporting positivity. Adapted with permission from Wolff et al [5] ©College of American Pathologists.

practice includes assessing the HER2 and centrometric probe for chromosome 17 (CEP17) signals in 20–60 tumor nuclei to determine the average HER2 and CEP17 copy numbers per cell, followed by calculating the HER2:CEP17 gene ratio. Cases are then reported as [6,11]:

- non-amplified negative (<1.80);
- borderline non-amplified negative (1.80–1.99);
- borderline amplified positive (2.00–2.20);
- amplified positive (>2.20); or
- HER2 gene copy number ≥6 may be considered positive if a single HER2 gene assessment method is employed.

For cases which fall in the borderline region, a second review with additional cells (20–40) is performed. An overall result is then reported, with the definitive HER2:CEP17 gene ratio cut-off defined as ≥2.00 (Figure 2.2) [1,6,11].

Testing in context

The review of HER2 expression, alongside ER and PR status, can aid in the treatment decision-making progress due to the (broadly speaking) inverse relationship between HER2 and ER/PR expression. For cases where distinguishing in situ from invasive breast cancer is necessary, additional stains and markers such as cytokeratin 5 can be invaluable [1]. To facilitate accurate quantification, it is useful if these markers are available for review at the time of HER2 protein and gene expression testing. The availability of the HER2 IHC-stained slide for ISH screening provides reviewers with the ability to define genuine areas of amplification associated with focal overexpression and report it accordingly.

Heterogeneity

Although it is not the predominant biologic pattern, intratumoral heterogeneity of the HER2 gene and its encoded protein may be observed. Intratumoral heterogeneity can be genuine and related specifically to clonal tumor cell populations within the mass, or it may have been introduced as an artifact through suboptimal sample handling at the pre-analytic stage [20]. Cases which present with bilateral carcinoma, if morphologically different, should be considered as individual tumor entities and tested and reported as such using standard HER2 reporting criteria.

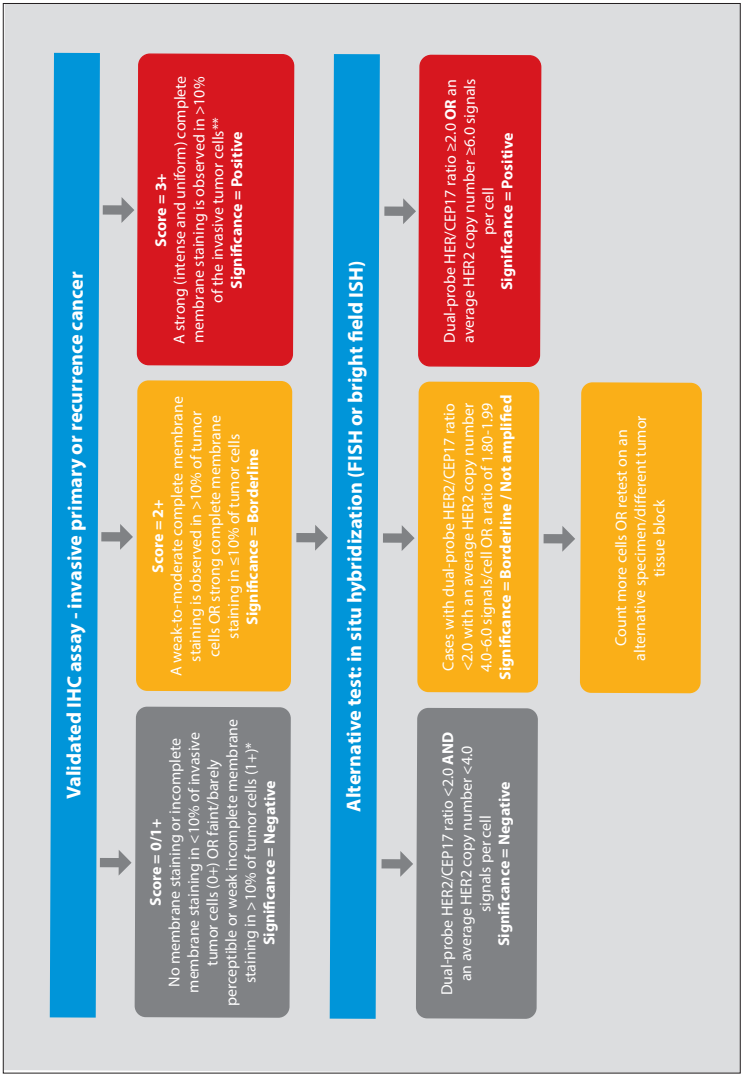

Figure 2.2 Recommended HER2 scoring algorithm for immunohistochemistry (IHC) and in situ hybridization (ISH). *Insufficient data are available to comment on moderate complete membrane staining in ≤10% of tumor cells or strong incomplete membrane staining in >10% of tumor cells. A repeat on another specimen/tissue block is advisable. **Membrane staining must be intense and uniform and resemble chicken-wire. Ignore incomplete or pale membrane staining in the percentage estimation. Reproduced with permission from Rakha et al [6] ©BMJ.

Intratumoral heterogeneity should be formerly documented on the report including any potential correlations between morphological, immunohistochemical, and in situ patterns. Cases involving both simple and complex heterogeneity patterns should be discussed within the context of multidisciplinary team meetings with the aim of correlating heterogeneic findings. Where possible, retesting on an alternate tissue block may prove useful.

Polysomy and co-amplification

Polysomy of CEP17 is when there is, on average, ≥3 signals or copies of the CEP17 probe per cell [1]. Cases which have high levels of both HER2 and CEP17 have been traditionally classified as negative for treatment purposes based on a resultant negative HER2:CEP17 gene ratio (<2.00) [6,11]. This topic remains an area of interest for researchers and clinicians, with ongoing investigations into the clinical significance of gene ratio versus single copy number, with a recent suggestion that cases with HER2 gene copy number ≥6 should be considered for treatment [5–7].

Distinctly different from polysomy, signal co-localization or co-amplification is described when the HER2 and CEP17 probes microscopically co-occupy spatially close regions on the chromosome, leading to an increase in the copy number of both HER2 and CEP17 signals. It is suggested that this observation may be due to the extension of the HER2 amplicon into the pericentromeric region of chromosome 17; in co-amplification, both the HER2 gene and this extension into the centromere visibly co-amplify [21]. This genotype has a distinct profile under fluorescence examination, producing an intense yellow 'fusion-like' color as a result of a color-merging of HER2 (red) and CEP17 (green) signals [21]; this pattern is more difficult to detect using chromogenic ISH methods due to lack of fluorescent color merging. Research recommendations suggest that these unusual and rare cases should be interpreted with caution, but patients may still be considered eligible for trastuzumab treatment (Figure 2.3) [21].

In order to help further accurately categorize gene amplification status, a number of models have been presented, such as those developed by Tse et al [22] and Mansfield et al [23] which utilize alternative markers to assess chromosome 17. Although in clinical practice there are few cases of gene co-amplification,

the Mansfield dual ISH model utilizes an alternative noncentromeric CEP (D17S122 locus [17p12]) in conjunction with the HER2 probe to provide a way of determining if co-amplification is due to specific pericentromeric extension of HER2 and not polysomy of chromosome 17 [23].

Alternatively, methods such as multiplex ligation-dependent probe amplification may prove useful [24]. However, as with other nonmorphologic-based methods, there is an inherent risk of viewing the result out of its pathologic context. A consensus on how these cases are handled clinically has not yet been defined. Both local and international breast cancer guidelines should be periodically reviewed, as they provide direction as to test interpretation based on current scientific and clinical data.

Recent research recommendations from the UK National External Quality Assessment Service for these challenging and infrequently seen cases suggest that they should be reported as amplified, with co-amplification of the centromere based on the HER2 copy number and associated balanced CEP17 copy number. Co-localization of the HER2 and CEP17 signals should also be indicated, with further genetic investigations performed to clarify the genetic profile [21]. The alternative chromosome 17 marker may be useful in defining the observations of both polysomy and co-amplification, both of which are currently reported and interpreted with an element of uncertainty.

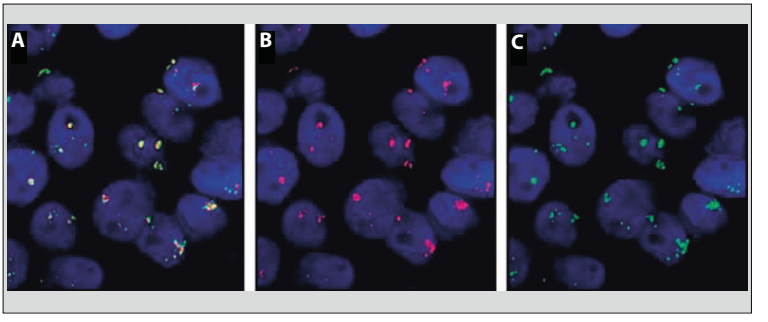

Figure 2.3 Co-localization of HER2 and CEP17 signals. (**A**) Co-localization of HER2 and CEP17 signals is represented in this figure by a yellow fusion signal. This differs from the independent (**B**) HER2 and (**C**) CEP17 signals. Reproduced with permission from Starczynski et al [21] ©American Society for Clinical Pathology.

Alternate HER2 testing methodologies

Although IHC and ISH methods are by far the most widely used methods in clinical practice, others can be used to augment clinical testing and for research investigations.

Serum HER2 monitoring

The monitoring of serum HER2 relies on the detection of the soluble shed HER2 p95 extracellular domain (ECD) [25]. The cleavage of the ECD to produce shed HER2 p95 occurs through interactions of the HER2 molecule with biologically active proteolytic enzymes present in the extracellular matrix [25,26]. Using an enzyme-linked immunosorbant assay, this method has mostly been used in research and trial settings in an attempt to monitor patient performance with HER2-targeted therapies (via association with concentration of shed extracellular domain). However, a recent comprehensive review by Leyland-Jones and Smith looked at over 60 independent investigations and concluded that, based on inconsistent data, HER2 ECD analysis should not be used for patient management purposes and clinicians should instead follow standard clinical parameters and national guidelines [25].

Quantitative reverse transcription polymerase chain reaction methods

Quantitative reverse transcription polymerase chain reaction (PCR) methods for determining amplification of the HER2 gene have been shown to produce results similar to those seen with ISH [27]. However, the main area of concern which has prevented these assays from widespread adoption in clinical practice is that they rely on either micro- or macrodissection of the tumor and normalization of background HER2 [28]. This can prove difficult in cases with mixed in situ and invasive components, or impossible when assessing areas of microinvasion which cannot be dissected. Thus, various PCR-based methods have shown discordant results [29]. The ability to review amplification or overexpression in a morphologic context still remains a key element to accurately assessing HER2 status.

HER2 somatic mutation analysis

As previously discussed, clinical analysis of the HER2 gene and its resultant protein has focused primarily on the genetic abnormalities of gene amplification, copy number variation, and resultant protein overexpression. The completion of the human genome project and the evolution of sequencing technology to accurately characterize mutations in the HER2 gene has led to HER2 genetic alterations as a rapidly developing area of interest. Current research suggests the need to include analyses of somatic HER2 mutations in clinical trials of HER2-targeted compounds [30], as well as for compounds which affect pathways that HER2 is a constituent part of.

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Chapter 3

HER2-positive breast cancer: neoadjuvant and adjuvant therapy

Nadia Harbeck

Neodjuvant therapy

In principle, anti-human epidermal growth factor receptor 2 (HER2) therapy can be started together with chemotherapy in the adjuvant and neoadjuvant settings. After neoadjuvant chemotherapy in combination with anti-HER2 therapy, trastuzumab is continued for a total of one year in the adjuvant setting. Trastuzumab is the only anti-HER2 agent approved for both the neoadjuvant and adjuvant setting. For neoadjuvant therapy, dual anti-HER2 blockade with trastuzumab and pertuzumab has recently been approved.

Achieving a pathological complete remission (pCR) under trastuzumab-based neoadjuvant therapy is correlated with an excellent outcome [1,2]. Thus, neoadjuvant trastuzumab therapy has a distinct advantage in that patients can be counseled more accurately about their prognosis based on the result at surgery. Moreover, participation in a post-surgery trial may improve outcome in non-pCR patients.

In the open-label Phase III NOAH trial, patients with newly diagnosed locally advanced or inflammatory HER2-positive breast cancer (n=235) were randomized to preoperative anthracycline-taxane chemotherapy with trastuzumab or chemotherapy alone. In a separate cohort, 99 patients with HER2-negative disease were also treated by the same chemotherapy. In the HER2-positive cohort, pCR rates were significantly higher with trastuzumab (in-breast pCR 43% vs 22%, P=0.0007; breast + lymph node [LN] pCR 38% vs 19%; P=0.001) [1]. Interestingly, pCR rates in the HER2-negative cohort were similar to those in the HER2-positive, non-trastuzumab group (in-breast pCR 17%; pCR breast +

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R.H. Alvarez et al. (eds.), *Handbook of HER2-Targeted Agents in Breast Cancer*, DOI 10.1007/978-3-319-28216-9_3

LN 16%) [1]. Given this high efficacy of preoperative trastzumab, subsequent neoadjuvant trials in HER2-positive disease did not have a non-trastuzumab control arm. The TECHNO trial was the first neoadjuvant trial in HER2-positive breast cancer which showed a significant correlation between pCR under a trastuzumab-containing regimen and improved outcome [3]. All patients received four doses of epirubicin + cyclophosphamide (EC) followed by four doses paclitaxel + trastuzumab every three weeks; pCR in breast + LN was reported in 38.7% of patients [3]. Patients who achieved a pCR had an excellent 3-year outcome with a significantly better disease-free survival (DFS) and overall survival (OS) than patients without a pCR (Figure 3.1) [3].

In the GeparQuattro Trial, preoperative EC followed by docetaxel (EC-Doc), + capecitabine, was combined with trastuzumab for eight cycles of chemotherapy. Looking at breast and axilla, a total pCR rate of 40.0% was found [4]. Additionally, a meta-analysis of the German Breast Group neoadjuvant trials (n=3332) demonstrated that simultaneous trastuzumab treatment in HER2-positive tumors increased odds of pCR 3.2-fold ($P<0.001$) [5]. In the GeparQuinto trial (n=620), preoperative EC-Doc was combined with either trastuzumab or lapatinib for the duration of chemotherapy; pCR was significantly better in the trastuzumab arm (30.3%) than in the lapatinib arm (22.7%; odd ratio [OR] 0.68; 95% confidence interval [CI], 0.47–0.97; $P=0.04$) [6]. Side effects differed between the two study groups, with trastuzumab being associated with more edema and dyspnea, and lapatinib with more diarrhea and skin rash. There were more treatment discontinuations (33% vs 14%) and more serious adverse events (87 vs 70) in the lapatinib group [6]. In view of this data, trastuzumab (+ chemotherapy) has become neoadjuvant standard in HER2-positive breast cancer.

Recent neoadjuvant trials have shown that dual HER2 blockade with a chemotherapy backbone is associated with higher pCR rates than single blockade using trastuzumab alone. In the NeoALTTO trial, patients were randomized to preoperative lapatinib (1500 mg); trastuzumab; or lapatinib (1000 mg) plus trastuzumab, with a backbone of paclitaxel monochemotherapy for 12 weeks following 6 weeks of targeted therapy [7]. After surgery, patients continued their targeted therapy for a total of 52 weeks. pCR was significantly higher in the lapatinib + trastuzumab

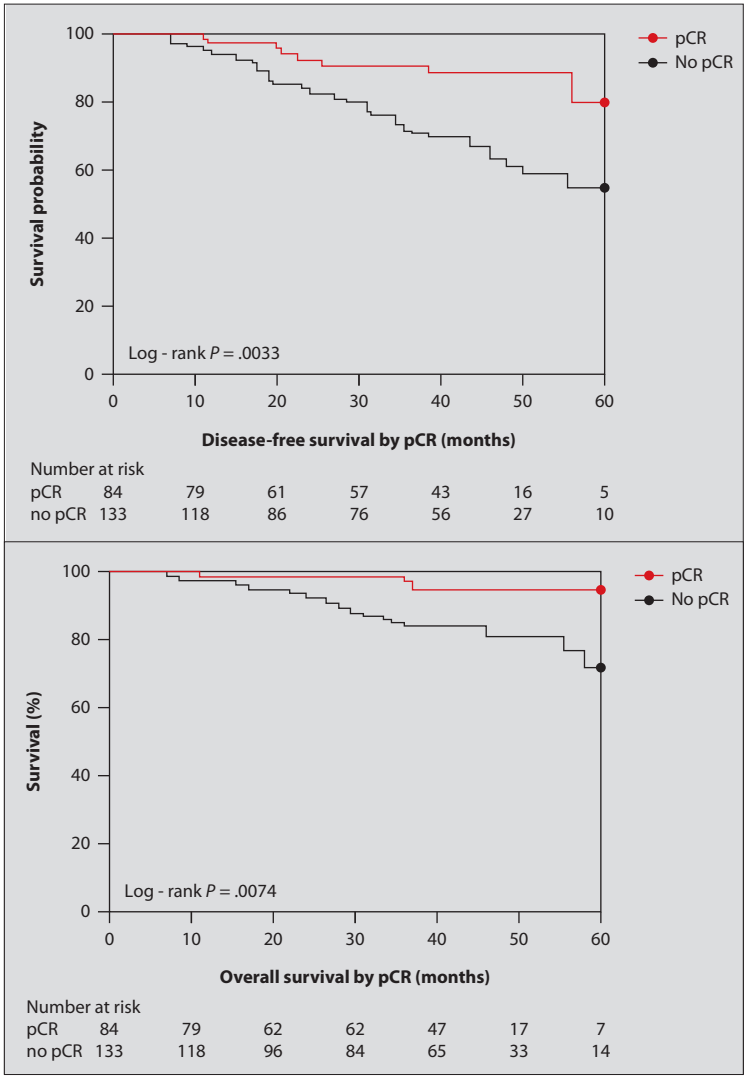

Figure 3.1 Disease-free and overall survival depending on pathological complete remission status after neoadjuvant trastuzumab + anthracycline-taxane chemotherapy. pCR, pathological complete remission. Reproduced with permission from Untch et al [3] ©ASCO.

group (51.3%) than in the trastuzumab-only group (29.5%; P=0.0001). pCR in the lapatinib group (24.7%) was similar to that in the trastuzumab group [7].

The first survival results of NeoALTTO after an event follow-up of 3.77 years confirmed a significantly better 3-year DFS (hazard ratio [HR] 0.38; 95% CI, 0.22–0.63; *P*=0.0003) and 3-year overall survival (0.35; 0.15–0.70; *P*=0.005) for patients who achieved a pCR compared to those who did not. Yet, there was no significant difference in DFS or OS according to treatment arm [8].

