

Handbook of HER2-Targeted Agents in Breast Cancer

Ricardo Alvarez

Javier Cortes

Mary Falzon

Michael Gandy

Luca Gianni

Nadia Harbeck

Martine Piccart

Handbook of HER2-Targeted Agents in Breast Cancer

Handbook of HER2-Targeted Agents in Breast Cancer

Ricardo H Alvarez

University of Texas MD Anderson
Cancer Center
Houston, Texas, USA

Javier Cortés

Vall d'Hebron Institute of Oncology
Barcelona, Spain

Leticia De Mattos-Arruda

Vall d'Hebron Institute of Oncology
Barcelona, Spain

Mary Falzon

University College London Hospital
London, UK

Angelica Fasolo

San Raffaele Scientific Institute
Milan, Italy

Michael Gandy

University College London
London, UK

Luca Gianni

San Raffaele Scientific Institute
Milan, Italy

Nadia Harbeck

Breast Center
University of Munich
Munich, Germany

Martine Piccart

Institut Jules Bordet
Université Libre de Bruxelles
Brussels, Belgium

Stefania Zambelli

San Raffaele Scientific Institute
Milan, Italy

Dimitrios Zardavas

Institut Jules Bordet
Université Libre de Bruxelles
Brussels, Belgium



Springer Healthcare

Published by Springer Healthcare Ltd, 236 Gray's Inn Road, London, WC1X 8HB, UK.

www.springerhealthcare.com

© 2013 Springer Healthcare, a part of Springer Science+Business Media.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means electronic, mechanical, photocopying, recording or otherwise without the prior written permission of the copyright holder.

British Library Cataloguing-in-Publication Data.

A catalogue record for this book is available from the British Library.

ISBN 978-1-907673-93-1

Although every effort has been made to ensure that drug doses and other information are presented accurately in this publication, the ultimate responsibility rests with the prescribing physician. Neither the publisher nor the authors can be held responsible for errors or for any consequences arising from the use of the information contained herein. Any product mentioned in this publication should be used in accordance with the prescribing information prepared by the manufacturers. No claims or endorsements are made for any drug or compound at present under clinical investigation.

Project editor: Katrina Dorn

Designer: Joe Harvey

Production: Marina Maher

Printed in Great Britain by Latimer Trend & Company Ltd

Contents

Author biographies	vii
1 Introduction and background biology	1
Introduction	1
Epidemiology	1
HER2 primary structure	2
Signal transduction through receptor tyrosine kinases	4
Receptor tyrosine kinases: site of therapeutic intervention	5
Signaling through HER-receptor family dimers leads to the activation of downstream cascades	5
Conclusions	10
References	10
2 HER2 testing	13
Introduction	13
Pre-analytic phase: sample handling	14
Analytic phase: HER2 testing methodologies	15
Post-analytic phase: screening and interpretation	17
Alternate HER2 testing methodologies	22
References	23
3 HER2-positive breast cancer: adjuvant and neoadjuvant therapy	25
Adjuvant setting	25
Neoadjuvant therapy	33
Current therapy standards for HER2-positive early breast cancer	38
Future directions and ongoing trials	38
References	39
4 HER2-positive metastatic breast cancer: first-line treatment	43
Introduction	43

Trastuzumab-based therapy	43
Pertuzumab	47
Ongoing first-line studies	51
HER2/hormone receptor co-positive tumors	54
Conclusions	55
References	56
5 HER2-positive metastatic breast cancer: second-line treatment	61
Introduction	61
Trastuzumab emtansine	62
Lapatinib in the metastatic setting	68
Investigations of trastuzumab-lapatinib combination therapy	70
Conclusions	70
References	71
6 Emerging targeted agents for HER2-positive breast cancer	75
Challenges and unmet needs in HER2-positive breast cancer	75
Novel HER2 tyrosine kinase inhibitors	77
PI3K/Akt/mTOR inhibition	80
Other signaling pathways	85
Boosting immunological response to HER2 blockade	88
Vascular endothelial growth factor inhibition	90
Conclusions	91
References	92

Author biographies

Ricardo H Alvarez, MD, MSc is Assistant Professor of Medicine at the University of Texas, MD Anderson Cancer Center in Houston, Texas. He obtained his medical degree at the University of La Plata Medical School, Argentina. He trained in medicine at the University of Texas Medical School in Houston and medical oncology at MD Anderson Cancer Center. He obtained the title of Master in Science from the Graduate School of Biomedical Science at the University of Texas. His main areas of research include advanced breast cancer, inflammatory breast cancer, anti-angiogenic therapy for breast cancer, and work in the laboratory with breast cancer xenograft models. His main clinical activity is in the drug development area in patients with advanced cancer.

Javier Cortés, MD, PhD received his degree in Medicine and Surgery from the Universidad Autónoma 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 University Hospital, Barcelona, Spain. She is also a PhD candidate at the Universitat Autònoma de Barcelona. She has been conducting translational research projects and cutting edge clinical trials

in breast cancer. She is a member of the American Society of Clinical Oncology (ASCO) and the European Society for Medical Oncology (ESMO), and an active member of the ESMO Young Oncologist Committee and Flims Alumni Club Steering Committee.

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 where he obtained his state registration as a Clinical Biomedical Scientist (BMS). Whilst practicing, he then went on to complete his Masters 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 National du Cancer, France (InCA) and to the European Commission. He sits in the Scientific Council of the IARC (International Agency for Research on Cancer, World Health Organization). In 1997 he co-founded the Michelangelo Foundation for the Advancement of Science and Treatment in Oncology, and is Chairman of the International Board of the Michelangelo Foundation Breast Cancer Study Group. In 2011 Dr. Gianni was awarded the “Gianni Bonadonna Award and Lecture” of the American Society of Clinical Oncology.

Professor 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 2012) and of the International Consensus on Advanced Breast Cancer (ABC 2011 & 2013). Moreover, she is an executive board member of the European Organization for Research and Treatment of Cancer (EORTC). 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 is President of the 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 just received the prestigious David A. Karnofsky Memorial Award at the 2013 American Society of Clinical Oncology (ASCO) meeting in Chicago.

Introduction and background biology

Angelica Fasolo, Stefania Zambelli, 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 are 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 is 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 23% (1.38 million) of the total new cancer cases and 14% (458,400) of the total cancer deaths in 2008 [1]. More than half of all cases and 60% of the

deaths are estimated to occur 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 [2–4]. 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 [5,6], which belongs to a family of transmembrane RTK kinases that mediate cell growth, differentiation, and survival [7,8]. HER2 overexpression is associated with aggressive tumor behavior [5] 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 [9,10]. In addition, approximately 50% of ductal carcinomas in situ (DCIS) display HER2 amplification. The concept that a portion of such DCISs eventually evolve into HER2 overexpressing infiltrating carcinomas is still the focus of discussion [11,12].