The NeoSphere trial has recently led to registration of the dual antibody blockade with trastuzumab and pertuzumab in the USA and Europe. In the Phase II trial, 417 patients were randomized to docetaxel + trastuzumab; pertuzumab; trastuzumab + pertuzumab; or trastuzumab + pertuzumab without preoperative chemotherapy. pCR rate was highest with docetaxel plus the dual antibody combination (45.8%) and lowest with the two antibodies alone (16.8%; Figure 3.2) [9]. As pre-specified in the protocol, survival was analyzed 5 years after randomization of the last patient. Again, all patients who achieved a pCR had a better outcome (3-year DFS: HR 0.68; 95% CI 0.36–1.26). Moreover, patients treated by docetaxel and dual blockade had a better 3-year DFS (92%; HR 0.60; 95% CI, 0.28–1.27) than those treated by docetaxel and trastuzumab alone (85%) [10].

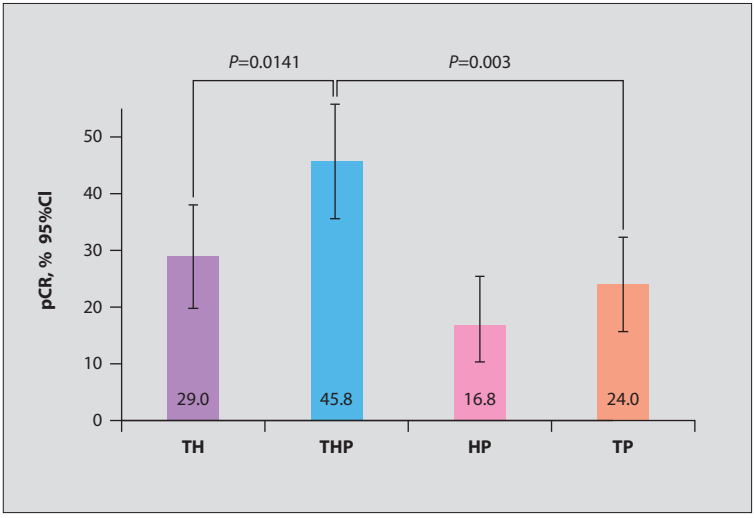

Figure 3.2 NeoSphere primary endpoint: in-breast pathologic complete response. Intent-to-treat population. CI, confidence interval; H, trastuzumab; P, pertuzumab; pCR, pathologic complete response; T, docetaxel. P-values from Cochran-Mantel-Haenszel test and adjusted for multiplicity.

Toxicity of three different chemotherapy schedules were evaluated in the TRYPHAENA trial [11]: fluorouracil-epirubicin-cyclophosphamide-docetaxel (FEC-Doc) + targeted agents during the whole chemotherapy versus in parallel with the taxane versus docetaxel + carboplatin (TcB) + targeted agents, together with dual HER2 blockade (trastuzumab + pertuzumab). Cardiac toxicity was low (3.9–5.6%) in all arms and diarrhea was the most frequent adverse event. In breast pCR (ypT0/is) was high in all arms: 61.6% (FEC-Doc + continuous trastuzumab + pertuzumab) vs. 57.3% (FEC-Doc + trastuzumab + pertuzumab), and 66.2% (TCb + trastuzumab + pertuzumab) [11].

There are still open clinical questions, such as optimal selection of patients for dual versus single blockade, as well as identification of patients who derive sufficient benefit from targeted therapy alone (eg, the 17% pCR rate in the trastuzumab + pertuzumab arm in NeoSphere [9]). A summary of the discussed neoadjuvant trials is found in Table 3.1 [1,3,4,6,8,9,11,12].

Adjuvant therapy

So far, trastuzumab is the only anti-human epidermal growth factor receptor 2 (HER2) drug registered in the early breast cancer (EBC) adjuvant setting. Moreover, the pivotal trials for registration of trastuzumab as the first anti-HER2 agent in early breast cancer (EBC) come from the adjuvant setting.

The first reports of the large adjuvant trastuzumab trials – HERceptin Adjuvant (HERA) [2], North Central Cancer Treatment Group (NCCTG) N9831 and National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31 [13] at the 2005 American Society of Clinical Oncology (ASCO) meeting – have set a gold standard for treatment of HER2-positive EBC. As a result, it has been determined that one year of adjuvant trastuzumab benefits patients with regard to DFS, OS, locoregional recurrence, and distant recurrence (all $P<0.001$) (Figure 3.3) [14]. No difference in the magnitude of benefit is seen between lobular and ductal histology [15].

Indication

In adjuvant trastuzumab trials, patients with uniform intense membrane staining in more than 10% of tumor cells or an HER2/chromosome enumeration probe 17 (CEP17) fluorescence in situ hybridization (FISH)

Trial	Patients (n)	Regimen	pCR rate	DFS rate	OS rate
Single blockade					
TECHNO [3]	217	EC → Pac+H	38.7%*	3-year: pCR 88.1% vs non-pCR 71.4% (*P*=0.0033)	3-year: pCR 96.3% vs non-pCR 85.0% (*P*=0.007)
NOAH [1]	334	A-Pac-CMF+H vs A-Pac-CMF	HER2+: 38% vs 19% (*P*=0.001)* HER2–: 16%*	3-year: 71% vs 56%	3-year: 87% vs 79%
GeparQuattro [4]	445 (HER2+ only)	EC+H+Doc vs EC+H+Doc-Cap vs EC+H +Doc → Cap	40.0%* (31.7% * w/o DCIS)	NA	NA
GeparQuinto [6]	620	EC-Doc+H vs EC-Doc+L	30.3% vs 22.7%	NA	NA
HannaH [12]	596	Doc-FEC+H(SC) vs Doc-FEC +H(IV)	45.4% vs 40.7%	1-yr: 95% in both arms	NA
Dual blockade					
NeoALTTO [8]	455	Pac+L vs Pac +H vs Pac+L+H	24.7% vs 29.5% vs 51.3%†	n.s. survival differences; 3-year pCR vs non-pCR HR 0.38; 95% CI, 0.22–0.63 (*P*=0.0003)	3-year HR 0.35; 0.15–0.70; (*P*=0.005)
NeoSphere [9]	417	Doc+ [H or P or H+P] vs H+P alone	Doc+H+P 45.8%**; Doc+H 29%**; Doc+P 24%**; H+P 16.8%**	3-yr Doc+H+P 92%; Doc+H 85%; HR 0.60; 0.28–1.27	NA
TRYPHAENA [11]	225	FEC - Doc+ [H+P] FEC - Doc+H+P TCbH+P	FEC-Doc + [H+P]: 61.6%** FEC - Doc+H+P: 57.3%** TCbH+P: 66.2%**	NA	NA

Table 3.1 Neoadjuvant trials with trastuzumab. A, doxorubicin; Cap, capecitabine; CMF, cyclophosphamide + methotrexate + fluorouracil; DCIS, ductal carcinoma in situ; DFS, disease-free survival; Doc, docetaxel; EC, epirubicin + cyclophosphamide; FEC, fluorouracil-epirubicin-cyclophosphamide; H, trastuzumab; IV, intravenous; L, lapatinib; NA, not available; n.s., non-significant; P, pertuzumab; Pac, paclitaxel; pCR, pathologic complete response; SC, subcutaneous; TCbH, trastuzumab + docetaxel + carboplatin. *pCR breast and lymph nodes; **pCR breast.

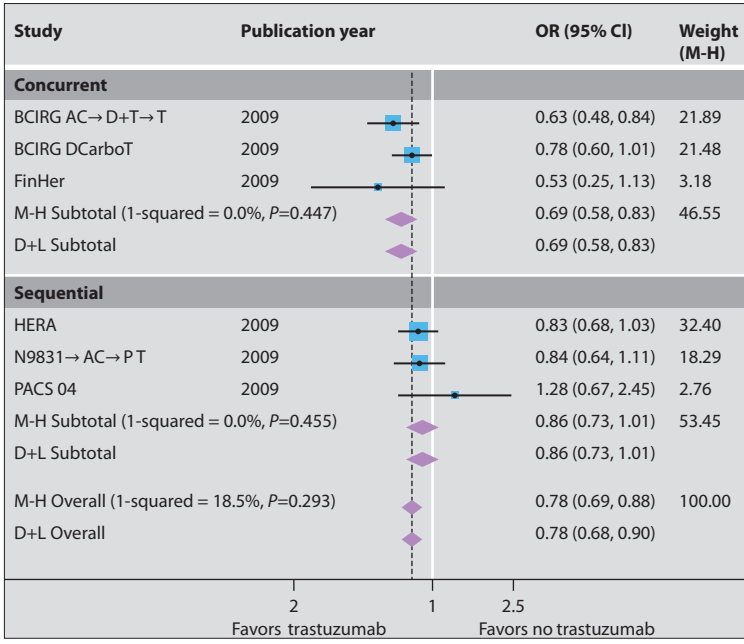

Figure 3.3 Forest plots of odds ratios for the association between adjuvant trastuzumab administration and overall survival by timing of trastuzumab initiation with respect to chemotherapy. The size of the square box is proportional to the weight that each study contributes in the meta-analysis. The overall estimate and confidence intervals are marked by a diamond. Symbols on the right of the solid line indicate OR >1 and symbols on the left of the solid line indicate OR <1. AC, cyclophosphamide + doxorubicin; Carbo, carboplatin; CI, confidence interval; D, docetaxel; D + L, DerSimonian and Laird (random-effects method); M-H, Mantel-Haenszel (fixed-effects method); OR, odds ratio; P, paclitaxel; T, trastuzumab. Reproduced with permission from Yin et al [14] ©PLoS.

amplification ratio of ≥2.0 are eligible for treatment, according to the US Food and Drug Administration (FDA) criteria. Thus, even though these criteria do not completely overlap with the 2007 ASCO-College of American Physicians (CAP) guidelines such patients should be able to receive trastuzumab in clinical practice [16]. In the NCCTG N9831 trial, approximately 1–4% of the patients would not have been eligible for the trial if the original ASCO/CAP guideline thresholds for HER2-positivity had been applied by both test results. Yet, retrospective analysis revealed similar benefit from adjuvant trastuzumab in both patient groups, either by 2007 ASCO/CAP criteria with a hazard ratio (HR) for DFS of 0.59, or by FDA criteria, with an HR of 0.60 [17]. The authors calculated a number needed to treat (NNT) to prevent one additional DFS event at

5 years with 10 and 11.2 patients, respectively, and recommend using the FDA-approved HER2 criteria for therapeutic decision making [6]. Meanwhile, ASCO/CAP have revised their criteria for HER2 positivity (see Chapter 2).

The benefit from trastuzumab in HER2-borderline or low tumors (ie, immunohistochemistry 1+ or 2+; HER2/CEP17 ratio <2.0) is currently being prospectively evaluated in the NSABP-B47 trial (NCT01275677). In the HERA trial, neither HER2 FISH ratio nor HER2 copy number or polysomy predicted differential benefit from adjuvant trastuzumab [18].

Regimen

Trastuzumab can be given at weekly (loading dose: 4 mg/kg body weight, then maintenance dose of 2 mg/kg) or at three-weekly intervals (loading dose: 8 mg/kg, then maintenance dose of 6 mg/kg) [19]. It can also be administered subcutaneously based on the data from the HannaH trial, which led to registration of trastuzumab (600 mg subcutaneous fixed dose) [20].

It can be given after adjuvant chemotherapy, as demonstrated in HERA [21], or with a regimen containing anthracycline and taxanes, such as 4 cycles doxorubicin or epirubicin + cyclophosphamide (AC or EC), followed by 12 cycles of paclitaxel weekly or 4 cycles of docetaxel three-weekly, as introduced by the combined analysis of the NSABP B-31, NCCTG N9831, and BCIRG 006 trials [22,23]. BCIRG 006 also introduced an additional anthracycline-free regimen: trastuzumab with six cycles of docetaxel + carboplatin (TCbH). While cardiac toxicity was substantially lower with TCbH when compared to AC-docetaxel + trastuzumab (AC-TH; $P<0.001$), a numerically lower efficacy rate in the TCbH arm versus the AC-TH arm was seen (5-year DFS: 81% with TCbH vs 84% with AC-TH; 5-year overall OS: 91% vs 92%) [23].

In the NCCTG N9831 trial, trastuzumab given concurrently with taxane was more effective than the sequential administration, with a 5-year DFS rate of 84.4% versus 80.1%, respectively, at a 6-year median follow-up [24]; HR for concurrent versus sequential trastuzumab was 0.77 (99.9% CI, 0.53–1.11), but statistically, the P-value (0.02) did not cross the pre-specified O'Brien-Fleming boundary (0.00116) for the interim analysis [24]. Also, in a meta-analysis for trastuzumab in EBC, concurrent use of trastuzumab was associated with a lower risk of death ($P<0.001$) [14]. The question of whether the higher incidence of

central nervous system metastasis (P=0.010) observed in the concurrent trastuzumab arm is due to longer patient survival remains to be further evaluated.

Trastuzumab can also be given safely together with anthracycline-containing chemotherapy, as demonstrated in the neoadjuvant setting [1,3]. However, starting trastuzumab with the anthracycline, instead of just with the taxane part of the neoadjuvant chemotherapy, does not seem to increase efficacy, as demonstrated by recent data from the American College of Surgeons Oncology Group (ACOSOG) Z1041 Alliance trial [25]. In the small PACS04 trial (n=528), starting trastuzumab after anthracycline- (and taxane-) containing chemotherapy did not result in a significant improvement of DFS (HR 0.86; 95% CI, 0.61–1.22; P=0.41) after a median follow-up of 47 months [26]. Moreover, 18% of patients had to discontinue trastuzumab due to cardiac events (any grade) [26]. Whether the size of this small trial or the sequential trastuzumab administration contributed to this negative trial has not been fully explored thus far. Still, international guidelines recommend administration of trastuzumab concurrently with the taxane chemotherapy [27,28].

Recently, a Phase II one-arm trial (n=406) demonstrated excellent 3-year invasive-disease-free survival of 98.7% (95% CI, 97.6–99.8%) after only 12x paclitaxel weekly adjuvant therapy together with trastuzumab which was then completed for 1 year in node-negative tumors ≤3 cm (Figure 3.4). Two of the patients (0.5%) had symptomatic congestive heart failure which normalized after trastuzumab discontinuation and 13 patients (3.2%), of whom 11 were able to resume trastuzumab therapy after a brief pause, experienced asymptomatic left ventricular ejection fraction (LVEF) declines [29].

Trastuzumab can be administered together with radiation therapy. Observed side effects included grade 3/4 dermatitis (51% of patients), esophagitis (12%), and grade ≥2 LVEF decrease after radiation therapy (6–10%) [30]. In a multivariate analysis, weekly trastuzumab administration (for LVEF decrease) and menopausal status (for dermatitis) were seen as independent prognostic factors. Regarding acute esophagitis toxicity, high cumulative trastuzumab dose (>1600 mg) was of borderline significance [30].

Duration

Based on the registration trials [12,13,23], 1-year of trastuzumab is now standard in the adjuvant setting. Recent results of the HERA trial showed that 2 years of

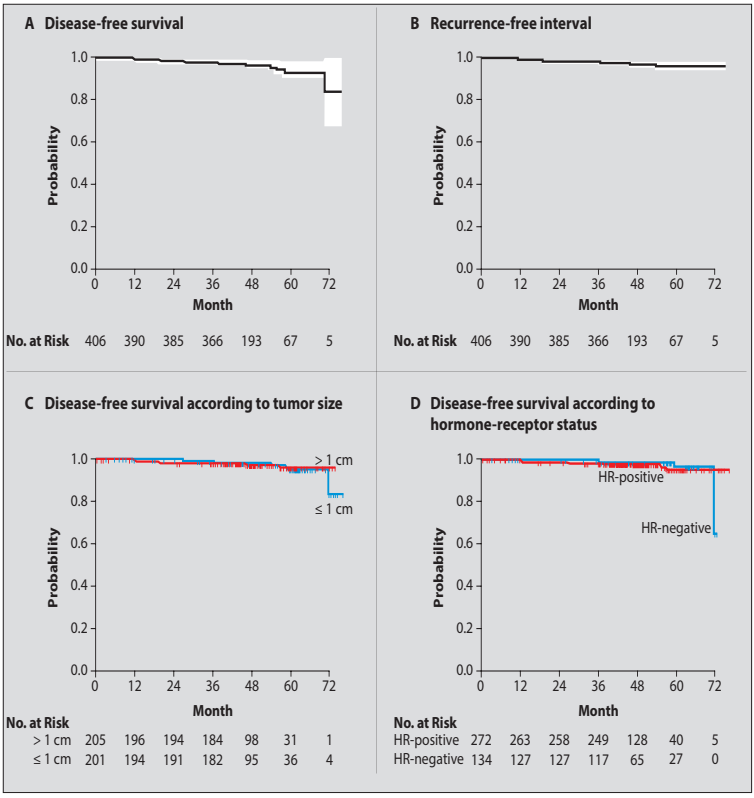

Figure 3.4 Neoadjuvant trials with trastuzumab: probabilities of disease-free survival and recurrence-free interval. (A,B) Probability of disease-free surival in intention-to-treat population. The white shading denotes the 95% confidence intervals. (**C,D**) Probability of disease-free survival according to tumor size and hormone receptor (HR; estrogen or progesterone receptor) status. Tick marks represent the time of censoring for patients who were recurrence-free. Reproduced with permission from Tolaney et al [29] ©Massachusetts Medical Society.

adjuvant trastuzumab is not more effective than 1 year (DFS HR 0.99; 95% CI, 0.85–1.14; *P*=0.86) [31]. Yet, adverse events and cardiac toxicity during treatment were more frequent in the 2-year group, with 20.4% vs 16.3% grade 3–4 adverse events, and 7.2% vs. 4.1% decreases in LVEF, respectively.

The Finland HERceptin (FinHER) trial was an adjuvant trial in both HER2-positive and HER2-negative EBC comparing docetaxel vs. vinorelbine, followed by FEC chemotherapy [32]. In the HER2-positive subgroup, patients receiving 9 weeks of trastuzumab, together with docetaxel or vinorelbine chemotherapy, seemed to benefit in terms of distant disease-free survival (DDFS), when compared

to those treated by chemotherapy alone (HR 0.65; 95% CI, 0.38–1.12; *P*=0.12; with adjustment for axillary lymph node involvement: HR 0.57; *P*=0.047). In an exploratory analysis, 9 weeks of trastuzumab (docetaxel + trastuzumab + FEC) seemed to significantly improve DDFS versus docetaxel + FEC (HR 0.32; *P*=0.029) or vinorelbin + trastuzumab + FEC (HR 0.31; *P*=0.020) [32]. Only one patient treated with trastuzumab developed cardiac failure during the 5-year follow-up, with median LVEF measurements of trastuzumab-treated patients remaining unaltered.

In the French PHARE trial (n=3384), 1 year of adjuvant trastuzumab was prospectively compared to a shortened 6-month treatment. After a median follow-up of 42.5 months, 2-year DFS was 93.8% in the standard and 91.1% (89.7–92.4) in experimental arm (HR 1.28; 95% CI, 1.05–1.56; *P*=0.29). Thus, the prespecified noninferiority margins were not reached. Yet, significantly more patients in the standard arm experienced a cardiac event than in the experimental arm (5.7% vs 1.9%; *P*<0.0001) [33]. The prospective PHARE trial failed to demonstrate noninferiority of shortened versus standard duration of trastuzumab. Given the higher rate of cardiotoxicity in the one-year arm, the results from further prospective trials looking at shorter trastuzumab duration in the adjuvant setting are eagerly awaited.