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 [13], 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.

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 [14,15]. It belongs to the ErbB family of transmembrane RTKs, which are a subclass of cell-surface growth-factor receptors with an intrinsic, ligand-controlled tyrosinekinase 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 four members of the epidermal growth factor receptor family

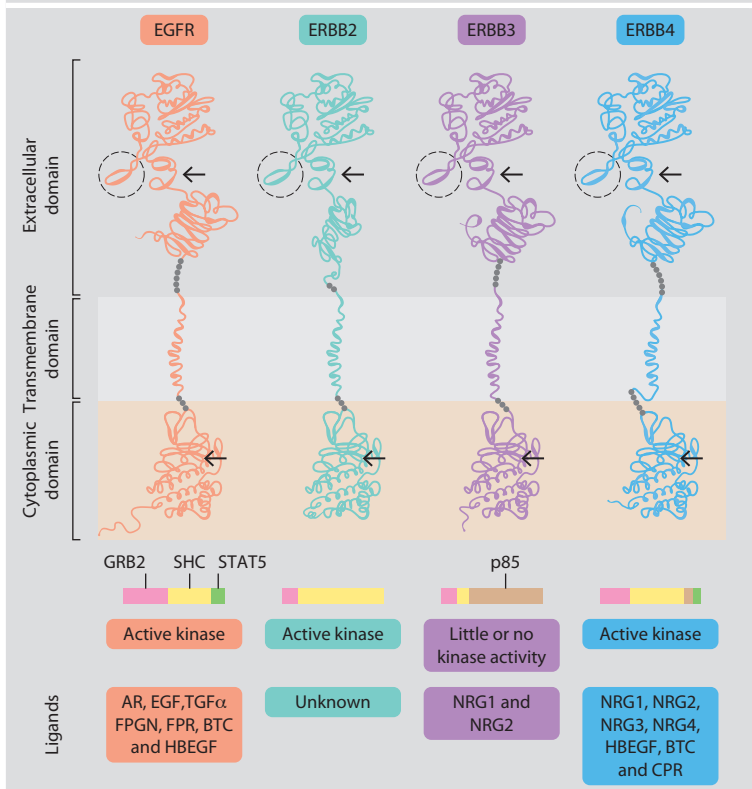


Figure 1.1 The four members of the epidermal growth factor receptor family. These are represented by their corresponding and highly homologous crystal structures. They have three major domains: the ligand-binding domain, the transmembrane domain and the kinase domain. The ligand-binding clefts are marked 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; TGF- α , transforming growth factor- α . Reproduced with permission from Yarden and Pines [18].

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 [16,17].

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, whereas HER3 does not possess kinase activity (Figure 1.1) [18].

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 [19].

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 because the cytoplasmic tail of HER3 contains binding sites for phosphatidylinositol 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 [20,21]. 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 [23]. 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 [24,25]. 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 [26], 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) [27,28]. In addition, compensatory pathways such as upregulation of ErbB3 or

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

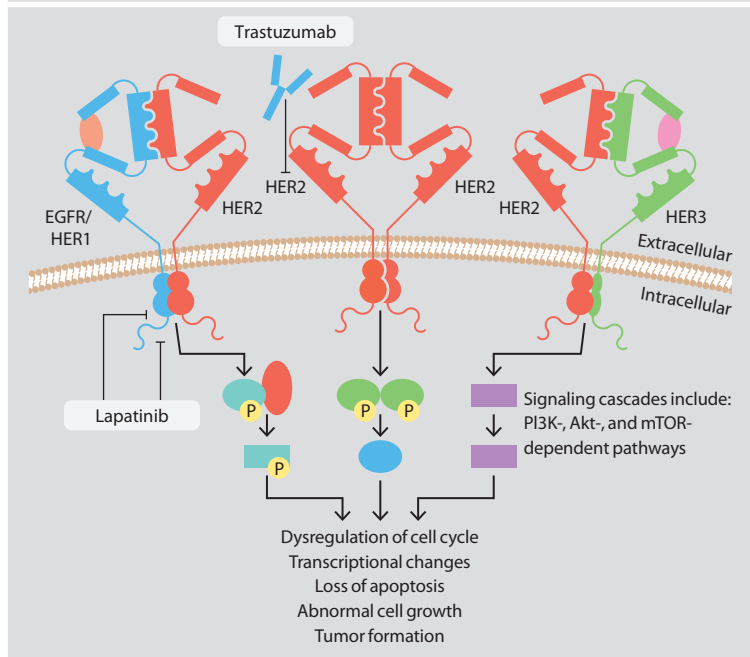


Figure 1.2 Signaling through HER-receptors family dimers leads to the activation of downstream cascades. Downstream signaling pathways include PI3K-, Akt-, mTOR- and MAPK-pathways, which control cell cycle, cell growth and survival, apoptosis, metabolism and angiogenesis. Signaling through HER2 homodimers 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 [22].

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 [29–31]. Similarly, resistance of breast cancer cells to lapatinib may involve overexpression of other RTKs or de-repression of the estrogen receptor (ER) pathway [32,33]. A more recent analysis showed that mutations in exon 21 of the *HER2* gene are also involved 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 [34].

The HER2:HER3 heterodimer

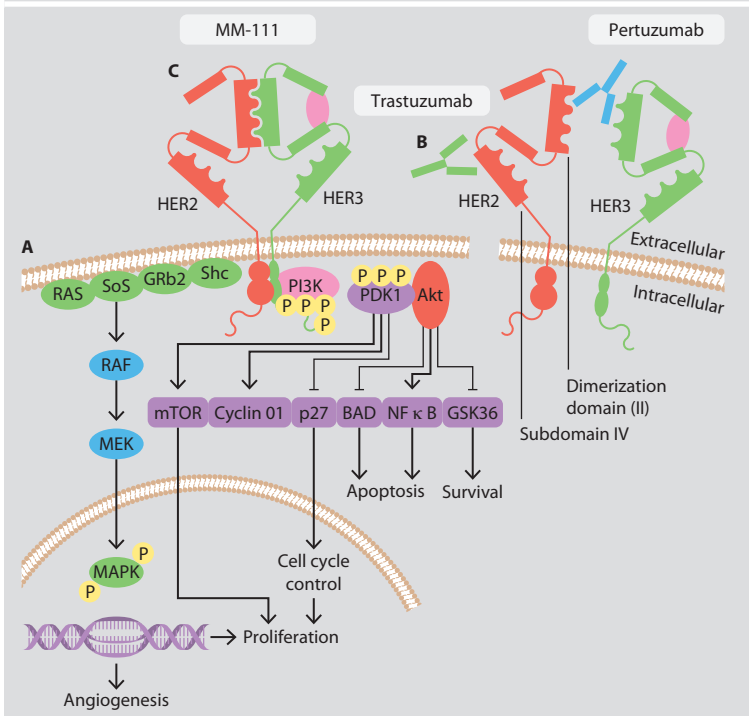


Figure 1.3A and B The HER2:HER3 heterodimer. (A) The HER2:HER3 heterodimer is a potent trigger of downstream signaling cascades, especially the PI3K/Akt cascade and the MAPK cascade. **B**, HER2:HER3 signaling can be inhibited by the monoclonal antibody pertuzumab, which prevents HER2:HER3 dimer formation by blocking the HER2 dimerization domain (subdomain II), which is distinct from the site of trastuzumab binding (subdomain IV). 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 [22].

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

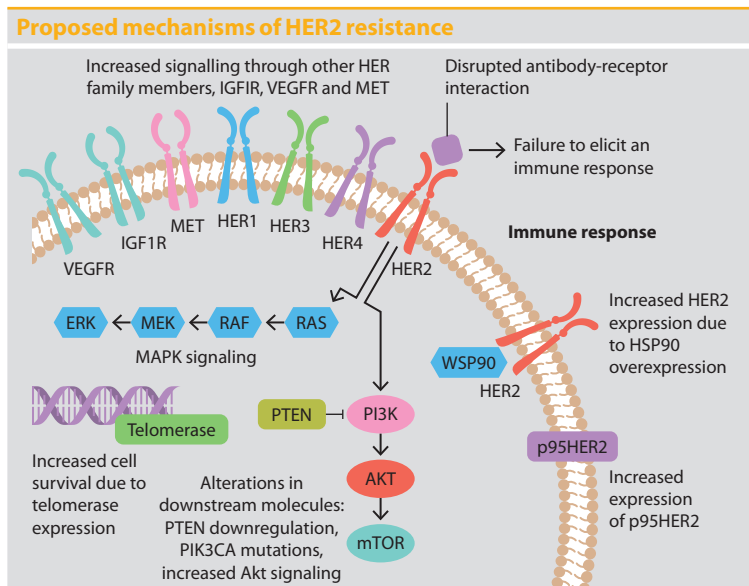


Figure 1.4 Proposed mechanisms of HER2 resistance. Akt, protein kinase B; HER, human epidermal growth factor receptor; HSP90, heat shock protein 90; IGF1R, insulin-like growth factor receptor 1; MAPK, mitogen-activated protein kinase; MET, mesenchymal epithelial transition factor; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; VEGFR, vascular endothelial growth factor receptor. Reproduced with permission from Mohd Shariar et al [40].

inhibition of HSP90 or the inhibition of telomerase was able to restore trastuzumab sensitivity [35,36]. 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 [37,38]. 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 [39]. 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).

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 re-growth after initial response to trastuzumab could be reversed upon introduction of pertuzumab as second antibody [41]. 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 cancer progressing on trastuzumab either alone or in combination with chemotherapy [42]. 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 [43]. 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 [44].

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 [45]. 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 [46], or inhibitors of mammalian target of rapamycin (mTOR) [47,48].

Conclusions

The thorough characterization of the biology of HER2 and of 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 drugs for oncology. 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.

References

- 1 Parkin DM, Bray F, Ferlay J. Global Cancer Statistics 2002. *CA Cancer J Clin*. 2005;55:74-108.
- 2 Jemal A, Center MM, Desantis C. Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiol Biomarkers Prev*. 2010;19:1893-1907.
- 3 Althuis MD, Dozier JD, Anderson WF. Global trends in breast cancer incidence and mortality 1973-1997. *Int J Epidemiol*. 2005;34:405-412.
- 4 Autier P, Boniol M, La Vecchia C. Disparities in breast cancer mortality trends between 30 European countries: retrospective trend analysis of WHO mortality database. *BMJ*. 2010;341:c3620.
- 5 Slamon DJ, Clark GM, Wong SG. Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987;235:177-182.
- 6 Sjögren S, Inganas M, Lindgren A. Prognostic and predictive value of c-erbB-2 overexpression in primary breast cancer, alone and in combination with other prognostic markers. *J Clin Oncol*. 1998;16:462-469.

- 7 Yarden Y, Sliwkowski M. Untangling the ErbB signaling network. *Nat Rev Mol Cell Biol.* 2001;2:127-137.
- 8 Gschwind A, Fischer OM, Ullrich A. The discovery of receptor tyrosine kinases: targets for cancer therapy. *Nat Rev Cancer.* 2004;4:361-370.
- 9 Menard S et al. HER2 as a prognostic factor in breast cancer. *Oncology.* 2001;61(suppl.2):67-72.
- 10 Ross JS et al. The HER-2/neu oncogene in breast cancer prognostic factor, predictive factor and target for therapy. *Stem Cells.* 1998;16:413-428.
- 11 Van de Vijver MJ et al. Neu-protein overexpression in breast cancer. Association with comedo-type ductal carcinoma in situ and limited prognostic value in stage II breast cancer. *N Engl J Med.* 1988; 319:1239-1245.
- 12 Lu J et al. 14-3-3 ζ cooperates with ErbB2 to promote ductal carcinoma in situ progression to invasive breast cancer by inducing epithelial-mesenchymal transition. *Cancer Cell.* 2009;16:195-207.
- 13 Lipton A, Kostler WJ, Leitzel K. Quantitative HER2 protein levels predict outcome in fluorescence in situ hybridization-positive patients with metastatic breast cancer treated with trastuzumab. *Cancer.* 2010;116:5168-5178.
- 14 Bargmann CI, Hung MC, Weinberg RA. The neu oncogene encodes an epidermal growth factor receptor-related protein. *Nature.* 1986;319(6050):226-230.
- 15 Coussens L, Yang-Feng TL, Liao YC. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science.* 1985;230:1132-1139.
- 16 Brandt-Rauf PW, Monaco R, Pincus MR. Conformation of the transmembrane domain of the epidermal growth factor receptor. *J Protein Chem.* 1994;13:227-231.
- 17 Gullick WJ, Bottomley AC, Lofts FJ. Three dimensional structure of the transmembrane region of the proto-oncogenic and oncogenic forms of the neu protein. *EMBO J.* 1992;11:43-48.
- 18 Yarden Y, Pines G. The ERBB network: at last, cancer therapy meets systems biology. *Nat Rev Canc.* 2012;12:553-563.
- 19 Schlessinger J. Signal transduction by allosteric receptor oligomerization. *Trends Biochem Sci.* 1988;13:443-447.
- 20 Pinkas-Kramarski R, Soussan L, Waterman H. Diversification of Neu differentiation factor and epidermal growth factor signalling by combinatorial receptor interactions. *EMBO J.* 1996;15:2452-2467.
- 21 Graus-Porta D, Beerli RR, Daly JM. ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. *EMBO J.* 1997;16:1647-1655.
- 22 Ahn ER, Vogel CL. Dual HER2-targeted approaches in HER2-positive breast cancer. *Breast Cancer Res Treat.* 2012;131:371-383.
- 23 Amit I, Wides R, Yarden Y. Evolvable signaling networks of receptor tyrosine kinases: relevance of robustness to malignancy and to cancer therapy. *Mol Syst Biol.* 2007;3:151.
- 24 Garrett JT, Arteaga, CL. Resistance to HER2-directed antibodies and tyrosine kinase inhibitors: mechanisms and clinical implications. *Cancer Biol Ther.* 2011;11:793-800.
- 25 Rexer BN, Engelman JA, Arteaga CL. Overcoming resistance to tyrosine kinase inhibitors: lessons learned from cancer cells treated with EGFR antagonists. *Cell Cycle.* 2009; 8:18-22.
- 26 Scaltriti M, Rojo F, Ocaña A, et al. Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. *J Natl Cancer Inst.* 2007;99:628-638.
- 27 Hubalek M, Brunner C, Matthä K, Marth C. Resistance to HER2-targeted therapy: mechanisms of trastuzumab resistance and possible strategies to overcome unresponsiveness to treatment. *Wien Klin Wochenschr.* 2010;160:506-512.
- 28 Pohlmann PR, Mayer IA, Mernaugh R. Resistance to trastuzumab in breast cancer. *Clin Cancer Res.* 2009;15:7479-7491.
- 29 Nahta R, Yu D, Hung MC, Hortobagyi GN, Esteva FJ. Mechanisms of disease: understanding resistance to HER2-targeted therapy in human breast cancer. *Nature Clin Pract Oncol.* 2006; 3:269-280.
- 30 Sergina NV, Rausch M, Wang D, et al. Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3. *Nature.* 2007;445:437-441.