Small tumors

Indication for (neo-) adjuvant trastuzumab in EBC should be independent of patient age and nodal status because there does not seem to be a difference in the magnitude of benefit between several clinically relevant subgroups [34]. All patients with a tumor size 1 cm (≥pT1c) should receive adjuvant trastuzumab based on the pivotal trial results [19–22,28,32]. However, no prospective data from the registration trials exist for small node-negative pT1a or pT1b tumors. Retrospective evidence suggests that small pT1a and pT1b HER2-positive pN0 tumors are rather aggressive and adjuvant trastuzumab therapy may be warranted in many of these patients, even accounting for potential cardiac side effects [35].

In a large retrospective series (n=965, all pN0 and pT1a or b) with a median follow-up of 74 months, HER2-positive tumors had a 5-year recurrence-free survival of 77.1% vs 93.7% for HER2-negative tumors (*P*<0.001); 5-year distant recurrence-free rates were 86.4% vs 97.2% (*P*<0.001). Compared to patients with

hormone receptor-positive tumors, patients with HER2-positive tumors had over five-times the rate of recurrences and over seven-times the rate of distant recurrences at 5 years [36]. In a retrospective multicenter series (n=97, median follow-up 29 months) with node-negative tumors (<1 cm), patients treated with adjuvant trastuzumab-based therapy had a lower recurrence rate (0% vs 9%; P=0.11), translating to a NNT of 11 to prevent one breast cancer-related event [36]. After an updated follow-up (median 41 months), the authors calculated a NNT of 19 to prevent one breast cancer-specific death. The number needed to harm for cardiac events was 14, with 3 patients showing altered LVEF after 5–7 months of trastuzumab therapy and no cardiac event in the untreated patients [37].

Similar findings were reported in a single center setting (n=43, median follow-up 4.3 years, all pN0) with no recurrences in patients with tumors <5 mm, but four recurrences in those with 5–10 mm tumors; three recurrences occurred in patients without adjuvant trastuzumab, and one recurrence occurred immediately after adjuvant trastuzumab [38]. Again, in another retrospective institutional cohort, adjuvant trastuzumab seemed to be beneficial in small node-negative HER2-positive tumors [39].

A recent analysis of epidemiological data from a large cancer registry did not see HER2 status as an independent prognostic factor for overall survival in small (pT1) node-negative tumors (n=9707). In this cohort, particularly in very small tumors ≤1 cm, hormone receptor status was the decisive factor for patient outcome [40]. Consequently, in patients with small node-negative tumors, an individual risk/benefit analysis needs to be performed. Unfortunately, the recent results about paclitaxel + trastuzumab with its short median follow-up of 4 years cannot add to the discussion about potential overtreatment of small HER2-positive tumors as numbers in subgroups were too small for risk/benefit analyses [29]. International guidelines such as German Gynecological Oncology Group (AGO) and National Comprehensive Cancer Network (NCCN) recommend trastuzumab in node-negative tumors larger than 0.5 cm (≥pT1b) [27,28].

Cardiac safety

Cardiac monitoring is obligatory during trastuzumab therapy in EBC, with LVEF assessments at baseline and every 3 months thereafter. At the moment, no evidence supports further cardiac monitoring in patients after chemotherapy and

trastuzumab treatment who have no cardiac symptoms and no signs of substantial (ie, >10% absolute decrease), but asymptomatic LVEF decline [41]. Cardiac toxicity with LVEF decline or clinical signs of cardiac failure may occur in as many as 4% of patients. Most cardiac events are observed within the first 2 years and late cardiac toxicities are rather rare. Significant risk factors for cardiac toxicities associated with trastuzumab therapy are: age >50 years, currently taking hypertension medication, low LVEF at baseline (<55%), or after 4 cycles of AC (<65%) [42].

In the NCCTG N9831 trial, 3-year cardiac event rates were 2.8% in the sequential trastuzumab arm and 3.3% in the concurrent arm [17]. Cardiac function improved in most congestive heart failure (CHF) cases after trastuzumab discontinuation and cardiac medication. Incidence of asymptomatic LVEF decreases that led to withholding trastuzumab was 8–10%; of these, in about 50% of cases, LVEF recovered and trastuzumab could be restarted [17]. In the HERA trial, trastuzumab was administered subsequently to adjuvant chemotherapy; 94.1% of patients were treated with anthracycline-containing chemotherapy [43]. At a median follow-up of 3.6 years, the overall incidence of cardiac endpoints was low in the trastuzumab arm (severe CHF 0.8%; confirmed significant LVEF decreases 3.6%). In the trastuzumab group, 59 of 73 patients with a cardiac endpoint reached acute recovery; 52 of these 59 patients were considered to have a favorable outcome from the cardiac endpoint by the cardiac advisory board. The incidence of discontinuation of trastuzumab due to cardiac disorders was 5.1% [43]. As discussed before, the lowest cardiac toxicity within the pivotal trials was observed with TCbH in the BCIRG 006 trial (CHF rate of 0.4% vs 2.0% with AC-TH; subclinical LVEF loss [>10% relative loss] 9.4% vs 18.6%, respectively). Interestingly, about 2% of patients randomized to AC-TH never received trastuzumab because of an unacceptable LVEF after the initial anthracycline treatment [23].

In conclusion, cardiac toxicity associated with adjuvant trastuzumab occurs in up to 4% of patients and mostly within the first 2 years. In most cases, it is mostly reversible after trastuzumab discontinuation and cardiac medication. An individual risk/benefit analysis is required in order to decide which patients should be treated with TCbH (or paclitaxel-trastuzumab) rather than with an anthracycline-taxane sequence [44]. An overview of adjuvant Phase II and III trials is found in Table 3.2.

Trial	Patients (n)	Regimen	DFS rate	OS rate	Cardiotoxicity
HERA [21]	3401	1 year of H vs observation after adjuvant chemotherapy	4-year: 78.6% H vs 72.2% observation	4-year: 89.3% vs 87.7%*	1–5% any cardiac end-point
NCCTG N9831 and NSABP B-31 [22]	N9831= 1944 B-31=2101	N9831: AC → Pac → H vs AC → Pac + H → H vs AC → Pac B-31: AC → Pac + H → H vs AC → Pac	4-year: 85.7% (HR 0.52; 95% CI 0.45–0.60; *P*<0.001)	4-year: 93.0% (HR 0.61; 95% CI 0.50–0.75; *P*<0.001)	3-year cardiac event rate 3.3–3.8% (concurrent Pac and H)
BCIRG 006 [23]	3222	AC-Doc vs AC-Doc + H vs TCH	5-year: 75% vs 84% vs 81%	5-year: 87% vs 92% vs 91%	CHF: 0.7% vs 2% vs 0.4%
FinHER [32]	HER2-neg: 1010 HER2-pos**: 232	HER2-neg: Doc + FEC vs Vin + FEC HER2-neg: Chemo + H vs chemo alone	Distant DFS H vs not: HR 0.65; 95% CI 0.38-1.12; *P*=0.12	Doc+H vs Doc: HR 0.42; 95% CI 0.13-1.33; *P*=0.14	Severe LVEF decline: 6.8% CHF: 0.9%
FNCLCC-PACS 04 [26]	528	1 year of H vs observation after adjuvant chemotherapy	3-year: 80.9% H vs 77.9% observation*	3-year: 95% H vs 96% observation*	Severe LVEF decline: 11.1% H vs 2.6% observation
NCT005-42451 [29]	406 pN0, pT≤3 cm	12 x Pac weekly + H for 1 year total	3-year: 98.7% (95% CI, 97.6–99.8)	n/a	0.5% symtompatic CHF; 3.2 % asymptomatic LVEF declines

Table 3.2 Adjuvant Phase II or III trastuzumab trials. AC, doxorubicin plus cyclophosphamide; CHF, congestive heart failure; CI, confidence interval; Doc, docetaxel; FEC, fluorouracil-epirubicin-cyclophosphamide; H, trastuzumab; HR, hazard ratio; Pac, paclitaxel; TCH, docetaxel-carboplatin-trastuzumab; Vin, vinorelbine; *Not significant; **Patients with HER2-positive tumors were further assigned to receive or not receive trastuzumab infusions.

Other anti-HER2 agents in the adjuvant setting

Adjuvant lapatinib

In patients who did have access to adjuvant trastuzumab after intial presentation of the pivotal trials, the TEACH trial (n=3161) evaluated one year of lapatinib (1500 mg/day) versus placebo started at any time after diagnosis. After a median follow-up of 48 months, 13% of patients experienced DFS events in the

lapatinib group versus 17% in the placebo group (HR 0.83; 95% CI, 0.70–1.00; *P*=0.053) [45]. A central HER2 review confirmed positive HER2 status in 79% of patients. In these patients, 13% experienced a relapse, compared to 17% on placebo (HR 0.82; 95% CI, 0.67–1.00; *P*=0.04) [45].

In the four-arm ALTTO trial, trastuzumab was compared with lapatinib, as well as the sequential and the concurrent use of both anti-HER2 agents in the adjuvant setting (n=8381). The lapatinib monotherapy arm was stopped prematurely due to inability to demonstrate non-inferiority. After a median follow-up of 4.5 years, the trial did not reach its primary endpoint and was not able to demonstrate significant superiority (*P*<0.025) of the combination versus trastuzumab alone [46]. A 16% reduction in the DFS hazard rate (HR 0.84; *P*=0.048) with trastuzumab + lapatinib versus trastuzumab alone and a 4% reduction (HR 0.96%; *P*=0.61) was observed. While cardiac toxicity was low (<1%) in all arms, lapatinib was associated with more diarrhea, rash, and hepatic toxicity [46].

Given the premature closure of the lapatinib monotherapy arm in the ALTTO study, the borderline significance of the therapy effect in the delayed adjuvant setting (TEACH) and the higher toxicities with lapatinib, adjuvant lapatinib should only be considered an option if adjuvant trastuzumab is not available. Moreover, analysis of the crossover population in HERA did demonstrate that starting adjuvant trastuzumab any time after adjuvant chemotherapy (median 22.8 months) is associated with improved outcome (DFS HR 0.68; 95% CI, 0.51–0.90; *P*=0.0077) [21].

Adjuvant neratinib

In the placebo-controlled ExteNET trial (NCT00878709), the irreversible pan-HER tyrosine kinase inhibitor neratinib was adminstered (240 mg orally daily for one year) after adjuvant chemotherapy and trastuzumab (n=2821) [47]. After 2 years, there was a numerically small but significant difference favoring the neratinib arm regarding iDFS (93.9 vs 91.6%; *P*=0.0046) and DDFS (95.1 vs 93.7%; *P*=0.0447). Diarrhea was the most frequent adverse event with 40% grade 3 events in the neratinib arm. Cardiotoxicity was low (1%) in both arms [47]. Given the unfavorable safety profile and the lack of a predictive marker, longer follow-up and more detailed analyses are needed.

Current therapy standards for HER2-positive early breast cancer

In HER2-positive EBC, one year of total anti-HER2 therapy started together (neo-) adjuvant chemotherapy is the current standard. Neoadjuvant administration of anti-HER2 therapy plus chemotherapy is preferred because patient management may then be individualized depending on pathological response at the time of surgery. Trastuzumab is still the only anti-HER2 therapy registered both for neoadjuvant and adjuvant therapy. For neoadjuvant settings, dual antibody blockade with trastuzumab + pertuzumab (together with chemotherapy) is now registered by the FDA and EMA.

Standard chemotherapy is an anthracycline-taxane sequential regimen (eg, 4 cycles of AC/EC), followed by 12 cycles of paclitaxel weekly or 4 cycles docetaxel three-weekly. TCbH is also a valid option, particularly if anthracycline toxicity needs to be avoided. For node-negative disease ≤3 cm, 12x paclitaxel and trastuzumab is now also an evidence-based option. Trastuzumab should already be started concurrently with taxane chemotherapy; sequential trastuzumab after chemotherapy should remain an exception. Benefits from trastuzumab seem to be independent of patient age, nodal status, histological type, and tumor size. After surgery, trastuzumab can be given simultaneously to radiation therapy. Due to the rapid progress in drug development and subsequent therapeutic concepts in HER2-positive breast cancer, participation in clinical trials is strongly recommended.

Future directions and ongoing trials

Subcutaneous trastuzumab has become available for clinical routine use. In the HannaH registration trial in the preoperative setting, patients were randomized to either intravenous trastuzumab or subcutaneous trastuzumab (fixed dose 600 mg administered over 5 minutes) at 3-weekly intervals with a backbone of docetaxel-FEC chemotherapy. After surgery, trastuzumab was continued up for to one year. Co-primary endpoints were serum trough concentration at pre-dose cycle 8 before surgery (noninferiority margin of 0.80 for ratio between groups) and pCR (noninferiority margin of –12.5% for difference between groups). Subcutaneous trastuzumab was noninferior to intravenous trastuzumab for both co-primary endpoints with a pCR of 45.4% versus 40.7% in the intravenous group. The incidence of grade 3–5

adverse events was similar between groups, yet, there were more serious adverse events (mostly infections) in the subcutaneous group (21% vs 12%) and 3 deaths versus 1 [20]. Early EFS analysis showed a similar survival rate of 95% one year post-randomization in both treatment groups [11]. Based on the HannaH trial demonstrating noninferiority, subcutaneous trastuzumab received approval by the European Medicines Agency in 2013. The PrefHER study demonstrated 88.9% (95% CI, 85.7–91.6%) patient preference for the subcutaneous formulation independent of the delivery mode (hand-held syringe vs single-use injection) [48].

Based on data demonstrating a strong correlation between improvement in pCR and a substantial improvement in patient outcome versus non-pCR patients in HER2-positive and other breast cancer subtypes, in 2012 the FDA has issued a directive outlining the potential for accelerated approval using neoadjuvant trials [49]. This will aid in using the neoadjuvant setting in HER2-positive breast cancer for rapid development of new anti-HER2 drugs and therapy concepts. In September 2013, pertuzumab was approved by the FDA as the first drug specifically for the neoadjuvant setting based on the totality of evidence including the results of the NeoSPHERE trial, the completion of the adjuvant APHINITY (NCT00490139) trial (results awaited in 2016 or 2017), and the substantial overall survival advantage seen in the CLEOPATRA trial [50]. Using pCR as a surrogate endpoint for survival, upcoming trials will try to increase pCR by combination of targeted agents (see Chapter 6) or try to explore which patients may benefit sufficiently from HER2 blockade alone without chemotherapy. In patients with pCR, one may be able to individualize post-surgical therapy and may even be able to shorten the total duration of anti-HER2 therapy. In non-pCR patients, however, treatment needs to be improved. The first trial aiming at outcome improvement in non-pCR patients, KATHERINE (NCT01772472) has almost finished recruitment. It is run by Roche (Basel, Switzerland) together with the NSABP (National Surgical Adjuvant Breast and Bowel Project) and the GBG (German Breast Group) and randomizes non-pCR patients to T-DM1 (see Chapter 1) versus continuation of trastuzumab for the remainder of the year.

Last but not least, given their substantially different pCR rates, endocrine sensitive HER2-positive tumors may need different therapy approaches than hormone-receptor negative HER2-positive tumors [51]. Recently, interim analysis

data from the WSG-ADAPT trial in hormone-receptor positive HER2-positive disease showed pCR rates >40% (breast and lymph nodes) after only 4 cycles of preoperative T-DM1. These results are comparable to those achieved by other trials with longer systemic chemotherapy using anthracyclines and taxanes, as well as dual blockade in this breast cancer subtype [52] (Figure 3.5). Moreover, the excellent survival results after 12x paclitaxel plus adjuvant trastuzumab [29] suggest overtreatment of a substantial proportion of patients by the administered chemotherapy. Consequently, de-escalation and individualization of anti-HER2 therapy according to individual risk and tumor biology will also need to be addressed in the future.

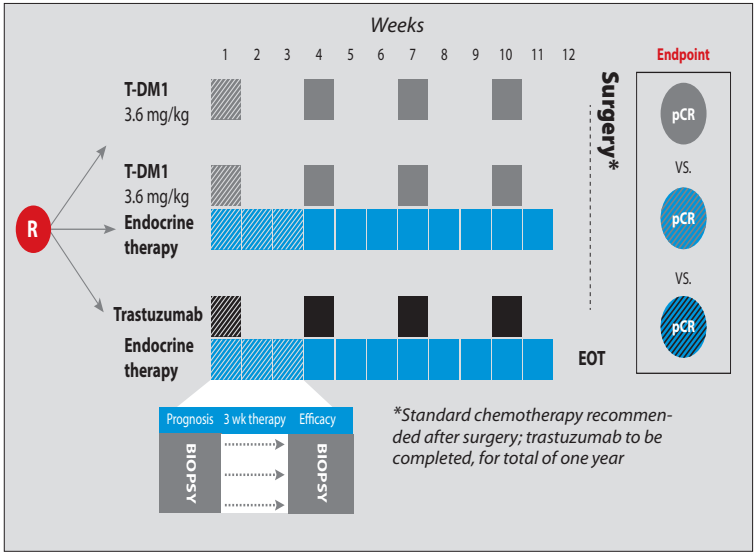

Figure 3.5 ADAPT HER2⁺/HR⁺ trial design. EOT, end of trial; pCR, pathological complete response; T-DM1, trastuzumab emtansine. Reproduced with permission from Harbeck et al [52] ©ASCO.

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Chapter 4

HER2-positive metastatic breast cancer: first-line treatment

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Introduction

Prognoses for patients with breast cancer overexpressing the human epidermal growth factor receptor 2 (HER2) have markedly improved with the administration of anti-HER2-targeted therapy. In the last decade, trastuzumab-based therapy has become the standard first-line treatment option for patients with HER2+ metastatic breast cancer [1–5]. However, trastuzumab- and pertuzumab-based therapy is now considered the new standard of care [6]. Lapatinib in combination with capecitabine is also an approved regimen after progression on a trastuzumab-containing chemotherapy [7]. Other anti-HER2-targeted therapies, such as trastuzumab emtansine (T-DM1) and neratinib have also emerged as important treatment possibilities.

Trastuzumab-based therapy

Trastuzumab has shown to be effective in patients with metastatic HER2+ breast cancer as a first-line therapy [8,9] either alone or in combination with selected chemotherapeutic agents [1,9]. In randomized clinical trials, trastuzumab has also been proven to be beneficial when combined with taxanes (with or without platinum compounds), vinorelbine, and capecitabine [1–4,10–12]. These combination regimens are

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recommended in the current National Comprehensive Cancer Network (NCCN) guidelines as options for first-line treatment [13].

Trastuzumab- and taxane-based first-line therapy

Trastuzumab and taxane dual-combination therapy

Pivotal trials have established the efficacy of the combination of taxanes with trastuzumab in patients with HER2-positive metastatic breast cancer (Table 4.1) [1–4,12]. In the H0648g trial, patients were allocated to receive chemotherapy alone or in combination with trastuzumab [1]. Anthracycline-naïve patients received anthracycline-based chemotherapy and patients already given anthracyclines received paclitaxel every 3 weeks. Patients in the trastuzumab-based therapy group had improvements in overall response rate (ORR), time to progression (TTP), and overall survival (OS) [1]. Additionally, in the M77001 study, docetaxel + trastuzumab showed superior clinical benefit compared with docetaxel administered as monotherapy [2,14].