- 31 Berns K, Horlings HM, Hennessy BT, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell*. 2007;12:395-402.
- 32 Liu L et al. Novel mechanism of lapatinib resistance in HER2-positive breast tumor cells: activation of AXL. *Cancer Res*. 2009;69:6871-6878.
- 33 Xia W, Bacus S, Hegde P, et al. A model of acquired autoresistance to a potent ErbB2 tyrosine kinase inhibitor and a therapeutic strategy to prevent its onset in breast cancer. *Proc Natl Acad Sci USA*. 2006;103:7795-7800.
- 34 Prempre T, Wongpaksa C. Mutations of HER2 gene in HER2-positive metastatic breast cancer. *J Clin Oncol*. 2006;24:13118.
- 35 Modi S, Stopeck A, Linden, et al. HSP90 inhibition is effective in breast cancer: a phase II trial of tanespimycin (17-AAG) plus trastuzumab in patients with HER2-positive metastatic breast cancer progressing on trastuzumab. *Clin Cancer Res*. 2011;17:5132-5139.
- 36 Goldblatt EM, Erickson PA, Gentry ER, Gryaznov SM, Herbert BS. Lipid-conjugated telomerase template antagonists sensitize resistant HER2-positive breast cancer cells to trastuzumab. *Breast Cancer Res Treat*. 2009;118:21-32.
- 37 Nahta R, O'Regan RM. Therapeutic implications of estrogen receptor signalling in HER2-positive breast cancers. *Breast Cancer Res Treat*. 2012;135:39-48.
- 38 Glück S, Arteaga CL, Osborne CK. Optimizing chemotherapy-free survival for the ER/HER2-positive metastatic breast cancer patient. *Clin Cancer Res*. 2011;17:5559-5561.
- 39 Sabnis G, Schayowitz A, Golubeva O, Macedo L, Brodie A. Trastuzumab reverses letrozole resistance and amplifies the sensitivity of breast cancer cells to estrogen. *Cancer Res*. 2009;69:1416-1428.
- 40 Mohd Shariar MS, Crown J, Hennessy BT. Overcoming resistance and restoring sensitivity to HER2-targeted therapies in breast cancer. *Ann Oncol*. 2012;23: 3007-3016
- 41 Scheuer W, Friess T, Burtscher H, Bossenmaier B, Endl J, Hasmann M. Strongly enhanced antitumor activity of trastuzumab and pertuzumab combination treatment on HER2-positive human xenograft tumor models. *Cancer Res*. 2009; 69:9330-9336.
- 42 Baselga J, Gelmon KA, Verma S, et al. Phase II trial of pertuzumab and trastuzumab in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer that progressed during prior trastuzumab therapy. *J Clin Oncol*. 2010; 1;28:1138-1144.
- 43 Gianni L, Pienkowski T, Im YH, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2012;13:25-32.
- 44 Baselga J, Cortés J, Kim SB, et al; for the CLEOPATRA Study Group. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *N Engl J Med*. 2012;366:109-119.
- 45 J Baselga, I Bradbury, H Eidtmann, et al; for the NeoALTTO Study Team, Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. *The Lancet*. 2012; 379: 633-640
- 46 TT Junttila, RW Akita, K Parsons, et al. Ligand-independent HER2/HER3/PI3K complex is disrupted by trastuzumab and is effectively inhibited by the PI3K inhibitor GDC-0941. *Cancer Cell*. 2009;15:429-440
- 47 Jerusalem G, Fasolo A, Dieras V, et al. Phase I trial of oral mTOR inhibitor everolimus in combination with trastuzumab and vinorelbine in pre-treated patients with HER2-overexpressing metastatic breast cancer. *Breast Cancer Res Treat*. 2011;125:447-455
- 48 O'Regan R, Ozguroglu M, Andre F, et al. Phase III, randomized, double-blind, placebo-controlled multicenter trial of daily everolimus plus weekly trastuzumab and vinorelbine in trastuzumab-resistant, advanced breast cancer (BOLERO-3). *J Clin Oncol*. 2013; 31(suppl): 505

HER2 testing

Mary Falzon, Michael Gandy

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 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 Assurance 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, there has been a recent update of the ASCO/CAP recommendations for HER2 testing [5].

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 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) [1,6,7]. Prolonged cold ischemic times can result in degradation of the HER2 protein and other cellular structures [8].
- *Fixation*: stabilization of tissue structures and genetic material in preparation for tissue processing [9]. A fixation time of 6–72 hours is recommended for breast cancer samples, depending on size of the sample [1,5].
- *Tissue processing*: complete dehydration, clearing, and paraffin embedding of the tissue sample in preparation for sectioning [1,7].
- *Slide preparation*: tissue sectioning at 3–4 μm and adherence onto charged microscope slides via incubation of the slide at 37°C overnight (or 60°C for 1 hour) in preparation for staining [7]. 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 [1,10,11].