Trastuzumab and taxane triple-combination therapy

Taxanes and trastuzumab have also been used in triple combinations with other agents. In a randomized Phase III trial, trastuzumab + paclitaxel + carboplatin demonstrated superior ORR (52% vs 36%, P=0.04) and progression-free survival (PFS; 10.7 months vs 7.1 months; hazard ratio [HR] 0.66; 95% CI, 0.59–0.73; P=0.03) compared with trastuzumab + paclitaxel [3]. However, the schedule of paclitacel (ie, every three weeks) was suboptimal. In another study, trastuzumab + paclitaxel + carboplatin was assessed in two different schedules (once weekly vs every three weeks); the weekly regimen had efficacy superior to that of the every-three-weeks regimen [15]. Thus, this triple combination can be considered in the clinical practice when a rapid response is warranted.

The addition of carboplatin to trastuzumab and docetaxel was not found to be superior to trastuzumab and docetaxel alone. However, one of the reasons for the inferior efficacy of this regimen was the reduced docetaxel dose in the triple-combination arm [4]. By contrast, capecitabine added to trastuzumab and docetaxel was demonstrated to have superior PFS (17.9 vs 12.8 months;

Drug combination	n	Regimen (mg/m²)	Response (%)			Median OS (m)	Median TTP (m)	Reference
			ORR	SD	PD			
Taxanes with or without trastuzumab	92	P 175 mg/m² q3wk + T 4 mg/kg loading, then 2 mg/kg/wk	41*	NR	NR	22.1	6.9*	[1]
	96	P 175 mg/m² q3wk	17	NR	NR	18.4	3.0	
	92	D 100 mg/m² q3wk + T 4 mg/kg loading, then 2 mg/kg/wk	61*	27	NR	31.2*	11.7*	[2]
	94	D 100 mg/m² q3wk	34	44	NR	22.7	6.1	
Triple therapy with taxanes and trastuzumab	112	T 8 mg/kg loading then 6 mg/kg + D 75 mg/m² q3wk + X 950 mg/m² b.i.d days 1–14 q3wk	70.5	25	3.6	0.75†	18.6*	[12]
	110	T 8 mg/kg loading then 6 mg/kg + D 100 mg/m² q3wk	72.7	16.4	9.1	0.66†	13.6	
	132	D 75 mg/m² q3wk + C AUC 6 mg/mL/min q3wk (8 cycles) + T 4 mg/kg loading then 2 mg/kg/wk, then T 6 mg/kg/wk alone until PD	72	15	8.3	37.4	10.4	[4]
	131	D 100 mg/m² q3wk + T 4 mg/kg loading then 2 mg/kg/wk, then T 6 mg/kg/wk alone until PD	72	18	8.4	37.1	11.0	
	98	T 4 mg/kg loading then 2 mg/kg/wk + 6 cycles: P 175 and C AUC6 q3wk, then T 2 mg/kg/wk alone until PD	52*	38	10	35.7	NR	[3]
	98	T 4 mg/kg loading then 2 mg/kg/wk + 6 cycles P 175 mg/m² q3wk, then T 2 mg/kg/wk alone until PD	36	43	21	32.2	NR	

Table 4.1 Trastuzumab- and taxane-based therapy as a first-line treatment strategy. AUC, area under the curve; b.i.d, twice daily; C, carboplatin; D, docetaxel; m, months; NR, not reported; ORR, overall response rate; OS, overall survival; P, paclitaxel; PD, progressive disease; q3wk, every 3 weeks; SD, stable disease; T, trastuzumab; TTP, time to progression; wk, weeks; X, capecitabine. *Statistically significant difference between treatment arms. †2-year survival probability.

HR 0.73; *P*=0.045) and longer TTP (18.6 vs 13.6 months; HR 0.70; *P*=0.033) versus trastuzumab + docetaxel, although ORR and OS rates were similar [12].

Other combinations involving trastuzumab and taxanes

Other combinations containing taxanes have been investigated and have demonstrated efficacy in the first-line setting. In two Phase II clinical trials, gemcitabine and trastuzumab were combined with either taxanes or platinum compounds, achieving an ORR of 52.5% and 66%, respectively [16,17].

Trastuzumab- and vinorelbine-based first-line therapy

In preclinical studies, vinorelbine was demonstrated to act synergistically with trastuzumab [18]. Given the high response rates with manageable toxicity observed with vinorelbine and trastuzumab in Phase II trials, two Phase III randomized studies investigated the combination of trastuzumab with either taxanes or vinorelbine [11,19].

The TRAVIOTA trial compared trastuzumab + weekly vinorelbine or taxane (paclitaxel or docetaxel) therapy and demonstrated comparable efficacy between both arms. However, as a consequence of poor accrual, the study was closed prematurely, with 81 evaluable patients instead of the original target of 250 [20]. Subsequently, the HERNATA trial has confirmed a role for vinorelbine + trastuzumab as an alternative first-line therapy combination [11]. In this Phase III clinical trial, the ORR was 59.3% in both the docetaxel + trastuzumab and vinorelbine + trastuzumab arms; the median TTP was 12.4 versus 15.3 months (HR 0.94; *P*=0.67) and the median OS was 35.7 versus 38.8 months (HR 1.01; *P*=0.98). The combination of vinorelbine and trastuzumab had significantly fewer adverse effects than docetaxel and trastuzumab [11].

Trastuzumab- and anthracycline-based first-line therapy

In the H0648g pivotal trial, the combination of trastuzumab with anthracycline-based chemotherapy was associated with a high rate (27%) of cardiac toxicity [1,21]. Liposomal forms of doxorubicin have been shown to provide efficacy similar to that of conventional doxorubicin with greater cardiac safety [22–24]. Several trials have evaluated pegylated and non-pegylated liposomal doxorubicin (NPLD) in combination with trastuzumab with or without taxanes [25–30]. For example, in the first-line setting, a Phase I/II trial investigating the combination of

NPLD, paclitaxel, and trastuzumab showed a general ORR of 98.1% and a median TTP of 22.1 months in the patients with metastatic disease (TTP not reached at time of publication in those with locally advanced nonoperable breast cancer) [27]. However, a recent randomized Phase III trial did not demonstrate significant clinical improvement with the addition of NPLD to paclitaxel and trastuzumab as first-line therapy [31]. The median PFS was 16.1 versus 14.5 months (HR 0.84; P=0.174) and the median OS was 33.6 versus 28.9 months (HR 0.79; P=0.083) for the arms with and without NPLD, respectively. Interestingly, for patients with estrogen receptor (ER)- and progesterone receptor-negative tumors, PFS was 20.7 months for those given NPLD and 14.0 months for those who were not (HR 0.68; 95% CI, 0.47–0.99). The incidence of congestive heart failure (New York Heart Association Class III/IV) was 3% with the arm that included NPLD [31].

Pertuzumab

Given that resistance to trastuzumab is common, new anti-HER2-targeted therapies with complementary and/or synergistic mechanisms of action have been investigated. One such therapy, pertuzumab, has dramatically changed the landscape of first-line HER2-positive breast cancer therapy. Pertuzumab is a HER2-targeted recombinant humanized monoclonal antibody that inhibits the ligand-dependent dimerization of HER2-HER3 [32]. Compared with trastuzumab, which binds to an epitope near the subdomain IV of HER2 [33], pertuzumab binds to HER2 at an epitope near the center of domain II (Figure 4.1) [34]. By blocking HER2 from interaction with itself or HER1, HER3, or HER4, pertuzumab hampers the activation of multiple HER signaling pathways [34].

Therapeutic efficacy

Pertuzumab was shown to be well tolerated and clinically active in Phase I trials in patients with advanced solid malignancies [35–37]. In the context of HER2-positive breast cancer, a dual anti-HER2 combination regimen containing pertuzumab and trastuzumab was initially investigated [38–40]. In the BO17929 study, all 66 patients had experienced progression on prior trastuzumab-based therapy. Treatment with pertuzumab + trastuzumab led to an ORR of 24.2%, a complete response rate of 7.6%, and a clinical benefit rate (CBR; total number of

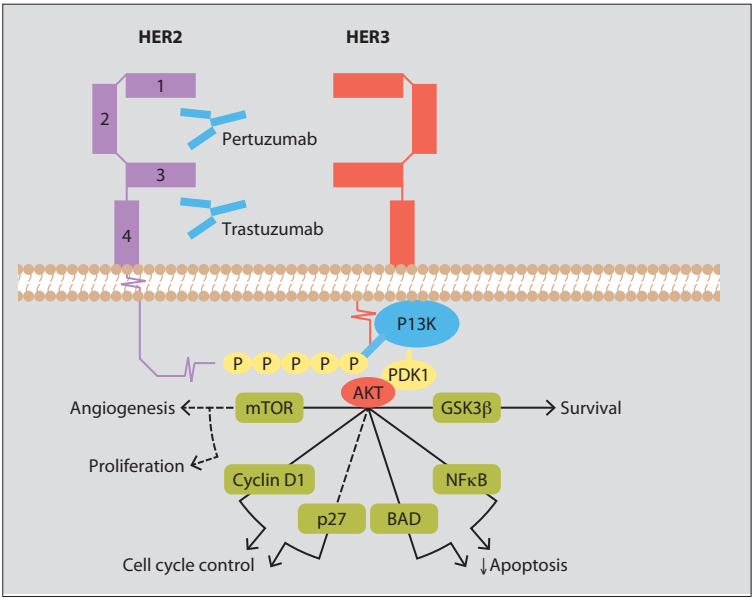

Figure 4.1 Pertuzumab- and trastuzumab-based therapy in HER2-positive metastatic breast cancer. Pertuzumab, a HER dimerization inhibitor, binds to an epitope on the subdomain II of HER2, preventing its ability to pair with other HER family members (HER1, HER3, and HER4) and with itself. By contrast, trastuzumab binds to an epitope on the subdomain IV of HER2. While blocking the formation of dimers, pertuzumab prevents the activation of key intracellular signaling pathways. AKT, protein kinase B; GSK3β, glycogen synthase kinase 3 beta; mTor, mammalian target of rapamycin; NFκB, nuclear factor kappa beta; P13K, phosphatidylinositol 3-kinase; PDK1, phosphoinositide-dependent kinase 1.

objective responses plus stable disease for >6 months) of 50%; median PFS was 5.5 months [38]. A separate cohort of the BO17929 study was designed to investigate the impact of reintroducing trastuzumab in patients who had progressed on both trastuzumab and pertuzumab monotherapy. The combination of pertuzumab and trastuzumab was shown to have superior activity versus pertuzumab monotherapy (ORR 17.6% vs 3.4%; CBR 41.2% vs 10.3%) [39].

First-line use of pertuzumab-based treatment in HER2-positive metastatic breast cancer

Pertuzumab and trastuzumab were tested in combination with docetaxel as first-line therapy in the randomized Phase III Clinical Evaluation of Pertuzumab and Trastuzumab (CLEOPATRA) registration study [6]. This study enrolled 808 patients (median age 54 years) with centrally confirmed HER2-positive metastatic or locally recurrent breast cancer and randomized them in a 1:1 ratio to receive

trastuzumab, docetaxel, and pertuzumab (n=402) or trastuzumab, docetaxel, and placebo (n=406). The primary endpoint, PFS (assessed independently), was significantly prolonged in the pertuzumab-containing arm (median PFS 18.5 months vs 12.4 months in the control arm; HR 0.62; 95% CI, 0.51–0.75; *P*<0.001) [6]. In addition, the ORR was superior for the pertuzumab-containing arm (80.2% vs 69.3%; *P*<0.001).

The confirmatory analysis of OS was reported after a median follow-up of 50 months [6]. In this final interim analysis, the combination of pertuzumab, trastuzumab, and docetaxel significantly improved OS for patients with HER2-positive metastatic breast cancer compared with trastuzumab and docetaxel (median OS 56.5 vs 40.8 months: HR 0.68; 95% CI, 0.56–0.84; *P*=0.001) [41]. Full efficacy results for CLEOPATRA can be found in Table 4.2 [6,41].

An exploratory post-hoc efficacy analysis was performed in elderly patients enrolled in the CLEOPATRA study (age ≥65 years). The pertuzumab-containing arm showed improved efficacy for the independently assessed PFS regardless of the patient's age (<65 years: median PFS 17.2 months vs 12.5 months for the control arm [HR 0.65, 95% CI, 0.53–0.80]; ≥65 years: median PFS 21.6 vs 10.4 months [HR 0.52, 95% CI, 0.31–0.86]) [42].

Based on the results of the CLEOPATRA study, the combination of pertuzumab + trastuzumab + a taxane has been incorporated into clinical practice

Study	n	Regimen (mg/m²)	Response (%)*					Median PFS (m)	Median OS (m)
			CR	PR	OR	SD	PD		
CLEOPATRA [6,41]	402	T 8 mg/kg loading then 6 mg/kg q3wk + D 75 mg/m² q3wk + P 840 mg q3wk followed by 420 mg q3wk	5.5	74.6	80.2	14.6	3.8	18.7	56.5
	406	T 8 mg/kg loading then 6 mg/kg q3wk + D 75 mg/m² q3wk + placebo	4.2	65.2	69.3	20.8	8.3	12.4	40.8

Table 4.2 Pertuzumab-based therapy as a first-line treatment for HER2-positive breast cancer. CR, complete response; D, docetaxel; m, months; NR, not reported; OR, objective response; OS, overall survival; P, pertuzumab; PD, progressive disease; PFS, progression-free survival; PR, partial response; q3wk, every 3 weeks; SD, stable disease; T, trastuzumab. *Overall response, as assessed at an independent review facility.

guidelines as a preferred first-line treatment for patients with HER2-positive metastatic breast cancer [13]. The efficacy and safety of an alternative option based on weekly paclitaxel in combination with trastuzumab and pertuzumab was confirmed in a single-arm, Phase II study [43].

Tolerability

In the BO17929 study, the administration of pertuzumab + trastuzumab was generally well tolerated [38,39]. The majority of the adverse events were grade 1 or 2; the most common were diarrhea (64% of patients), fatigue (33%), nausea (27%), and rash (26%) [38]. In the CLEOPATRA study, the most common adverse events were more frequent in the pertuzumab arm than in the control arm (diarrhea, 66.8% vs 46.3%; alopecia, 60.9% vs 60.5%; neutropenia, 52.8% vs 49.6%) [6]. These adverse events were generally grades 1 and 2. The incidence of grade 3 or 4 febrile neutropenia in all geographic areas was approximately 10% for both treatment groups; however, for patients in Asia, the incidence of grade 3 or 4 febrile neutropenia was significantly higher in the pertuzumab arm (26% vs 12% for the control arm) [6].

Treatment with pertuzumab combined with trastuzumab [36,37] or with trastuzumab and docetaxel [6] was not associated with an increase in adverse cardiac events. In the CLEOPATRA study, left ventricular systolic dysfunction at any grade was shown to be more frequent in the control arm than in the pertuzumab arm (8.6% vs 6.6%) [6]. A specific analysis of safety in the elderly population (based on a cut-off age of 65 years) was performed and the incidence of diarrhea, dysgeusia, and fatigue was higher for those aged ≥65 years in both arms. By contrast, the incidence of neutropenia and febrile neutropenia was less frequent for those in that age group [42]. For patients with good performance status, pertuzumab should be used irrespective of the patient's age.

Advantages of using trastuzumab and pertuzumab for the treatment of HER2-positive breast cancer

The robust activity of pertuzumab and trastuzumab administered in combination has been demonstrated in several HER2-positive tumor models [44,45]. In the clinic, the combination of pertuzumab and trastuzumab appears to be more effective than pertuzumab monotherapy [39]. Thus, pertuzumab has

been recognized as the first HER-dimerization inhibitor with a mechanism of action complementary to that of trastuzumab, which allows for a more complete blockade of HER2-driven signaling pathways. Pertuzumab-based therapy is now considered the new standard of care for the first-line treatment of HER2-positive metastatic breast cancer [6,46].

Other first-line anti-HER2 combination strategies

Given the interactions between HER2 and other molecular pathways, there is a compelling rationale for combining pertuzumab with other therapeutic approaches and simultaneously targeting multiple pathways [10].

T-DM1 an anti-HER2 antibody-drug conjugate that combines trastuzumab ('T') with the highly potent cytotoxic antimicrotubule (maytansinoid) emtansine ('DM1'), targets HER2-expressing cells to specifically deliver the cytotoxic agent [47]. As a first-line therapy, T-DM1 showed superior efficacy versus trastuzumab + docetaxel in terms of PFS (14.2 vs 9.2 months; HR 0.59; 95% CI 0.36–0.97; *P*=0.035) and also had a favorable safety profile [48]. Pertuzumab and T-DM1 may have synergistic and complementary mechanisms of action.

The randomized Phase III study (MARIANNE) investigated the role of pertuzumab in combination with T-DM1 as another first-line strategy for dual HER2 blockade [49]. In this multicenter trial, which has PFS as a primary endpoint, patients were randomized to receive T-DM1 + pertuzumab, T-DM1 + placebo, or trastuzumab + a taxane [49]. The interim analysis of the MARIANNE trial showed that patients with HER2-positive metastatic breast cancer treated with T-DM1 + pertuzumab had similar PFS compared with those treated with trastuzumab plus a taxane-based chemotherapy. After a median follow-up of 35 months, both T-DM1-containing regimens showed noninferior PFS, but not superiority, over trastuzumab + taxane. The median PFS was 15.2 months in the T-DM1 plus pertuzumab arm (HR 0.87, 95% CI, 0.69, 1.08; *P*=0.14), 14.1 months with T-DM1 alone (HR 0.91, 95% CI, 0.73, 1.13; *P*=0.31) compared with 13.7 months with trastuzumab + taxane [49]. The overall survival data were not yet reached. Though the trial met its noninferiority endpoint, showing a similar PFS in the first-line setting between the two combination therapies along with T-DM1 alone, it failed to demonstrate that T-DM1 outperforms trastuzumab + chemotherapy.

The mammalian target of rapamycin (mTOR), an important protein kinase that regulates multiple signaling pathways, is involved in trastuzumab resistance [50,51]. Everolimus is an oral inhibitor of mTOR, which has been investigated in trastuzumab-resistant HER2-positive metastatic breast cancer [51]. In patients previously treated with trastuzumab and taxanes, the addition of everolimus to trastuzumab and paclitaxel was safe and well tolerated and demonstrated promising efficacy [52].

In the previously reported BOLERO-3, everolimus added to trastuzumab and vinorelbine significantly improved PFS for patients with trastuzumab-resistant previously treated cancer [53]. In the first-line setting, the BOLERO-1 Phase III trial evaluated the combination of everolimus with trastuzumab and paclitaxel [54]. The primary endpoint was investigator-assessed PFS in the whole population and in the hormone-negative subpopulation. The study enrolled 719 patients [54]. In the full study population, PFS was comparable between the arms: 14.95 months with the addition of everolimus and 14.49 months with placebo (HR=0.89; P=0.1166). In the hormone receptor–negative subpopulation, however, everolimus-treated patients achieved a median PFS of 20.27 months vs 13.08 months with placebo (HR=0.66; P=0.0049) [54].

Ongoing first-line studies

In the first-line setting, several ongoing studies are investigating pertuzumab with other anti-HER2-targeted therapies and cytotoxic chemotherapeutic agents [49, 52, 55–69]. In addition, new anti-HER2 agents (eg, neratinib, dasatinib) are being investigated as first-line strategies and might change the paradigm for the treatment of patients with HER2-positive breast cancer. Table 4.3 illustrates the current clinical trials that are enrolling HER2-positive breast cancer patients for treatment in the first-line.

HER2/hormone receptor co-positive tumors

Given the crosstalk between the ER and HER pathways and the associated endocrine therapy resistance of HER2-positive tumors [70,71], the concomitant inhibition of both ER and HER2 pathways has been posited to be a more effective treatment strategy than ER inhibition alone. In fact, the combination of aromatase inhibitors with anti-HER2 therapies is an option for some patients who coexpress both HER2 and the ER, although its efficacy seems to be inferior

Clinical trial / NCT ID	Phase of development	Regimen	Primary objective(s)	Estimated enrollment
Pertuzumab-based therapy				
PERUSE (pertuzumab global safety study) [55]	IIIb	Pertuzumab + trastuzumab + taxane	Safety and tolerability	1500
NCT01491737 [56]	II	Trastuzumab + AI ± pertuzumab + chemotherapy*	PFS	250
		Trastuzumab + AI ± chemotherapy*		
EORTC (NCT01597414) (elderly patients) [57]	II	Pertuzumab + trastuzumab†	PFS	80
		Pertuzumab + trastuzumab + metronomic chemotherapy†		
NCT01565083 [58]	II	Pertuzumab + trastuzumab + vinorelbine	ORR	210
NCT01276041 (0 or 1 prior treatment in the metastatic setting) [59]	II	Pertuzumab + trastuzumab + paclitaxel	6 months progression-free	69
NCT01730833 [60]	II	Pertuzumab + trastuzumab + albumin-stabilized nanoparticle formulation of paclitaxel	PFS and ORR	45
HELENA (NCT01777958) [61]	Observational	Pertuzumab + trastuzumab after adjuvant trastuzumab	PFS	478
Miscellaneous				
NEFERTT (NCT00915018) [62]	II	Trastuzumab + paclitaxel	PFS	480
		Neratinib + paclitaxel		

Table 4.3 Ongoing clinical trials for the treatment of HER2-positive breast cancer in the first-line setting (*continues overleaf*). AI, aromatase inhibitor; NCT ID, National Clinical Trials Identifier (ClinicalTrials.gov); ORR, overall response rate; OS, overall survival; PFS, progression-free survival; T-DM1, trastuzumab emtansine. *Induction chemotherapy at the investigator's discretion. †After progression, patients will be given the option of receiving T-DM1.

Clinical trial / NCT ID	Phase of development	Regimen	Primary objective(s)	Estimated enrollment (n)
Miscellaneous (continued)				
NCT01306942 [63]	I/II	Dasatinib + trastuzumab + paclitaxel	Phase I: maximum tolerated dose and recommended Phase II dose Phase II: ORR	60
NCT00520975 [64]	III	Induction: trastuzumab + placebo + paclitaxel ± carboplatin Maintenance: trastuzumab + placebo	PFS	489
		Induction: trastuzumab + bevacizumab + paclitaxel ± carboplatin Maintenance: trastuzumab + bevacizumab		
ICORG (NCT01526369) [65]	III	Paclitaxel + trastuzumab	PFS	600
		Paclitaxel + trastuzumab + lapatinib		
NCT00496366 [66]	II	Lapatinib + capecitabine	ORR	11
NCT01835236 [67]	II	Pertuzumab + trastuzumab (first- line) → T-DM1 (second-line)	OS at 24 months	208
		Pertuzumab + trastuzumab + paclitaxel (or vinorelbine) (first-line) → T-DM1 (second-line)		
NCT01269346 [68]	II	Eribulin + trastuzumab	ORR	52
NCT00033514 [69]	I/II	Erlotinib + trastuzumab	ORR	58

Table 4.3 Ongoing clinical trials for the treatment of HER2-positive breast cancer in the first-line setting (*continued*).

to that of chemotherapy plus anti-HER2-targeted therapy. For example, in the first-line setting, the addition of lapatinib to letrozole was evaluated in 1286 patients with metastatic breast cancer. In the HER2/hormonal receptor co-positive subgroup (n=219), adding lapatinib was associated with an important increase in the median PFS (8.2 vs 3.0 months; *P*=0.019), ORR (28% vs 15%; *P*=0.021); and CBR (48% vs 29%; *P*=0.003) [72].

In another study, trastuzumab and anastrozole combination therapy was compared with anastrozole monotherapy in 208 postmenopausal patients and showed a superior median PFS (4.8 vs 2.4 months; *P*=0.0016) and CBR (42.7% vs 27.9%; *P*=0.026) [73]. Median OS rates were 28.5 and 23.9 months in the combination and single-agent arms, respectively (*P*=0.325). Both combination therapies involving lapatinib + letrozole and trastuzumab + anastrozole were manageable and well tolerated [72,73].

Conclusions

Anti-HER2-targeted agents combined with chemotherapy are the current standard of care for the first-line treatment of patients with HER2-positive breast cancer (Table 4.4). Pertuzumab provides a more comprehensive inhibition of HER2-driven signaling pathways and has mechanistic advantages when combined with trastuzumab. Based on the outstanding results of the CLEOPATRA study, pertuzumab + trastuzumab + docetaxel should be offered as a preferred first-line treatment for patients with HER2-positive metastatic breast cancer.

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Regimen	Content	Frequency
Pertuzumab + trastuzumab + docetaxel	Pertuzumab 840 mg IV followed by 420 mg IV Trastuzumab 8 mg/kg IV as a 90-minute infusion D1 followed by 6 mg/kg IV as a 30-minute infusion weekly Docetaxel 75 mg/m^2 IV D1	Every 3 weeks
Pertuzumab + trastuzumab + paclitaxel	Pertuzumab 840 mg IV followed by 420 mg IV Trastuzumab 4 mg/kg IV as a 90-minute infusion D1 followed by 2 mg/kg IV as a 30-minute infusion weekly *or* Trastuzumab 8 mg/kg IV as a 90-minute infusion D1 followed by 6 mg/kg IV as a 30-minute infusion weekly Paclitaxel 80 mg/m^2 IV D1	Every 3 weeks Weekly
Paclitaxel + trastuzumab	Paclitaxel 90 mg/m^2 IV D1 Trastuzumab 4 mg/kg IV as a 90-minute infusion D1 followed by 2 mg/kg IV as a 30-minute infusion weekly *or* Trastuzumab 8 mg/kg IV as a 90-minute infusion D1 followed by 6 mg/kg IV as a 30-minute infusion every 3 weeks	Weekly
Docetaxel + trastuzumab	Docetaxel 80–100 mg/m^2 IV D1 or Docetaxel 35 mg/m^2 IV D1 Trastuzumab 4 mg/kg IV as a 90-minute infusion D1 followed by 2 mg/kg IV as a 30-minute infusion weekly *or* Trastuzumab 8 mg/kg IV as a 90-minute infusion D1 followed by 6 mg/kg IV as a 30-minute infusion every 3 weeks	Every 3 weeks or weekly
Paclitaxel + carboplatin + trastuzumab	Paclitaxel 175 mg/m^2 IV D1 Carboplatin AUC6 IV D1 or Paclitaxel 80 mg/m^2 IV D1, 8, 15 Carboplatin AUC2 IV D1, 8, 1	Every 3 weeks
Paclitaxel + carboplatin + trastuzumab	Trastuzumab 4 mg/kg IV as a 90-minute infusion D1 followed by 2 mg/kg IV as a 30-minute infusion weekly or Trastuzumab 8mg/kg IV as a 90-minute infusion D1 followed by 6 mg/kg as a 30-minute infusion every 3 weeks	28-day cycle
Vinorelbine + trastuzumab	Vinorelbine 25 mg/m^2 IV D1 Trastuzumab 4 mg/kg IV as 90-minute infusion D1 followed by 2 mg/kg IV as a 30-minute infusion weekly	Weekly
Lapatinib + letrazole	Lapatinib 1500 mg PO daily Letrozole 2.5 mg PO daily	Continuous

Table 4.4 HER2-targeted therapy for HER2-positive metastatic breast cancer in the first-line setting. AUC, area under the curve; D, day; IV, intravenous; PO, orally.

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Chapter 5

HER2-positive metastatic breast cancer: second-line treatment

Ricardo H Alvarez

Introduction

The human epidermal growth factor receptor 2 (HER2) is a transmembrane tyrosine kinase that is overexpressed in approximately 20% of invasive breast cancers, primarily due to gene amplification [1]. Over the last 15 years, several HER2-targeted therapies have been developed and have significantly improved the outcome of patients with metastatic breast cancer (MBC).

Trastuzumab was the first HER2-targeted agent to be approved by the United States Food and Drug Administration (FDA) for the treatment of both early stage and metastatic HER2-overexpressing breast cancer [2,3]. Since the approval of trastuzumab, the field has continued to develop rapidly. Lapatinib, an orally bioavailable small molecule dual HER2- and epidermal growth factor receptor (EGFR) HER1-specific tyrosine kinase inhibitor, received FDA approval in 2007 (in combination with capecitabine) for the treatment of patients with advanced or metastatic HER2-positive breast cancer who had received prior therapy with an anthracycline, a taxane, and trastuzumab, and (in combination with letrozole) for the treatment of postmenopausal patients with metastatic hormone receptor-positive, HER2-positive breast cancer for whom hormonal therapy is indicated [4]. More recently, pertuzumab, a recombinant humanized monoclonal antibody that inhibits the ligand-dependent dimerization of HER2, demonstrated an overall survival (OS) benefit when administered in combination with trastuzumab and docetaxel; it was approved in 2012 in the first-line setting for patients who have not received prior chemotherapy or anti-HER2 therapy

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[5,6]. Finally, trastuzumab emtansine (T-DM1) demonstrated an OS benefit when compared with capecitabine + lapatinib in patients who had previously been treated with trastuzumab and a taxane, and was approved in 2013 [7,8].

These recent advances have not only enabled a better treatment for patients with HER2-positive breast cancer, but have also generated additional questions regarding the order, timing, and effective use of HER2-directed therapies. In this chapter, we will discuss the current clinical data on second-line HER2-targeted agents.

Inhibiting HER2 therapeutically

HER2-amplification in breast cancer is an excellent example of the oncogene addiction hypothesis, which argues that some cancers are driven by a single oncogene that harbors an activating mutation or is overexpressed through gene amplification as a consequence of this single 'gene driver.'

Trastuzumab emtansine

The hypothesis that a HER2 antibody-cytotoxic drug conjugate would deliver the cytotoxic agent directly to the tumor cells, thereby reducing the side effects on normal cells while preserving the anti-HER2 activity of the antibody, has been confirmed. T-DM1 is an antibody drug conjugate (ADC) that incorporates the HER2-targeting antitumor properties of trastuzumab with the cytotoxic activity of the microtubule-inhibitory agent DM1 (derivative of maytansine) [8].

The challenges posed by the development of ADCs are formidable. Over the past 30 years, many cell surface proteins that have selective aberrant expression on malignant cells or are aberrantly highly expressed on the surface of malignant cells have been identified. As a result of such improvement, gemtuzumab ozogamicin was granted accelerated US FDA approval for the treatment of acute myelogenous leukemia in 2000, becoming the first commercially available ADC. However, in 2010, it was withdrawn from the market because it failed to meet its prospective efficacy target. Brentuximab ventodin reached FDA approval in 2011 for the treatment of refractory Hodgkin's lymphoma and, currently, more than 40 ADCs are in clinical trials.

The use of ADCs – cytotoxic drugs connected by chemical linkers to monoclonal antibodies specific for tumor-associated antigens – offers the potential

for the targeted delivery of potent cytotoxic drugs to cancer cells [9–11]. The development of an effective ADC includes three major challenges [12]:

- the identification of a target uniquely expressed in the cancer cells;
- a cytotoxic agent that is potent at low concentration; and
- a linker that can deliver a cytotoxic agent to the cancer cell without releasing the drug into the systemic circulation.

Antibody-drug conjugate: trastuzumab

Clinical trials have demonstrated significant improvement in disease-free survival and OS in patients with HER2-positive early breast cancer [13,14]. Similarly, in the pivotal trastuzumab trial in the first-line metastatic setting, trastuzumab + chemotherapy improved median OS compared with chemotherapy alone (25.1 vs 20.3 months; P=0.046) [5,15]. Importantly, 72% of patients randomized to receive chemotherapy alone subsequently received trastuzumab. This crossover is likely to underestimate the survival benefit associated with the addition of trastuzumab to chemotherapy in this patient population.

The mechanism by which trastuzumab exerts its actions is not fully understood. However, several mechanisms have been proposed, including inhibition of phosphoinositide-3-kinase (PI3K)/AKT and mitogen-activated protein kinase (MAPK) signaling transduction pathways [16,17]; prevention of HER2 ectodomain cleavage [18]; inhibition of angiogenesis [19]; antibody-dependent cell-mediated cytotoxicity [20,21]; and induction of apoptosis [22].

Cytotoxic drug: derivative of maytansine

Several standard chemotherapy agents that have been conjugated to monoclonal antibodies and these ADCs were found to be effective in the preclinical setting [23–25]. However, they were inefficient in the clinical setting because of their inability to achieve therapeutic levels of the cytotoxic agent within the tumor cells [26]. Maytansinoids, which are derivatives of the cytotoxic, antimitotic drug maytansine, are a class of agents that disrupt microtubule function. Maytansine and its congeners have been isolated from mosses, higher plants, and *Actinosynnema pretiosum*, an actinomycete [27]. Many of these compounds are antitumor agents of great potency and bind directly to microtubules to inhibit polymerization in a way similar to that seen with vinca alkaloids. During the 1970s, multiple Phase I and II studies investigated the efficacy of maytansine

in breast cancer. However, the non-selective toxicity prevented further clinical development and, ultimately, the compound was abandoned because it exhibited a poor therapeutic window [28–30].

However, improving the therapeutic index of maytansine through conjugation to monoclonal antibodies that mediate its targeted delivery to cancer cells was considered a promising approach. Efforts to link maytansine covalently to monoclonal antibodies led to the development of DM1, which has an in vitro cytotoxicity 3–10 times greater than maytansine and 10–200 times greater than taxanes or vinca alkaloids [31–33].

Stable linker: MCC

The linker which bridges a toxin and an antibody needs to be able to maintain molecular stability while in circulation but must also be amenable to cleavage while inside cancer cells [30]. Common linkers used in ADCs for cancer therapy include protease cleavable linkers, acid-labile hydrazones, and disulfides, which facilitate the release of the cytotoxic agent in a pH-dependent manner or by disulfide reduction [31,34]. The thioether (MCC) linker protein, to fit both requirements mentioned above, was thus selected as the conjugate linking trastuzumab to DM1 to create trastuzumab-MCC-DM1 (T-DM1), or trastuzumab emtansine (Figure 5.1) [35,36].

T-DM1 allows intracellular delivery of DM1 specifically to HER2-overexpressing cells, thereby improving the therapeutic index and minimizing exposure of normal tissue to this agent. T-DM1 is internalized upon binding to HER2-positive tumor cells and is thought to go through intracellular proteolytic degradation, in turn releasing the active maytansinoid metabolite (lysine-N-MCC-DM1) [36].

Trastuzumab emtansine clinical trials

After impressive preclinical results, a Phase I study conducted in 24 patients with advanced, heavily pretreated HER2-positive breast cancer (median of four prior chemotherapeutic agents for metastatic disease) assessed the safety and tolerability of ascending doses administered every 3 weeks [37]. The maximum tolerated dose (MTD) of T-DM1 was 3.6 mg/kg, with transient grade 4 thrombocytopenia observed as a dose-limiting toxicity. The toxicity profile was favorable, with common drug-related adverse events (AEs) being

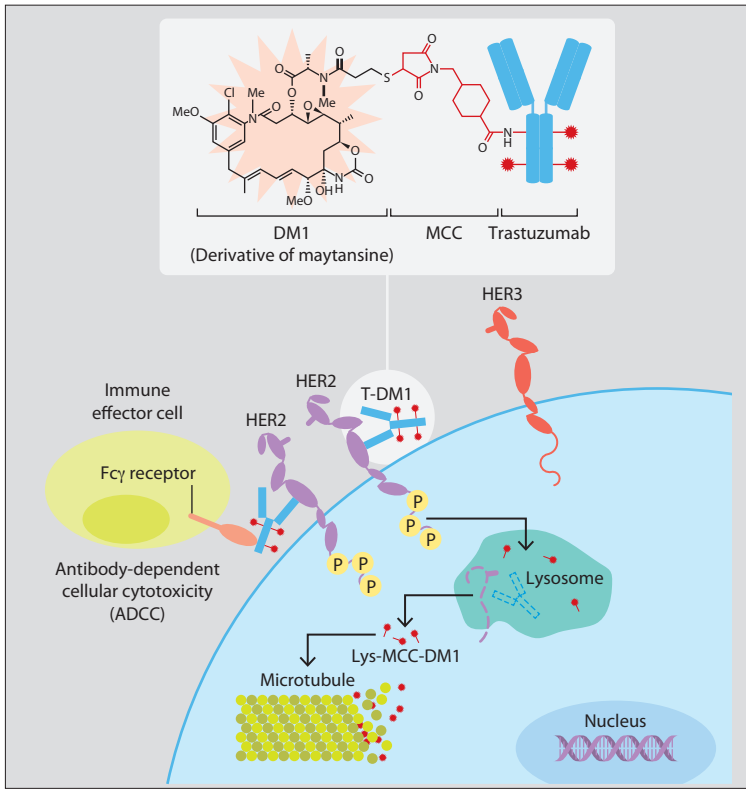


Figure 5.1 Structure and mechanism of action of trastuzumab emtansine. HER2, human epidermal growth factor receptor 2; T-DM1, trastuzumab emtansine.

thrombocytopenia, elevated transaminases, fatigue, nausea, and anemia [37]. Nearly all patients who received T-DM1 at doses >1.2 mg/kg experienced declines in platelet levels with nadirs observed at around Day 8; recovery was generally rapid (by approximately Day 15). Preliminary signs of antitumor activity were observed, with a clinical benefit result (CBR), defined as the objective response plus stable disease at 6 months, of 73% in the 15 patients treated at the MTD. Six patients had an objective partial response, five of which were later confirmed.

Based on these results, two Phase II studies of T-DM1 were conducted in heavily pretreated patients with HER2-positive MBC. The first trial was a single-arm study of 112 patients who had progressed on prior trastuzumab and chemotherapy [38]. T-DM1 was associated with an overall response rate (ORR) of 25.9% (95% CI, 18.4–34.4%). The median duration of response was

9.4 months by investigator assessment and had not yet been reached according to the independent review. The median progression free survival (PFS) was 4.6 months [38]. T-DM1 was well tolerated; most AEs were grade 1 or 2, and the most common grade ≥3 AEs were hypokalemia (8.9%), thrombocytopenia (8.0%), and fatigue (4.5%). Thrombocytopenia was not associated with serious hemorrhage, and there was no dose-limiting cardiotoxicity [38].