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 [12]; 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 [10]. All types of epithelial-derived invasive primary breast cancers should be tested at diagnosis for HER2 status by immunohistochemistry (IHC) in the first instance [13]. HER2 testing is not currently performed routinely for ductal carcinoma in situ [10].

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 [14]. 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 [13].

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 [10,11]. 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 [10]. 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 [10]. These target the HER2 protein and gene, respectively, and are clinically validated, commercially available, and subject to regional regulatory clearance and control (eg, FDA) [1,15,16]. 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 [1,2,15-17].

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 [18]. 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.

Immunohistochemical staining for HER2-positive breast cancer

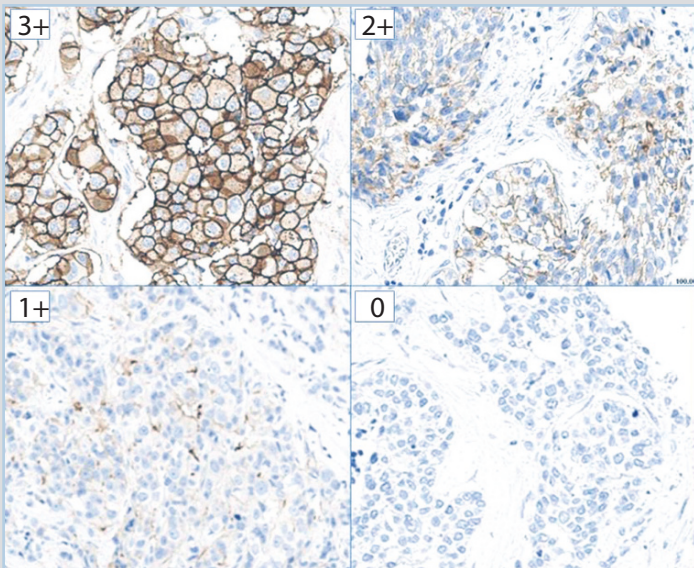


Figure 2.1 Immunohistochemical staining for HER2-positive breast cancer. This figure illustrates the 4 distinct immunohistochemical grades: 0, 1+, 2+, 3+.

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) [5,10]. 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.

Recommended immunohistochemical scoring method		
Score to report	HER2 protein overexpression 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.

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 [10]. Standard practice includes assessing the HER2 and centromeric 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 [10]:

- non-amplified negative (<1.80);
- borderline non-amplified negative (1.80 – 1.99);
- borderline amplified positive (2.00 – 2.20); or
- amplified positive (>2.20).

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 [1,10].

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 [19].

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) [10]. 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].

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 [20]. 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 [20]; 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 2.2) [20].

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 [21] and Mansfield et al [22] which utilize alternative markers to assess chromosome 17. Although in clinical practice there

Co-localization of HER2 and CEP17 signals

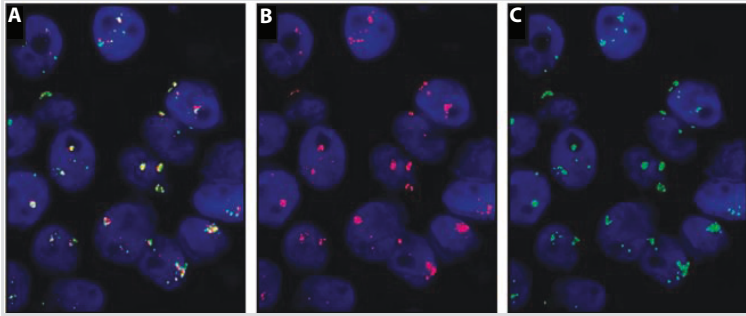


Figure 2.2. Co-localization of HER2 and CEP17 signals. (A) Co-localization of HER2 and CEP17 signals is represented in this figure by a yellow fusion signal. This differs from the independent (B) HER2 and (C) CEP17 signals. Reproduced with permission from Starczynski et al [20].

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 [22].

Alternatively, methods such as multiplex ligation-dependent probe amplification may prove useful [23]. 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 [20]. 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.

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) [24]. 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 [24,25]. Using an enzyme-linked immunosorbent 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 [24].

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 [26]. 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 macro-dissection of the tumor and normalization of background HER2 [27]. 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 [28]. 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 [29], as well as for compounds which affect pathways that HER2 is a constituent part of.

References

- 1 Wolff AC, Hammond MEH, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol.* 2007;25:118-145.
- 2 Pedersen K, Angelini P-D, Laos S, et al. A naturally occurring HER2 carboxy-terminal fragment promotes mammary tumor growth and metastasis. *Mol Cell Biol.* 2009;29:3319-3331.
- 3 Negri T, Tarantino E, Orsenigo M, et al. Chromosome band 17q21 in breast cancer: significant association between *beclin 1* loss and *HER2/NEU* amplification. *Genes Chromosomes Cancer.* 2010;49:901-909.
- 4 Pinhel I, Hills M, Drury S, et al; for the NCRI Adjuvant Breast Cancer Trial Management Group. ER and HER2 expression are positively correlated in HER2 non-overexpressing breast cancer. *Breast Cancer Res.* 2012;14:R46.
- 5 Wolff AC, Hammond ME, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *J Clin Oncol.* 2013;31:3997-4013
- 6 Hammond MEH, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). *Arch Pathol Lab Med.* 2010;134:e48-e72.
- 7 Müller S, Matthiesen SH, Nielsen KV. Preparation of FFPE tissue slides for solid tumor FISH analysis. In: Kumar GL, Rudbeck L, eds. *Education Guide: Immunohistochemical Staining Methods.* 5th edition. Carpinteria, CA: Dako North America; 2009:67-73.
- 8 Yildiz-Aktas IZ, Dabbs DJ, Bhargava R. The effect of cold ischemic time on the immunohistochemical evaluation of estrogen receptor, progesterone receptor, and HER2 expression in invasive breast carcinoma. *Mod Pathol.* 2012;25:1098-1105.
- 9 Farmilo AJ, Stead RH. Fixation and processing. In: Kumar GL, Rudbeck L, eds. *Education Guide: Immunohistochemical Staining Methods.* 5th edition. Carpinteria, CA: Dako North America; 2009:29-33.
- 10 Walker RA, Bartlett JMS, Dowsett M, et al. HER2 testing in the UK: further update to recommendations. *J Clin Pathol.* 2008;61:818-824.