In the second Phase II study, T-DM1 was administered at doses of 3.6 mg/kg intravenously every 3 weeks to 110 patients with HER2-positive MBC who had previously been treated with anthracycline, trastuzumab, taxane, capecitabine, and lapatinib therapy [39]. An ORR of 34.5% (95% CI, 26.1–43.9%; all partial responses), a median duration of response of 7.2 months (95% CI, 4.6 months to not estimable), and a median PFS of 6.9 months (95% CI, 4.2–8.4 months) were seen with T-DM1 monotherapy. In patients with confirmed HER2-positive tumors (n=80), the ORR was 41.3% (95% CI, 30.4–52.8%), and the median PFS was 7.3 months (95% CI, 4.6–12.3 months). The most frequent grade ≥3 AEs were thrombocytopenia (9.1%), fatigue (4.5%), and cellulitis (3.6%) [39].

Following the successful results of the Phase II studies, EMILIA, a randomized, Phase III clinical trial, was developed to assess the efficacy and safety of TDM-1 versus capecitabine + lapatinib in nearly 991 patients with advanced HER2-positive breast cancer who had previously been treated with trastuzumab and a taxane [7]. The study had a median follow-up of approximately 13 months (19 months for OS) and showed a significantly longer median PFS in patients receiving T-DM1 compared with those receiving capecitabine + lapatinib (9.4 months vs 6.4 months; hazard ratio [HR] 0.65; 95% CI, 0.55–0.77; $P<0.001$. Median OS at the second interim analysis crossed the stopping boundary for efficacy (30.9 months vs 25.1 months; HR 0.68; 95% CI, 0.55–0.85; $P<0.001$) (Figures 5.2 and 5.3) [7]. The ORR was higher with T-DM1 (43.6% vs 30.8% with capecitabine + lapatinib; $P<0.001$). In terms of toxicity, rates of grade 3 or 4 AEs were lower for T-DM1 overall (41% vs 57%). T-DM1 was associated with higher incidences of thrombocytopenia and increased liver enzyme levels and lower incidences of diarrhea, nausea, vomiting, and palmar-plantar erythrodysthesia than capecitabine + lapatinib [7].

A recent publication of an analysis of patient-reported outcomes from EMILIA demonstrated that T-DM1 treatment resulted in a statistically significant delay in clinically meaningful symptom worsening when compared with capecitabine + lapatinib (7.1 months vs 4.6 months; HR =0.796; $P=0.012$) [40].

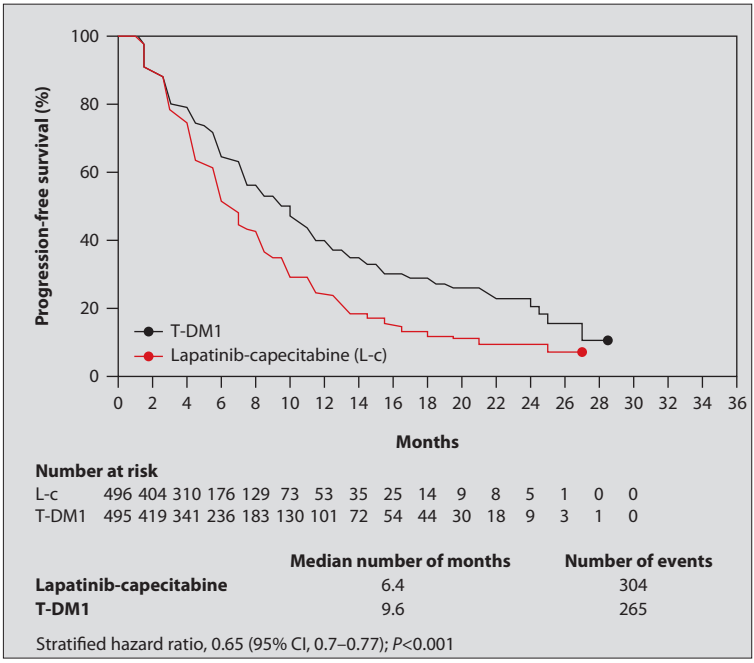
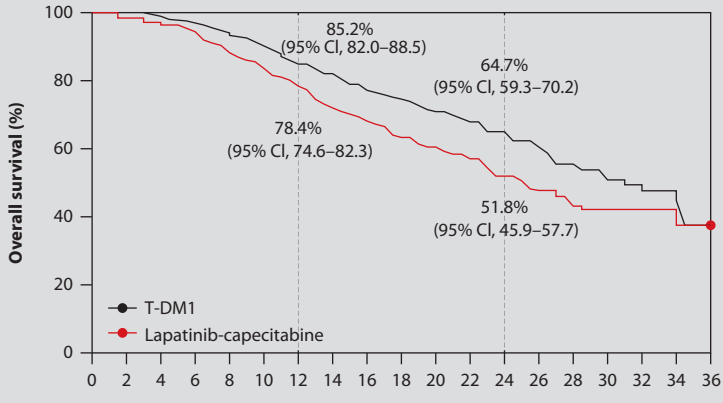


Figure 5.2 EMILIA study: progression-free survival. CI, confidence interval.; T-DM1, trastuzumab emtansine. Reproduced with permission from Verma et al [7] ©NEJM.

Brain metastasis are common in patients with HER2-positive MBC, with up to half of patients experiencing central nervous system (CNS) metastases. Guidelines for the management of CNS metastases in patients with HER2-positive breast cancer was published by ASCO [41]. In a retrospective, exploratory analysis from the EMILIA study, patients with CNS metastasis demonstrated that the rate of CNS progression in patients with HER2-positive MBC was similar for T-DM1 and for capecitabine + lapatinib, and higher overall in patients with CNS metastases at baseline compared with those without CNS metastases at baseline [42]. In patients with treated symptomatic CNS metastases at baseline, T-DM1 was associated with a significantly improved OS compared with capecitabine + lapatinib [42]. The results of EMILIA study provide solid evidence for T-DM1 as the treatment of choice in the second-line setting for HER2-positive MBC and were used as the basis for T-DM1 approval [7].

MARIANNE (NCT01120184) is a Phase III clinical trial that recruited more than 1,000 patients with HER2-positive MBC who had not received any



Number at risk

L-c	496	471	453	435	403	368	297	240	204	159	133	110	86	63	45	27	17	7	4
T-DM1	495	485	474	457	439	418	349	293	242	197	164	136	111	86	62	38	28	13	5

	Median number of months	Number of events
Lapatinib-capecitabine	25.1	182
T-DM1	30.9	149

Stratified hazard ratio, 0.68 (95% CI, 0.55–0.85); $P<0.001$
Efficacy stopping boundary $P=0.0037$ or hazard ratio 0.73

Figure 5.3 EMILIA study. Overall survival results for lapatinib + capecitabine versus T-DM1. CI, confidence interval; T-DM1, trastuzumab emtansine. Reproduced with permission from Verma et al [7] ©NEJM.

chemotherapy in the metastatic setting. In this study, patients were randomly assigned to receive taxanes/trastuzumab, T-DM1, or T-DM1 + pertuzumab. However, this study did not include a comparator arm with taxanes, trastuzumab, and pertuzumab, which is the current standard first-line therapy for HER2-positive MBC. Approximately 37% of patients had de novo metastatic disease. As reported during the 2015 ASCO presentation, the study met the PFS noninferiority endpoint, but not the superiority endpoint [43]. OS was similar across treatment arms. The detailed data have not yet been published and the trial sponsor recently announced that the MARIANNE trial did not reach its primary endpoint [43].

The TH3RESA (NCT01419197) global trial was launched to determine the effectiveness of T-DM1 in heavily pretreated patients (beyond second-line) who had HER2-positive MBC. In this study, 606 patients with HER2-positive MBC

who previously received trastuzumab, lapatinib, and a taxane, were randomly selected to receive either T-DM1 or physician's choice of therapy [45]. The final results showed a 3-month improvement in the median PFS in the T-DM1 group. Response rates were improved in the T-DM1 group (31% in T-DM1 vs 9% in the control group). T-DM1 resulted in significant prolongation of PFS compared to treatment with physician's choice (median 6.2 months vs 3.3 months) and reduced the risk of disease progression or death by 47% (HR=0.528; $P<0.0001$). There was a suggestion of an improved overall survival rate for patients treated with T-DM1, although the data are not mature. Importantly, T-DM1 was better tolerated than other standard chemotherapies. On the basis of these results, T-DM1 is now considered a new standard treatment after multiple lines of therapy for patients who have HER2-positive MBC (Table 5.1).

There is a great interest in testing the efficacy of T-DM1 for early-stage breast cancer. An ongoing single-arm Phase II trial (NCT01196052) is evaluating the efficacy of T-DM1 in the adjuvant or neoadjuvant setting [46]. After completion of an anthracycline-based adjuvant/neoadjuvant chemotherapy regimen

Study	Patient population and sample size	Study phase	Treatment arms	Results
EMILIA [7]	Pretreated HER2$^+$ + MBC (n=991)	III	T-DM1 vs capecitabine + lapatinib	PFS: 9.6 vs 6.4 months OS: 30.9 vs 25.1 months OS: 44% vs 31%
TH3RESA [45]	Pretreated HER2$^+$ + MBC (n=602)	III	T-DM1 vs physician choice	PFS: 6.2 vs 3.3 months OS: No reached vs 14.9 months
MARIANNE [43]	First-line HER2 + MBC (n=1,092)	III	T-DM1 + placebo vs T-DM1 + pertuzumab vs trastuzumab + taxane	PFS: 14.1 vs 15.2 vs 13.7months ORR: 59.7% vs. 64.2% vs 67.9%

Table 5.1 Major completed trials of trastuzumab emtansine (T-DM1). MBC, metastatic breast cancer; PFS, progression-free survival; ORR, overall response rate; OS, overall survival.

(doxorubicin + cyclophosphamide [AC] or 5-fluorouracil + epirubicin + cyclophosphamide [FEC]), 153 patients will be treated with T-DM1 instead of the conventional taxanes/trastuzumab combination for 17 cycles.

In the ATEMPT trial (NCT01853748), 500 patients with resected stage I breast cancer will be randomly assigned to T-DM1 versus 12-week paclitaxel + trastuzumab, followed by trastuzumab regimen [47]. In the KAITLIN trial (NCT01966471), 2,500 patients will be randomly assigned after adjuvant AC/FEC to either a taxane, trastuzumab + pertuzumab, or T-DM1 + pertuzumab.

A randomized Phase III study (KATHERINE; NCT01772472) will evaluate the efficacy of T-DM1 in patients who have residual disease after neoadjuvant trastuzumab-containing regimens [48]. In this study, patients are randomly assigned to continuation of trastuzumab (standard treatment) or T-DM1. This study has a planned enrollment of more than 1,400 patients.

Finally, the KRISTINE trial (NCT02131064) will examine the combination of docetaxel, carboplatin, trastuzumab, and pertuzumab (one of the FDA-approved neoadjuvant pertuzumab-containing regimens) versus T-DM1 + pertuzumab [49]. All of these trials will provide important information relating to the integration of T-DM1 in the treatment of HER2-positive early-stage breast cancer.

Lapatinib in the metastatic setting

Lapatinib is an oral, selective, reversible small-molecule dual tyrosine kinase inhibitor of both the HER1- (ErbB1) and HER2- (ErbB2) signaling pathways. In vitro studies have confirmed that lapatinib treatment inhibits growth [50,51] and can lead to apoptosis [37] in human tumor cells overexpressing HER2 [52].

ErbB receptors form hetero- or homodimers after ligand binding, causing autophosphorylation of specific tyrosine residues within the conserved catalytic kinase domains of these receptors [53,54]. These phosphorylated tyrosine residues are docking sites for phosphotyrosine-binding domain- and Src-homology 2-containing proteins, which link activated ErbB receptors to MAPK and PI3K pathways [55]. Lapatinib treatment has been shown to inhibit the growth of HER2-overexpressing human breast cancer cells that do not respond to trastuzumab after long-term conditioning [37]. These studies also demonstrated the reduction of the volume of HER2-overexpressing human breast cancer xenografts in vivo [44].

Promising evidence of clinical activity was demonstrated in a Phase I study of lapatinib in advanced refractory solid tumors that over expressed HER1 and/or HER2 (n=67) [56]. The most common drug-related AEs were diarrhea (42%) and rash (31%). No grade 4 toxicities were reported. Four patients with trastuzumab-resistant MBC had partial responses and 24 patients experienced stable disease [56].

The activity of lapatinib monotherapy in patients with advanced HER2-positive or MBC that progressed on a first- or second-line trastuzumab-containing regimen was evaluated in a multicenter Phase II study (n=78) [57]. Lapatinib was well tolerated at either 1250 mg or 1500 mg, the median daily dose was 1467 mg (range 940–1500 mg) and the median duration of exposure was 8.4 weeks (range 1–70 weeks). The investigator-assessed ORR and CBR were 7.7% and 14.1%, respectively. In addition, five patients (6%) had stable disease for ≥24 weeks [57]. Responding patients were mostly estrogen receptor (ER)-negative/ progesterone receptor (PR)-negative. However, because the number of responders was limited, the relationship between the ER/PR relationship and response to lapatinib therapy is unclear [57].

Lapatinib was approved primarily based on results from a Phase III, randomized, open-label study comparing lapatinib + capecitabine with capecitabine alone in patients with advanced HER2-positive or MBC that had progressed after prior treatment with anthracyclines, taxanes, and trastuzumab [58]. Patients received lapatinib at 1250 mg/day continuously plus capecitabine 2000 mg/m^2 per day on Days 1–14 of a 21-day cycle or capecitabine 2500 mg/m^2 per day on Days 1–14 of a 21-day cycle. The primary endpoint was the time to tumor progression (TTP) [58]. At an interim analysis, the median TTP of the combination therapy was 8.4 months, compared with 4.4 months with capecitabine alone, a difference of 4.0 months (HR 0.49; 95% CI, 0.34–0.71; P<0.001). However, there was no significant difference in the median OS times between the two groups [58]. At the time of closure of accrual, the difference in the TTP between groups was over 50% lower, at 1.9 months (6.2 months vs 4.3 months; HR 0.57; 95% CI, 0.43–0.77; P<0.001) [59]. The OS duration also did not differ significantly between groups at this time point (15.6 months vs 15.3 months) or in the final analysis of mature survival data (75.0 vs 64.7 weeks) HR 0.87;

95% CI, 0.71–1.08; *P*=0.210) [60]. However, the study design did not allow for sufficient power to detect a survival benefit [60].

Trastuzumab-lapatinib combination therapy

Trastuzumab and lapatinib have complementary mechanism of HER2 blockade, and preclinical studies have found both increased antibody-dependent cellular cytotoxicity and enhanced induction of apoptosis in these agents [61–63]. In the metastatic setting, the combination of trastuzumab + lapatinib was assessed in the Phase III EGF104900 study: 296 patients who were pretreated with trastuzumab to receive lapatinib + trastuzumab or lapatinib alone [64]. A significant prolongation of median PFS (11.1 vs 8.1 weeks; HR 0.74; 95% CI, 0.58-0.94; *P*=0.011) and OS (14 vs 9.5 months; HR: 0.74; 95% CI, 0.57–0.7; *P*=0.026) were seen with the combination therapy versus lapatinib alone, despite significant crossover. There was a 10% improvement in the absolute OS rate at 6 months and a 15% improvement at 12 months in the lapatinib + trastuzumab arm compared with the lapatinib arm. The combination regime was well tolerated, with the most common AEs being diarrhea, nausea, fatigue, rash, and vomiting. Eleven patients given lapatinib + trastuzumab and three patients given lapatinib monotherapy experienced cardiac events [57]. This combination recently received European Medicines Agency (EMA) approval in 2013.

Conclusions

The identification and effective targeting of HER2 has redefined our approach to treating breast cancer and has raised hope that targeted therapies can effectively treat this tumor subtype with less (or even no) chemotherapy. Trastuzumab has significantly improved the prognosis for patients with HER2-positive breast cancer in the early stage as well as in the metastatic setting. However, not all patients will respond to trastuzumab and those who do not will almost inevitably experience tumor progression. The development of anti-HER2 ADCs provides further treatment options for these patients, achieving the selected delivery of potent chemotherapy coupled with HER2 inhibition. In addition, dual HER2 blockade has resulted in clinical success in both the neoadjuvant and metastatic settings. Nevertheless, many questions need to be answered:

- How can we better select the optimal HER2-blockade strategy for the individual patient? (To answer this question, we need a strong predictive biomarker, which is not available at present)
- How we can identify those patients who can be effectively treated with anti-HER2 therapy without chemotherapy?
- What is the optimal sequence of chemotherapy + anti-HER2 treatment in our current therapeutic armamentarium?
- In a financially constrained environment, how will new anti-HER2 treatments impact the cost of health care?

The future is promising, with more effective treatments in development for patients with HER2-positive breast cancer (see Chapter 6).

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Chapter 6

Emerging targeted agents for HER2-positive breast cancer

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Challenges and unmet needs

Advances in understanding the biology of human epidermal growth factor receptor 2 (HER2)-positive breast cancer has led to the successful clinical development of HER2-targeted agents. Trastuzumab represents the archetype of molecular-targeted agents in the setting of solid tumors, with efficacy proven in metastatic, neoadjuvant, and adjuvant settings of HER2-positive breast cancer [1]. Lapatinib, a dual epidermal growth factor receptor (EGFR)/HER2 reversible tyrosine kinase inhibitor (TKI), is currently approved in combination with either capecitabine for patients with HER2-positive metastatic breast cancer refractory to taxanes, anthracycline, and trastuzumab, or with letrozole for postmenopausal women with HER2-positive metastatic breast cancer for whom hormonal therapy is indicated [2]. Pertuzumab, a monoclonal antibody targeting the dimerization domain II of HER2, is an approved agent for the first-line treatment of HER2-positive metastatic breast cancer, in combination with docetaxel and trastuzumab [3]. Lastly, a conjugate of trastuzumab with the microtubule inhibitory agent emtansine (T-DM1) was approved in 2013 for the treatment of patients with HER2-positive metastatic breast cancer who previously received trastuzumab and a taxane, separately or in combination, as well as in the neoadjuvant setting [4].

Meanwhile, there is a subset of patients with early-stage disease who experience recurrence, despite the administration of adjuvant trastuzumab. Moreover, in the metastatic setting, primary or secondary resistance to HER2-targeted

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agents inevitably develops, with affected patients experiencing continued disease progression [5]. This clinical reality mandates the development of additional targeted compounds. The elucidation of the different molecular mechanisms mediating resistance to HER2 blockade (Table 6.1) [6–11] holds the promise to effectively address this gap in treatment. In this chapter, we will provide a thorough overview of the emerging targeted agents that block HER2 (and other signaling pathways) that are currently under clinical development. The biologic rationale, as well as available clinical efficacy data, will be provided.

Class of mechanism resistance	Molecular mediator	Description
Impaired trastuzumab access to HER2	HER2 p95/HER2 shedding	HER2 shedding results in the formation of soluble ECD and HER2 p95. The latter is a constitutively active, truncated form of HER2, lacking the ECD (the binding site for trastuzumab) while retaining its signaling activity [6,7]
	Transmembrane masking proteins (MUC4 and CD44)	Transmembrane molecules that mask the HER2 epitope recognized by trastuzumab interfere with trastuzumab's antibody-binding capacity [8]
Alternative oncogenic signaling pathway activation	PI3K pathway	PIK3CA mutations and/or PTEN loss activate PI3K signaling, despite trastuzumab administration [8]
	IGF pathway	A selective IGF-1R and HER2 crosstalk has been documented in trastuzumab resistance [8]. Additionally, HER2/HER3/IGF-1R heterotrimers have been associated with trastuzumab resistance [9]
	Met pathway	Prevention of trastuzumab-mediated p27 induction [10]
	Src	Src activation functions as a common node downstream of multiple resistance mechanisms [11]
	HER3	HER3/EGFR heterodimerization occurs upon HER2 blockade. HER3 signaling is not fully abrogated by dual HER2 blockade

Table 6.1 Mechanisms of trastuzumab resistance in HER2-positive breast cancer. CD44, cluster of differentiation 44, ECD, extracellular domain; EGFR, epidermal growth factor receptor; HER2/3, human epidermal receptor 2/3; IGF, insulin growth factor; IGF-1R, IGF receptor 1; MUC4, mucin-4; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog.

Novel HER2 tyrosine kinase inhibitors

Neratinib

Currently under clinical development, neratinib is an orally available, irreversible TKI that blocks HER1, HER2, and HER4. A Phase II study evaluated neratinib in patients with HER2-positive metastatic breast cancer, either trastuzumab-pretreated (cohort A, n=66) or trastuzumab-naïve (cohort B, n=70) [12]. Substantial antitumor activity was noted, with the 16-week progression free survival (PFS) rate reaching 59% and 78% and median PFS reaching 22.3 and 39.6 weeks for cohorts A and B, respectively. Toxicities were manageable; diarrhea, nausea, vomiting, and fatigue were the most common grade 3/4 adverse events (AEs), and no neratinib-related grade 3/4 cardiotoxicity was reported [12].

A Phase I/II trial evaluated neratinib in combination with trastuzumab in 45 trastuzumab-pretreated patients with HER2-positive metastatic breast cancer and produced promising antitumor activity [13]. The 16-week PFS rate was 47% and the overall response rate (ORR) was 27%; median PFS was 19 weeks. Treatment with neratinib was relatively well tolerated [13]. Additionally, the triple combination of neratinib + trastuzumab + paclitaxel was evaluated in a Phase I trial of ten patients with HER2-positive metastatic breast cancer pretreated with trastuzumab and a taxane. One complete response (CR) and three partial responses (PR) were reported; diarrhea was the most frequent grade 3/4 AE [14].

Neratinib monotherapy was compared to lapatinib + capecitabine as a second-line treatment option for HER2-positive metastatic breast cancer in a randomized Phase II clinical trial. Despite its high antitumor activity, with an ORR of 29%, neratinib was inferior to the combination of lapatinib + capecitabine in terms of median PFS (the primary endpoint of the trial), which was 4.5 and 6.8 months for neratinib and lapatinib + capecitabine, respectively (hazard ratio [HR]=1.19; 95% CI, 0.89–1.60) [15].

Furthermore, a Phase I/II study assessed the combination of neratinib with capecitabine in 72 patients with HER2-positive breast cancer [16]. Promising antitumor activity was reported: the ORR reached 57% and 64% for patients with or without lapatinib pre-treatment, respectively, with the respective median PFS reaching 35.9 and 40.3 weeks. The toxicity reported was manageable, with the most common drug-related AEs being diarrhea (88%) and palmar–plantar erythrodysesthesia syndrome (48%) [16]. It should be noted

that neratinib has been recently evaluated in the adjuvant setting for patients with early-stage HER2-positive breast cancer, through the Phase III Extended Adjuvant Breast Cancer (ExteNET) trial [17]. This was a Phase III study that randomized 2,840 women with HER2-positive breast cancer and prior adjuvant trastuzumab and chemotherapy to receive either 240 mg/day of neratinib for 1 year or placebo (1,420 patients in each arm). The primary endpoint of the trial was invasive disease-free survival (iDFS). At 24 months, patients who received neratinib had an iDFS rate of 93.9%, as compared to 91.6% in the placebo group (HR=0.67, 95% CI, 0.50–0.91; P=0.009). No data were presented concerning overall survival because of the short follow-up period of the ExteNET study, and a follow-up is anticipated [17].

Currently, there are several ongoing trials evaluating neratinib in HER2-positive breast cancer (Table 6.2) [18–20]. Notably, a Phase III study investigating neratinib + capecitabine compared with lapatinib + capecitabine in patients

Trial	Phase	Description	Patients (n)	Primary endpoint(s)	Secondary endpoint(s)
NCT01494662 [18]	II	Efficacy of neratinib in the treatment of CNS metastases	Patients with CNS metastases; any prior systemic therapy (45)	ORR	PFS, OS, CNS response, first site of disease progression, safety, association of CTCs and OS, clinical outcomes
NEFERTT (NCT00915018) [19]	II	Safety and efficacy of neratinib + paclitaxel vs trastuzumab + paclitaxel	Trastuzumab-naïve (for metastatic disease) (480)	PFS	OS, ORR, duration of OR, CBR, AEs, HRQoL, frequency of CNS lesions, time to CNS lesions
NALA (NCT01808573) [20]	III	Efficacy of neratinib + capecitabine compared with lapatinib + capecitabine	Patients who have received two or more prior HER2-targeted regimens	PFS	ORR, CBR, duration of response, safety, health outcomes assessments

Table 6.2 Selected ongoing clinical trials with neratinib. AEs, adverse events; CBR, clinical benefit rate; CNS, central nervous system; CTCs, circulating tumor cells; DLTs, dose-limiting toxicities; HRQoL, health-related quality of life; OR, objective response; ORR, overall response rate; OS, overall survival; PFI, progression-free interval; PFS, progression-free survival.

with HER2-positive metastatic breast cancer who have received two or more prior HER2-targeted regimens has recently been initiated [20].

Afatinib

Afatinib is another oral, irreversible, pan-HER TKI that blocks EGFR/ HER1, HER2, and HER4. Afatinib has been assessed as monotherapy in a Phase II single-arm trial in 41 trastuzumab-pretreated patients with HER2-positive metastatic breast cancer who received a median of three prior chemotherapy lines in the metastatic setting (range 0–15) [21]. Promising antitumor activity was noted: 19 patients (46%) had clinical benefit (CR, PR, or stable disease [SD]); median PFS reached 15.1 weeks (95% CI, 8.1–16.7 weeks); and median overall survival (OS) was 61.0 weeks (95% CI, 56.7 weeks to 'not evaluable'). Its toxicity profile was consistent with the findings reported from the other targeted agents of the same family, with diarrhea and rash being the most common AEs [21].

A Phase I study assessed the concept of dual HER2 blockade in the setting of HER2-positive metastatic breast cancer, through the combination of afatinib with trastuzumab, with 18 heavily pre-treated patients receiving the combination [22]. The maximum tolerated dose (MTD) of afatinib was 20 mg daily, combined with weekly administered standard dosed trastuzumab, with diarrhea being the dose-limiting toxicity (DLT). The most frequently reported AEs were diarrhea (94%), skin rash (56%), and fatigue (56%). In terms of antitumor efficacy, the ORR and disease control rates reached 11% and 39%, respectively, with the median PFS being 111 days (95% CI, 56–274) [22].

In a randomized, open-label, neoadjuvant Phase II study, afatinib (n=10) was compared with trastuzumab (n=11) and lapatinib (n=8) in 29 patients with locally advanced HER2-positive breast cancer (median tumor size = 6.4 cm). After the 6-week treatment period, objective response was assessed using Response Evaluation Criteria for Solid Tumors (RECIST 1.0) and the best ORRs were 80%, 75%, and 36.4% for the patients treated with afatinib, lapatinib, and trastuzumab, respectively [23]. Concerning toxicities observed during the trial, all patients treated with afatinib experienced drug-related AEs, with the most common being diarrhea and cutaneous toxicities such as dermatitis acneiform and paronychia, compared to 6 out of 8 treated with lapatinib (most common AEs: diarrhea and rash) and 5 out of 11 treated with trastuzumab (vomiting and arthralgia).

There are several ongoing trials evaluating afatinib in HER2-positive breast cancer (Table 6.3) [24–27]. Unfortunately, a Phase III randomized study (LUX-Breast 1) comparing afatinib plus vinorelbine to trastuzumab plus vinorelbine as first- or second-line treatment in the metastatic setting was prematurely terminated.

Trial	Phase	Description	Patients (n)	Primary endpoint(s)	Secondary endpoint(s)
LUX-Breast 1 (NCT01125566) [24]	III	Efficacy of afatinib + vinorelbine vs trastuzumab + vinorelbine	Trastuzumab-pretreated (508) Prematurely stopped by an independent data monitoring and safety committee at n≈500	PFS	Best RECIST assessment, OS, tumor shrinkage, time to deterioration, HRQoL, safety, PK
LUX-Breast 2 (NCT01271725) [25]	II	Safety and efficacy of afatinib, alone or in combination with paclitaxel or vinorelbine	Trastuzumab-pretreated in the (neo) adjuvant setting (85)	OR assessed by RECIST 1.1	Best OR, duration of OR, PFS, safety
LUX-Breast 3 (NCT01441596) [26]	II	Safety and efficacy of afatinib vs afatinib + vinorelbine vs investigator's choice of treatment	CNS metastases, pretreated with trastuzumab and/or lapatinib (120)	Patient benefit at 12 weeks	PFS, OS
DAFNE (NCT01594177) [27]	II	Safety and efficacy of afatinib + trastuzumab followed by afatinib + trastuzumab + paclitaxel in patients receiving taxane + anthracycline neoadjuvant chemotherapy	Neoadjuvant (65)	pCR	RR by physical examination, conservation rate, safety, TR

Table 6.3 Selected ongoing clinical trials with afatinib. CNS, central nervous system; HRQoL, health-related quality of life; OR, objective response; OS, overall survival; pCR, pathologic complete response; PFS, progression-free survival; PK, pharmacokinetics; RECIST, Response Evaluation Criteria in Solid Tumors; RR, response rate; TR, translational research.

PI3K/Akt/mTOR inhibition

mTOR inhibitors

The phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway is one of the most commonly deregulated oncogenic signaling pathways in the setting of breast cancer, with aberrations affecting most of its molecular components [28], primarily:

- overexpression of PI3K-activating receptor tyrosine kinases (RTKs), including HER2;
- activating events of positive PI3K pathway regulators (eg, *PIK3CA* mutations); and
- inactivating events of negative PI3K pathway regulators (eg, loss of phosphatase and tensin homologue [PTEN]).

Activation of this intracellular transduction system has been shown to mediate resistance to HER2 blockade. A study employing a large-scale RNA interference genetic screen in a HER2-overexpressing breast cancer cell line treated with trastuzumab revealed that PI3K pathway activation, conferred by either loss of PTEN or *PIK3CA* mutations, mediates resistance to trastuzumab [29]. Confirmatory clinical results were presented in the same study, from a cohort of 55 patients with HER2-positive metastatic breast cancer who received trastuzumab-based regimens; patients with tumors bearing either PTEN loss or *PIK3CA* mutations had a poorer clinical outcome [29].

Presently, the mTOR inhibitor everolimus is the most clinically advanced compound targeting this signaling pathway in patients with HER2-positive metastatic breast cancer. A Phase Ib clinical trial assessed the triple regimen of everolimus, paclitaxel, and trastuzumab administered weekly in 33 patients with HER2-positive metastatic breast cancer who had received a median of two prior lines of chemotherapy in the metastatic setting (range, 0–17 lines) [30]. Encouraging efficacy results were presented, with a median PFS of 34 weeks (95% CI, 29.1–40.7 weeks) and an overall clinical benefit rate (CBR) ≥24 weeks reaching 74%. Grade 3/4 neutropenia was the most frequently reported AE related to study treatment (52%) [30]. A different Phase Ib study evaluated the combination of everolimus, vinorelbine, and trastuzumab in 50 heavily pretreated patients with HER2-positive metastatic breast cancer [31]. Antitumor activity was noted, with an ORR of 19.1%, a disease control rate ≥6 months of 83%, and a median PFS of 30.7

weeks (95% CI, 28.0–44.9 weeks). In terms of toxicity, neutropenia (92%) and stomatitis (70%) of any grade were the two most frequently reported AEs [31].

Two Phase I/II studies evaluating the combination of everolimus + trastuzumab in the metastatic setting of HER2-positive breast cancer were conducted concurrently, with their results presented in a pooled analysis (n=47). The CBR was 34% and median PFS reached 4.1 months, with fatigue, infection, and mucositis being the most frequent AEs [32].

Temsirolimus, another mTOR blocking agent, has been assessed in combination with neratinib in a Phase I study for patients with HER2-positive metastatic solid tumors, including breast cancer [33]. The most common drug-related toxicity was diarrhea, reaching 93% for all grades, while it constituted also the most frequent DLT observed in the study. Grade 3 diarrhea was reported in 22% of the patients, with other common AEs being nausea (53%), vomiting (42%), stomatitis (53%), hypokalemia (30%), rash (38%), and some cases of cytopenias (≥ grade 3 toxicity rates were 8% for anemia, 18% for lymphopenia, 5% for neutropenia, and 7% for thrombocytopenia). Concerning drug efficacy, of 15 patients with this histology enrolled in the trial, there were two objective responses in patients with HER2-amplified breast cancer [33].

BOLERO-3 randomized 569 women with HER2-positive locally advanced or metastatic breast cancer who were previously treated with a taxane and were resistant to trastuzumab to receive everolimus (n=284) or placebo (n=285) in combination with trastuzumab and vinorelbine (Figure 6.1) [34]. Final PFS results showed that the addition of everolimus to trastuzumab and vinorelbine reduced the risk of disease progression by 22% after a median follow-up of 20.2 months (HR=0.78; 95% CI, 0.65–0.95; $P<0.0067$), with median time to progression being 7.00 months in the everolimus arm and 5.78 months in the placebo arm [34]. OS findings were not reported. Regarding toxicity, the most common AEs reported were neutropenia (73% vs 62% of the patients in the everolimus and placebo group respectively), leucopenia (38% versus 29%), anemia (19% vs 6%), febrile neutropenia (16% vs 4%), stomatitis (13% vs 1%), and fatigue (12% vs 4%).

The results of BOLERO-1, international collaborative Phase III trial assessing everolimus in the first-line setting of HER2-positive metastatic breast cancer, were recently published [35]. BOLERO-1 randomized 719

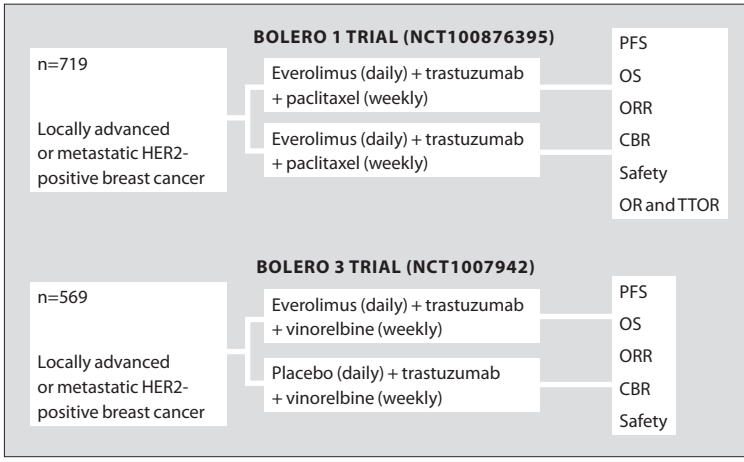

Figure 6.1 Everolimus in HER2-positive breast cancer: the BOLERO-1 and -3 clinical trials. CBR, clinical benefit rate; OS, overall survival; ORR, overall response rate; PFS, progression-free survival; PRO, patient-reported outcome; TTOR, time to objective response.

patients with newly diagnosed HER2-positive metastatic breast cancer 2:1 to receive trastuzumab and paclitaxel with or without everolimus. The study did not meet its primary endpoint because the PFS was comparable between the two arms: 14.95 and 14.49 months for the arms with and without everolimus, respectively (HR=0.89; P=0.1166) [35]. In a subgroup analysis based on the hormone receptor status, there was a 7-month improvement in the PFS with the addition of everolimus for patients with hormone receptor negative, HER2-positive disease (PFS 20.27 months vs 13.08 months; HR=0.66; P=0.0049). This improvement in PFS did not meet the prespecified level of significance, which was set at P<0.0044. Concerning the reported toxicities, they were similar to the BOLERO-3 trial [34,35]. In particular, stomatitis and diarrhea were the most frequently reported AEs, observed more frequently in the everolimus arm (67% vs 32% and 56% vs 47%, respectively).

Other PI3K/Akt/mTOR-blocking agents

mTOR inhibitors block the mTORC1 complex of the PI3K/Akt/mTOR signaling pathway, while the mTOCR2 complex remains uninhibited [36]. Moreover, mTORC1 inhibition downregulates ribosomal protein S6 kinase

α-1 (S6K-α-1)-dependent autoinhibitory feedback mechanisms. As a way to overcome these hurdles, various direct PI3K-blocking agents are under clinical development, including:

- dual PI3K/mTOR inhibitors;
- pan-PI3K inhibitors;
- isoform-selective PI3K inhibitors (p110α-selective inhibitors being the most relevant for breast cancer);
- AKT inhibitors;
- mTORC1/2 inhibitors; and
- 3-phosphoinositide-dependent protein kinase-1 (PDK1) inhibitors.

Preclinical studies have provided evidence of antitumor activity for these inhibitors in the setting of HER2-positive breast cancer, and some of these agents have now entered clinical trials (Table 6.4) [37–44]. For example, a study by Junttila et al showed that HER2-overexpressing breast cancer cells bearing PIK3CA mutations E545K and H1047R were sensitive to GDC-0941, a pan-PI3K inhibitor [45]. Another study found that the transduction of HER2-overexpressing breast cancer cell lines with either of these PIK3CA mutations rendered them resistant to lapatinib; this resistance was reversed upon administration of NVP-BEZ235, a dual PI3K/mTOR inhibitor [46].