- 11 Bartlett JMS, Starczynski J, Atkey N, et al. HER2 testing in the UK: recommendations for breast and gastric in-situ hybridisation methods. *J Clin Pathol*. 2011;64:649-653.
- 12 Chavez-MacGregor M, Clarke CA, Lichtensztajn D, Hortobagyi GN, Giordano SH. Male breast cancer according to tumor subtype and race: a population-based study. *Cancer*. 2013;119:1611-1617.
- 13 National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology. Breast Cancer. Version 3.2013. NCCN website. www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Accessed November 19, 2013.
- 14 Lindström LS, Karlsson E, Wilking UM, et al. Clinically used breast cancer markers such as estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 are unstable throughout tumor progression. *J Clin Oncol*. 2012;30:2601-2608.
- 15 Guidelines for Scoring Herceptest. Dako website. www.dako.com/38602_19feb10_herceptest_scoring_guidelines-breast_ihc.pdf. Accessed November 11, 2013.
- 16 PathVysion Product Probe Description. Abbott Molecular website. www.abbottmolecular.com/us/products/oncology/fish/breast-cancer-pathvysion-her2.html. Accessed November 19, 2013.
- 17 Herceptin (trastuzumab) development timeline. Genentech website. www.gene.com/media/product-information/herceptin-development-timeline. Accessed November 19, 2013.
- 18 Leong AS-Y, Formby M, Haffajee Z, Clarke M, Morey A. Refinement of immunohistologic parameters for Her2/neu scoring validation by FISH and CISH. *Appl Immunohistochem Mol Morphol*. 2006;14:384-389.
- 19 Seol H, Lee HJ, Choi Y, et al. Intratumoral heterogeneity of *HER2* gene amplification in breast cancer: its clinicopathological significance. *Mod Pathol*. 2012;25:938-948.
- 20 Starczynski J, Atkey N, Connelly Y, et al. *HER2* gene amplification in breast cancer: a rogues' gallery of challenging diagnostic cases: UKNEQAS interpretation guidelines and research recommendations. *Am J Clin Pathol*. 2012;137:595-605.
- 21 Tse CH, Hwang HC, Goldstein LC, et al. Determining true *HER2* gene status in breast cancers with polysomy by using alternative chromosome 17 reference genes: implications for anti-*HER2* targeted therapy. *J Clin Oncol*. 2011;29:4168-4174.
- 22 Mansfield AS, Sukov WR, Eckel-Passow JE, et al. Comparison of fluorescence in situ hybridization (FISH) and dual-ISH (DISH) in the determination of *HER2* status in breast cancer. *Am J Clin Pathol*. 2013;139:144-150.
- 23 Bravaccini S, Rengucci C, Medri L, Zoli W, Silvestrini R, Amadori D. Detection of *HER2* and Topo 2 in breast cancers: comparison between MLPA and FISH approaches. *J Clin Pathol*. 2012;65:183-185.
- 24 Leyland-Jones B, Smith BR. Serum *HER2* testing in patients with *HER2*-positive breast cancer: the death knell tolls. *Lancet Oncol*. 2011;12:286-295.
- 25 Molina MA, Codony-Servat J, Albanell J, Rojo F, Arribas J, Baselga J. Trastuzumab (Herceptin), a humanized anti-*HER2* receptor monoclonal antibody, inhibits basal and activated *HER2* ectodomain cleavage in breast cancer cells. *Cancer Res*. 2001;61:4744-4749.
- 26 Jacquemier J, Spyrtos F, Esterni B, et al. SISH/CISH or qPCR as alternative techniques to FISH for determination of *HER2* amplification status on breast tumors core needle biopsies: a multicenter experience based on 840 cases. *BMC Cancer*. 2013;13:351.
- 27 Millson A, Suli A, Hartung L, et al. Comparison of two quantitative polymerase chain reaction methods for detecting *HER2/neu* amplification. *J Mol Diagn*. 2003;5:184-190.
- 28 Dabbs DJ, Klein ME, Mohsin SK, Tubbs RR, Shuai Y, Bhargava R. High false-negative rate of *HER2* quantitative reverse transcription polymerase chain reaction of the Oncotype DX test: an independent quality assurance study. *J Clin Oncol*. 2011;29:4279-4285.
- 29 Bose R, Kavuri SM, Searleman AC, et al. Activating *HER2* mutations in *HER2* gene amplification negative breast cancer. *Cancer Discov*. 2013;3:224-237.

HER2-positive breast cancer: adjuvant and neoadjuvant therapy

Nadia Harbeck

Adjuvant setting

So far, trastuzumab is the only anti-human epidermal growth factor receptor 2 (HER2) drug registered in the early breast cancer (EBC) adjuvant setting. The first reports of the large adjuvant trastuzumab trials – HERceptin Adjuvant (HERA) [1], North Central Cancer Treatment Group (NCCTG) N9831, and National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31 [2] 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 disease-free survival (DFS), overall survival (OS), locoregional recurrence, and distant recurrence (all $P < 0.001$) (Figure 3.1) [3]. No difference in the magnitude of benefit is seen between lobular and ductal histology [4].

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) 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 [5]. In the NCCTG N9831 trial,

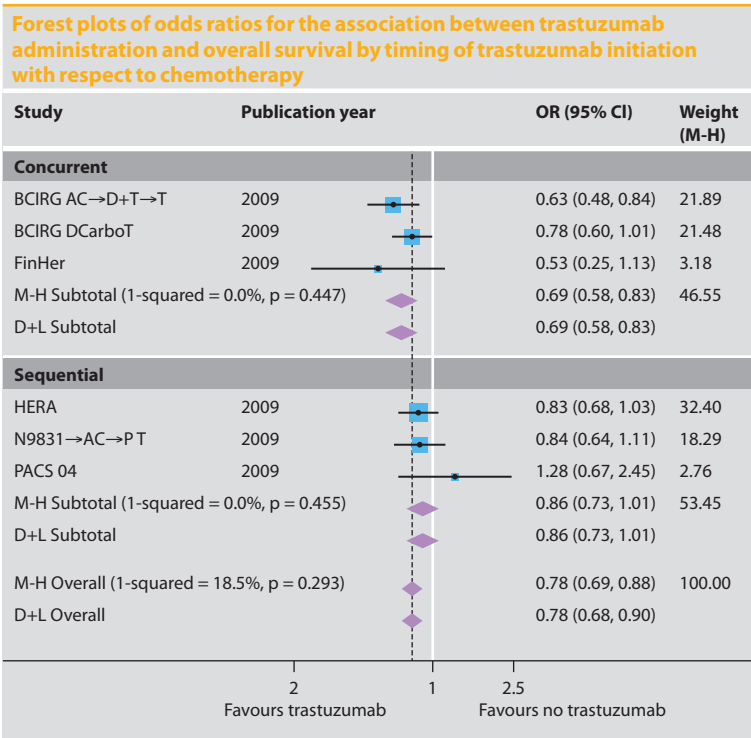


Figure 3.1 Forest plots of odds ratios for the association between adjuvant trastuzumab administration and overall survival by timing of trastuzumab initiation with respect to chemotherapy. The size of the square box is proportional to the weight that each study contributes in the meta-analysis. The 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 [3].