Preliminary antitumor activity from early clinical trials has already been reported. A Phase I trial assessed the combination of MK-2206, an Akt inhibitor, with trastuzumab in 32 patients with trastuzumab and/or lapatinib-pretreated HER2-positive metastatic breast cancer; findings showed one patient with CR, one with PR (unconfirmed), and four with prolonged SD for ≥4 months [47]. Concerning toxicity findings, the most frequently reported treatment-related AEs were fatigue, hyperglycemia, and cutaneous toxicity (ie, rash), consistent with prior findings for this agent class. Another Phase I study evaluated three different doses of daily oral BEZ235, a dual PI3K/mTOR inhibitor, in combination with weekly trastuzumab in 19 patients with trastuzumab-resistant HER2-positive breast cancer bearing molecular alterations of PIK3CA and/or PTEN. Of the 19 patients, 15 were evaluable for efficacy; 4 had SD for ≥4 cycles (16 weeks), and there was 1 case of PR in a patient with pulmonary and brain metastases [48].

Trial	Phase	Description	Patients (n)	Primary endpoint(s)	Secondary endpoint(s)
NCT02152943 [37]	I	Toxicity of everolimus combined with letrozole and trastuzumab	Pretreated with ER+/ HER2-positive metastatic	MTD, CBR of triple combination	N/A
PIKHER2 (NCT01589861) [38]	Ib/II	Safety and efficacy of buparsilib in combination with lapatinib	Trastuzumab-pretreated, HER2-positive/ PI3K-activated (106)	MTD, ORR	Safety, clinical benefit, PFS, PK
NCT01042925 [39]	I/II	Dose escalation of XL147 in combination with trastuzumab or trastuzumab + paclitaxel	Trastuzumab-pretreated (42)	MTD, objective tumor response	PFS, PK, PD
NCT02038010 [40]	Ib	Dose escalation of BYL719 with T-DM1	Pretreated HER2-positive metastatic (28)	DLT and MTD of BYL-719	PK data generation
NCT02167854 [41]	I	Dose escalation of BYL719 combined with LJM716 and trastuzumab	Pretreated HER2-positive metastatic (48)	MTD of BYL719	Toxicity
NCT01132664 [42]	Ib/II	Safety and efficacy of buparsilib in combination with trastuzumab	Trastuzumab-pretreated (88)	AEs, DLT	ORR
NCT01285466 [43]	Ib	Dose escalation of BEZ235 and buparsilib in combination with paclitaxel or paclitaxel + trastuzumab	Metastatic or locally advanced HER2-positive (110)	DLTs	AEs, ORR, PK, impact of treatment on biomarkers of PI3K pathway
NCT00928330 [44]	Ib	Safety, tolerability, PK, and activity of GDC-0941 in combination with trastuzumab or T-DM1	Trastuzumab-pretreated (57)	AEs, changes in cardiac function, changes in vital signs and clinical laboratory results	PK, PFS, ORR, DR

Table 6.4 Selected ongoing clinical trials with everolimus and other PI3K/Akt/mTOR blocking agents. AE, adverse events; BCS, breast-conserving surgery; CBR, clinical benefit rate; CNS, central nervous system; DLT, dose-limiting toxicities; DR, duration of response; HRQoL, health-related quality of life; MTD, maximum-tolerated dose; OCRR, objective clinical response rate; ORR, overall response rate; OS, overall survival; pCR, pathologic complete response; PD, pharmacodynamics; PFS, progression-free survival; PK, pharmacokinetics; RR, response rate; TTP, time to progression.

Other signaling pathways

HER3 inhibition

HER3, the third member of the ErbB type I transmembrane RTKs, has recently emerged as a rational therapeutic target for HER2-positive breast cancer. While HER3 lacks kinase activity, it is the preferred dimerization partner with the other ErbB receptors, with the HER2/HER3 heterodimer being the most potent PI3K signaling pathway activator [49]. In HER2-overexpressing breast cancer cells, preferential phosphorylation of HER3 has been documented, with HER3 being as important as HER2 in terms of maintaining cellular proliferation [50]. A 2012 study showed that the administration of an anti-HER2 monoclonal antibody preventing HER2/HER3 heterodimerization stimulated the formation of EGFR/HER3 heterodimers in high and low HER2-expressing cancer cells [51].

Importantly, another study found that dual HER2 blockade with trastuzumab and lapatinib does not fully abrogate HER3 signaling, whereas the addition of an anti-HER3 monoclonal antibody to the trastuzumab + lapatinib combination in a HER2-positive breast cancer xenograft model resulted in reduced tumor growth and prolonged survival compared with trastuzumab + lapatinib alone [52]. Based on this evidence, early-phase clinical trials are currently ongoing with anti-HER3 monoclonal antibodies (eg, U3-1287/AMG 888) (Table 6.5) [53–56].

Src inhibition

The Src family of tyrosine kinases fuels malignant progression through induction of cellular proliferation, metastatic dissemination, and angiogenesis [57,58]. Src has been shown to be an important mediator of intracellular transduction of HER2 signaling [59]. Additionally, there is evidence that Src is a modulator of response to trastuzumab, functioning as a common node downstream of different resistance pathways for both de novo and acquired trastuzumab resistance. The addition of a Src-blocking agent has reversed these two types of resistance in vivo [11]. A quantitative proteomics approach coupled with a focused siRNA screen revealed that proteins associated with the Src kinase pathway are upregulated in trastuzumab-resistant HER2-overexpressing breast cancer cell lines [60]. Early-phase clinical trials assessing the efficacy of Src small-molecule inhibitors (eg, dasatinib) are currently recruiting patients in the setting of HER2-positive breast cancer (Table 6.5).

Trial	Phase	Description	Patients (n)	Primary endpoint(s)	Secondary endpoint(s)
HER3-blocking agents					
NCT01512199 [53]	Ib/II	Safety and efficacy of U3-1287 (AMG 888) in combination with trastuzumab + paclitaxel	Trastuzumab-naïve (86)	MTD, PFS	PK, ORR, DCR
Src-blocking agents					
NCT01306942 [54]	I/II	Safety and efficacy of dasatinib in combination with trastuzumab + paclitaxel	Trastuzumab-naïve (60)	MTD	Safety, CBR, TTP, PFS, DR
IGF-blocking agents					
NCT00684983 [55]	II	Safety and efficacy of capecitabine and lapatinib ± cixutumumab	Trastuzumab-pretreated (154)	PFS	OS, TTTF, CTR, DR, AEs
Met-blocking agents					
NCT01138384 [56]	I/II	Safety and efficacy of foretinib in combination with lapatinib	Trastuzumab-pretreated (19)	MTD, toxicity	PK, preliminary efficacy

Table 6.5 Selected ongoing clinical trials with other emerging targeted agents in HER2-positive breast cancer. AEs, adverse events; CBR, clinical benefit rate; CTR, confirmed tumor response; DCR, disease control rate; DR, duration of response; MTD, maximum tolerated dose; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PK, pharmacokinetics; TTP, time to progression; TTTF, time to treatment failure.

Insulin growth factor inhibition

The insulin growth factor (IGF) signaling pathway represents an intracellular transduction system commonly deregulated in breast cancer [61], and has emerged as another rational therapeutic target in the setting of HER2-positive disease. Selective crosstalk of IGF-1R and HER2 has been documented in trastuzumab-resistant HER2-overexpressing breast cancer cells, which were resensitized to trastuzumab upon IGF-1R blockade [62]. A different study showed the formation of HER2/HER3/IGF-1R heterotrimeric complexes as a potential mechanism of resistance to trastuzumab; the formation of these complexes was disrupted by the knockdown of IGF-1 receptor (IGR-1R) and HER3 expression by short-hairpin RNA [9]. An interesting finding is that IGF-mediated trastuzumab resistance is associated with a decrease of

p27KipI protein levels through heightened ubiquitination involving the PI3K pathway [63].

Confirmatory results for the IGF-signaling pathway mediating resistance to trastuzumab were generated from a neoadjuvant trial assessing the combination of trastuzumab + vinorelbine in 48 patients with locally advanced HER2-positive breast cancer. Single and multigene biomarkers assessment studies were conducted, and positive IGF-1R membrane expression assessed by immunohistochemistry was associated with a lower response rate than negative expression (50% vs 97%; P=0.001) [64]. A variety of IGF-blocking agents are currently under clinical development, with some being assessed in the setting of HER2-positive breast cancer (Table 6.5).

Met inhibition

Met is another signaling pathway contributing to the malignant progression of breast cancer, with overexpression of both the c-Met receptor and its corresponding ligand, hepatocyte growth factor (HGF), noted in one study in almost half of breast cancer cases [65]. Importantly, Met has been found to be often co-expressed with HER2 in HER2-overexpressing breast cancer cell lines and primary tumors, providing a growth advantage [10]. The same study showed that HGF mediates resistance to trastuzumab through abrogation of p27 induction; Met depletion by small-interfering RNA or pharmacologic inhibition sensitized HER2-overexpressing breast cancer cells to trastuzumab [10]. Moreover, preclinical evidence supports the involvement of Met in lapatinib resistance, which could be reversed with the addition of a dual Met/HER2 inhibitor [66]. Currently, several Met-blocking agents (anti-Met monoclonal antibodies such as onartuzumab; multitargeted TKIs: foretinib, fabozantinib; and small-molecule inhibitors such as BMS777607, PF-02341066, and ARQ197) are under clinical development in breast cancer, with one of them (foretinib) being assessed in the setting of HER2-positive disease (Table 6.5).

Boosting immunological response to HER2 blockade

One of the main mechanisms by which trastuzumab exerts its antitumor activity is its immunologic action, with an abundance of evidence supporting a trastuzumab-triggered antibody-dependent-cellular-cytotoxicity (ADCC)

[67–69]. Moreover, adaptive immune responses involving CD8+ cytotoxic lymphocytes and myeloid differentiation have been implicated in trastuzumab-mediated antitumor activity [70,71].

Boosting the immunologic response to HER2 blockade has emerged as a potential strategy to improve clinical outcomes in this setting, with one exploratory approach being antibody modifications to enhance immune effector functions. Preclinical evidence showed that in a HER2-amplified breast cancer xenograft mouse model, afucosylated trastuzumab increased antitumor activity (mediated through heightened ADCC) compared with conventional trastuzumab, resulting in an increase in median PFS from 23.4 to 48 days [72]. Additional evidence for increased ADCC induced by afucosylated trastuzumab was found in an analysis of peripheral blood mononuclear cells from 30 volunteers, including 20 patients with breast cancer [73].

Another promising strategy to boost immunologic response to HER2 blockade is the co-administration of agents that impede the programmed death protein 1 (PD-1) receptor pathway. The PD-1 receptor is an inhibitory T-cell receptor utilized by cancer cells as part of their wide repertoire of immune suppression mechanisms, and is activated upon the binding of programmed cell death ligand 1/2, which can be expressed by both cancer and stromal cells [74,75]. Results from a recent study noted a synergy between trastuzumab and an anti-PD-1 monoclonal antibody in a HER2-overexpressing transgenic breast cancer mouse model, providing the rationale and evidence for subsequent clinical testing [71]. Of note, there is currently an ongoing trial sponsored by the International Breast Cancer Study Group (IBCSG), supported by BIG, that is evaluating the addition of MK-3475, an anti-PD1 monoclonal antibody, to trastuzumab, for patients with metastatic HER2-positive disease that experience trastuzumab resistance (NCT02129556) [76].

Lastly, efforts to target HER2 overexpression for therapeutic vaccination, which induces long-lasting antitumor activity by the immune system, are underway. The US National Cancer Institute has listed HER2 as a candidate vaccine antigen [77], with multiple HER2-targeting vaccines under clinical development (Table 6.6) [78–81]. A joint analysis of two clinical trials assessed a vaccine generated from E75, an HER2-derived peptide, which was administered with granulocyte macrophage colony-stimulating factor in 186 conventionally treated patients with breast cancer (both HER2-positive and HER2-negative) at high risk

for recurrence. There was a statistically significant prophylactic effect of vaccination at 20 months of follow-up (recurrence rate of 5.6% in vaccinated patients vs 14.2% in the nonvaccinated controls; P=0.04), but it lost its significance at 26 months of median follow-up [82]. A Phase I study also assessed a HER2 multipeptide vaccine in ten patients with metastatic HER2-negative breast cancer. Five patients had stable disease and one had a PR, providing proof of evidence that patients with bulky disease are immunocompetent and thus susceptible to respond to vaccination [83]. Whether such strategies will result in objective tumor responses in the metastatic setting is yet to be documented.

Trial	Phase	Description	Patients (n)	Primary endpoint(s)	Secondary endpoint(s)
NCT01355393 [78]	I/II	Safety and efficacy of a HER-2/neu peptide vaccine in combination with rintatolimod ± sargramostim	Optimally treated, Stage II-IV (98)	IR, safety	DFS, OS
NCT00791037 [79]	I/II	Safety of adoptive T cell therapy following in vivo priming with a HER2 ICD peptide-based vaccine	Trastuzumab-pretreated (20)	Safety	IR, development of CD4+ and CD8+ epitope spreading, response of skeletal disease
NCT01632332 [80]	I	Safety and immunogenicity of a multi-epitope HER2 peptide vaccine	Optimally treated, Stage II-III (24)	Safety, IR	DFS
NCT01526473 [81]	I	Safety and antitumor activity of AVX901	Trastuzumab-pretreated (12)	Safety	IR

Table 6.6 Selected ongoing clinical trials with HER2 vaccines in HER2-positive breast cancer. DFS, disease-free survival; ICD: intracellular domain; IR, immune response; OS, overall survival.

Vascular endothelial growth factor inhibition

Vascular endothelial growth factor (VEGF), a potent inducer of angiogenesis, is another therapeutic target for HER2-positive breast cancer. In vitro experiments showed that HER2 overexpression in breast cancer cell lines is associated with increased VEGF mRNA expression [84], with a subsequent study finding

that this HER2-mediated VEGF upregulation involves two different promoter regions, the core promoter through SP1 binding sites and the hypoxia responsive element [85]. In mice, transfection of human breast cancer cells with constitutively active HER2 kinase resulted in tumors of increased microvessel density, and was linked to increased VEGF protein synthesis [86]. In a clinical setting, HER2 and VEGF expression were found to be positively correlated in two breast cancer studies, the first a cohort of 611 consecutive unselected patients ($P<0.001$) [87] and the second a trial of 107 patients ($P<0.01$) [88].

Based on this evidence, clinical trials have assessed the antitumor activity of bevacizumab, an anti-VEGF humanized monoclonal antibody, in HER2-positive breast cancer. In the neoadjuvant setting, a Phase II single-arm trial studied bevacizumab in combination with trastuzumab, nab-paclitaxel, and carboplatin in 28 patients with locally advanced HER2-positive breast cancer. Objective response rates reached 86% and pCR was achieved in 54%; however, bevacizumab-related complications such as wound-healing delays and left ventricular ejection fraction decreases were noted postoperatively [89]. Bevacizumab was assessed in combination with fluorouracil, epirubicin, and cyclophosphamide (cycles 1–4) and with docetaxel and trastuzumab (cycles 5–8) preoperatively in a Phase II study of primary inflammatory HER2-positive breast cancer (BEVERLY-2). Out of 52 patients, 33 had a centrally confirmed pCR (63.5%); the common AEs were asthenia (69%), nausea (69%), alopecia (65%), and mucosal inflammation (63%) [90].

Another interesting study conducted in the neoadjuvant setting of HER2-positive breast cancer was the AVATAXHER trial [91]. This was a Phase II study that enrolled 142 evaluable patients with early-stage HER2-positive breast cancer that received initially two cycles of preoperative docetaxel plus trastuzumab. Before each of the first two cycles, an FDG-PET assessment was performed, with the change in the standardized uptake value (SUV) being used to predict pCR in each patient. Patients that were evaluated as responders continued to receive standard of care, whereas patients deemed to be non-responders (n=73) were randomized (2:1) to receive another four cycles of docetaxel/trastuzumab with (Group A, n=48) or without adding bevacizumab (Group B, n=25). pCR cases were achieved in 37 PET responders (53.6%, 95% CI, 41.2–65.7), 21 of those in group A (43.8%, 29.5–58.8), and six of those in group B (24.0%, 9.4–45.1), indicating that early PET evaluation can predict pCR in this setting, with

predicted non-responders benefiting from the addition of bevacizumab. Of note, the frequency of grade 3–4 AEs were similar in all three groups. The most common grade 3–4 AEs were neutropenia (4 in PET responders, 5 in group A, and 3 in group B), febrile neutropenia (1, 3, and 1, respectively), and myalgia (4, 0, and 1, respectively) [91].

Bevacizumab was combined with lapatinib in a Phase II trial of 52 patients with HER2-positive metastatic breast cancer, most of whom had received prior chemotherapy (96%) and/or trastuzumab (90%) in the metastatic setting. Median PFS reached 24.7 weeks, the CBR was 30.8%, and the most common AEs were diarrhea, rash, and fatigue [92]. A Phase II single-arm study assessed the combination of bevacizumab + trastuzumab in 50 patients with locally recurrent or metastatic HER2-positive breast cancer. Bevacizumab + trastuzumab therapy led to an ORR of 48%, median time to progression of 9.2 months, and a median OS of 43.8 months [93]. Another Phase II single-arm trial assessed the combination of bevacizumab with trastuzumab + capecitabine as first-line treatment in 88 patients with HER2-positive metastatic breast cancer. Antitumor activity was noted, with an ORR of 73% and a median PFS of 14.4 months; the main grade ≥3 AEs were hand-foot syndrome (22%), diarrhea (9%), and hypertension (7%) [94].

Lastly, the Phase III AVEREL trial randomized 424 patients with HER2-positive locally recurrent and/or metastatic breast cancer to receive docetaxel + trastuzumab with or without bevacizumab as first-line treatment [95]. The study did not meet its primary endpoint, with the investigator-assessed PFS showing a prolongation in the bevacizumab arm without reaching statistical significance (HR=0.82; 95% CI, 0.65–1.02; P=0.078). Additionally, there was no difference in time to treatment failure (9.8 vs 7.7 months; HR=0.94; 95% CI, 0.76–1.15; P=0.539) and investigator-assessed ORR (74.3% vs 69.9%; P=0.349) between the bevacizumab and nonbevacizumab treatment arms [95]. Interestingly, patients with high baseline VEGF-A levels derived a greater benefit from bevacizumab than those with low levels (HR=0.70 vs 0.83). Median PFS in patients with high baseline plasma VEGF-A was 16.6 months in those given bevacizumab and 8.5 months in those not given bevacizumab [95]. These findings should be viewed as hypothesis-generating, with the VEGF-A level as a potential biomarker to predict response to bevacizumab in this patient population.

Conclusions

HER2-positive breast cancer oncology represents one of the most dynamically evolving fields. Trastuzumab has proven efficacy in all settings of HER2-positive disease, opening the way for the clinical development of an abundance of HER2-targeted agents, as exemplified by the success stories of lapatinib and, more recently, pertuzumab and trastuzumab-DM1. However, resistance to HER2 blockade is a clinical reality, mandating the development of new targeted agents. Second-generation TKIs, namely afatinib and neratinib, have shown antitumor activity in Phase I/II trials and are moving further in Phase III clinical evaluation. Moreover, molecular elucidation of HER2 signaling, along with emerging knowledge of alternate oncogenic signaling pathways mediating resistance to HER2 inhibition, have led to several other classes of targeted compounds currently under clinical investigation for HER2-positive breast cancer. Results from ongoing clinical trials are eagerly awaited, with predictive biomarkers guiding treatment selection among this abundance of riches still to be identified.

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