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 [6]. 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 recently published revised 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 [7].

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) [8]. It can be given after adjuvant chemotherapy, as demonstrated in HERA [9], 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 [10,11]. BCIRG 006 also introduced an additional anthracycline-free regimen: trastuzumab with 6 cycles of docetaxel and carboplatin (TCH). While cardiac toxicity was substantially lower with TCH when compared to AC-docetaxel + trastuzumab (AC-TH; $P<0.001$), a numerically lower efficacy rate in the TCH arm versus the AC-TH arm was seen (5-year DFS: 81% with TCH vs 84% with AC-TH; 5-year overall OS: 91% vs 92%) [11].

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 [12]; 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 [12]. Also, in a meta-analysis for trastuzumab in EBC, concurrent use of trastuzumab was associated with a lower risk of death ($P<0.001$) [3]. 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 the anthracycline-containing chemotherapy, as demonstrated in the neoadjuvant setting [13,14]. 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 [15]. 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 [16]. Moreover, 18% of patients had to discontinue trastuzumab due to cardiac events (any grade) [16]. 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 [17,18].

In the adjuvant setting, trastuzumab can be administered together with radiation therapy. Observed side effects included grade 3/4 dermatitis (51% of patients), esophagitis (12%), and grade >2 left ventricular ejection fraction (LVEF) decrease after radiation therapy (6–10%) [19]. 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 [19].

Duration

Based on the registration trials [1,2,11], 1-year of trastuzumab is now standard in the adjuvant setting. Recent results of the HERA trial showed that 2 years of adjuvant trastuzumab is not more effective than 1 year (DFS HR 0.99; 95% CI, 0.85–1.14; $P=0.86$) [20]. 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 HER-negative EBC comparing docetaxel versus vinorelbine, followed by fluorouracil-epirubicin-cyclophosphamide (FEC) chemotherapy [21]. In the HER2-positive subgroup, patients receiving nine 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, nine weeks of trastuzumab (docetaxel + trastuzumab – FEC) seemed to significantly improve DDFS versus DOC – FEC (HR 0.32; $P=0.029$) or vinorelbine + trastuzumab – FEC (HR 0.31; $P=0.020$) [21]. 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 non-inferiority 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%; $P<0.0001$) [22]. The prospective PHARE trial failed to demonstrate non-inferiority 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 [23]. All patients with a tumor size 1 cm ($>pT1c$) should receive adjuvant trastuzumab based on the pivotal trial results [8–10,18,21]. 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 [24].

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 [25]. 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 [25]. After an updated follow-up (median 41 months), the authors calculated a NNT of 19 to prevent one breast-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 [26].

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 [27]. Again, in another retrospective institutional cohort, adjuvant trastuzumab seemed to be beneficial in small node-negative HER2-positive tumors [28]. Consequently, in patients with small node-negative tumors, an individual risk/benefit analysis needs to be performed. International guidelines such as AGO and NCCN recommend trastuzumab in node-negative tumors larger than 0.5 cm (>pT1b) [17,18].

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 [29]. 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%) [30].

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 [6]. Cardiac function improved in most congestive heart failure (CHF) cases after trastuzumab discontinuation and cardiac medication. Incidence of asymptomatic LVEF decreases which led to withholding trastuzumab was 8–10%; of these, in about 50% of cases, LVEF recovered and trastuzumab could be restarted [6]. In the HERA trial, trastuzumab was administered subsequently to adjuvant chemotherapy; 94.1% of patients were treated with anthracycline-containing chemotherapy [31]. At a median follow-up of 3.6 years, the overall incidence of cardiac end points 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 end point reached acute recovery; 52 of these 59 patients were considered to have a favorable outcome from the cardiac end point by the cardiac advisory board. The incidence of discontinuation of trastuzumab due to cardiac disorders was 5.1% [31]. As discussed before, the lowest cardiac toxicity within the pivotal trials was observed with TCH 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 [11].

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 TCH rather than with an anthracycline-taxane sequence [32]. A summary of adjuvant Phase III trials is found in Table 3.1.

Adjuvant Phase III trastuzumab trials					
Trial	Patients (n)	Regimen	DFS rate	OS rate	Cardiotoxicity
HERA [9]	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 [10]	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 [11]	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 [18]	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 [16]	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

Table 3.1 Adjuvant Phase 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.

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) vs 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 vs 17% in the placebo group (HR 0.83, 95% CI, 0.70–1.00; $P=0.053$) [33]. 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% 0.67–1.00; $P=0.04$) [33].

Given the borderline significance of the therapy effect and higher toxicities with lapatinib, adjuvant lapatinib should only be considered an option in settings where trastuzumab is not available. Moreover, analysis of the cross-over 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; $P=0.0077$) [9].

Neoadjuvant therapy

In principle, trastuzumab can be started together with chemotherapy in the adjuvant and neoadjuvant settings. After neoadjuvant chemotherapy in combination with trastuzumab, trastuzumab is continued for a total of one year in the adjuvant setting. Achieving a pathological complete remission (pCR) under trastuzumab-based neoadjuvant therapy is correlated with an excellent outcome [13,34]. Thus, neoadjuvant trastuzumab therapy has a distinct advantage in that patients can be counselled 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.

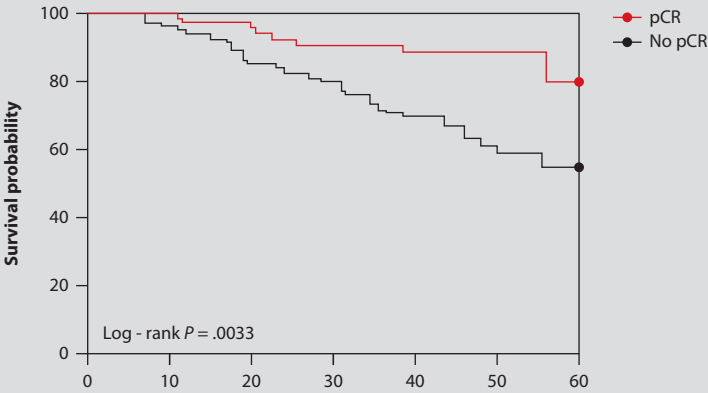
For example, in the open-label Phase III NOAH trial, patients with newly diagnosed locally advanced or inflammatory HER2-positive breast cancer ($n=235$) were randomized to preoperative anthracycline-taxane chemotherapy with trastuzumab or chemotherapy alone. In a separate cohort, 99 patients with HER2-negative 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%; $P=0.001$) [13]. 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 + LN 16%) [13]. Given this high efficacy of preoperative trastuzumab, 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 [34]. All patients received four doses of EC followed by four doses paclitaxel + trastuzumab every three weeks;

pCR in breast + LN was reported in 38.7% of patients [34]. Patients who achieved a pCR had an excellent 3-year outcome with a significantly better DFS and OS than patients without a pCR (Figure 3.2) [34].

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 pCR rate of 40.0% was found [14]. 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 ($P<0.001$) [35]. 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%; OR 0.68; 95% CI, 0.47–0.97; $P=0.04$) [36]. 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 [36]. In view of the above data, the current neoadjuvant standard in the HER2-positive disease remains trastuzumab (plus chemotherapy).

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 [37]. After surgery, patients continued their targeted therapy for a total of 52 weeks. pCR was significantly higher in the lapatinib plus trastuzumab 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 [37]. In the Phase II NeoSphere 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 3.3) [38].

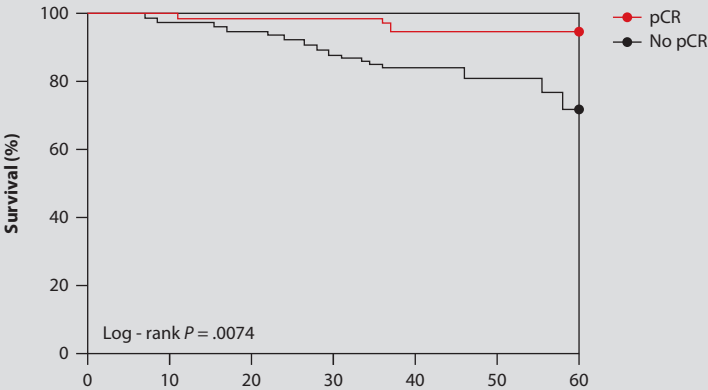
Disease-free and overall survival depending on pCR status after neoadjuvant trastuzumab + anthracycline-taxane chemotherapy



Disease-free survival by pCR (months)

Number at risk

pCR	84	79	61	57	43	16	5
no pCR	133	118	86	76	56	27	10



Overall survival by pCR (months)

Number at risk

pCR	84	79	62	62	47	17	7
no pCR	133	118	96	84	65	33	14

Figure 3.2 A and B 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 [34].

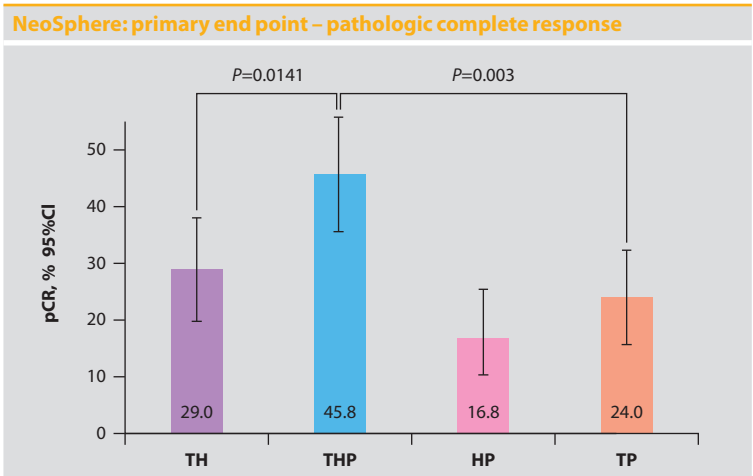


Figure 3.3 NeoSphere: primary end point – pathologic complete response. Intent-to-treat population. CI, confidence interval; H, trastuzumab; P, pertuzumab; pCR, pathologic complete response; T, docetaxel. *P*-values from Cochran-Mantel-Haenszel test and adjusted for multiplicity.

Toxicity of three different chemotherapy schedules were evaluated in the TRYPHAENA trial [39]: (FEC-Doc plus targeted agents during the whole chemotherapy vs in parallel with the taxane vs TCb + targeted agents) together with dual HER2 blockade (trastuzumab + pertuzumab) was evaluated. 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 H + P) versus 57.3% (FEC-Doc + H + P), and 66.2% (TCH + P) [39]. Whether these improved pCR rates by dual HER2 blockade translate into improved patient outcomes will be seen once follow-up data of the respective trials become available. 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 [38]). A summary of the neoadjuvant trials is found in Table 3.2.

Neoadjuvant trials with trastuzumab					
Trial	Patients (n)	Regimen	pCR rate	DFS rate	OS rate
Single blockade					
TECHNO [34]	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 [13]	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 [14]	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 [36]	620	EC-Doc+H vs EC-Doc+L	30.3% vs 22.7%	NA	NA
HannaH [40]	596	Doc-FEC+H(SC) vs Doc-FEC +H(IV)	45.4% vs 40.7%	NA	NA
Dual blockade					
NeoALTTO [37]	455	Pac+L vs Pac +H vs Pac+L+H	24.7% vs 29.5% vs 51.3%†	NA	NA
NeoSphere [38]	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%**	NA	NA
TRYPHAENA [39]	225	FEC - Doc+ [H+P] FEC - Doc+H+P TCH+P	FEC-Doc + [H+P]: 61.6%** FEC - Doc+H+P: 57.3%** TCH+P: 66.2%**	NA	NA

Table 3.2 Neoadjuvant trials with trastuzumab. A, doxorubicin; Cap, capecitabine; CMF, cyclophosphamide + methotrexate + fluorouracil; DCIS, ductal carcinoma in situ; Doc, docetaxel; EC, epirubicin + cyclophosphamide; H, trastuzumab; IV, intravenous; L, lapatinib; NA, not available; P, pertuzumab; Pac, paclitaxel; pCR, pathologic complete response; SC, subcutaneous. *pCR breast and lymph nodes; **pCR breast.

Current therapy standards for HER2-positive early breast cancer

In HER2-positive EBC, one year of trastuzumab in the context of (neo-) adjuvant chemotherapy is the current standard. Neoadjuvant administration is preferred because patient management may then be individualized depending on pathological response at time of surgery. 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. TCH is also a valid option, particularly if anthracycline toxicity needs to be avoided. 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

Recently, 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 end points were serum trough concentration at pre-dose cycle 8 before surgery (non-inferiority margin of 0.80 for ratio between groups) and pCR (non-inferiority margin of -12.5% for difference between groups). Subcutaneous trastuzumab was non-inferior to intravenous trastuzumab for both co-primary end points with a pCR of 45.4% versus 40.7% in the intravenous group. The incidence of grade 3–5 adverse events (AEs) was similar between groups, yet, there were more serious adverse events (mostly infections) in the subcutaneous group (21% vs 12%) and 3 deaths vs 1 [40]. Based on the HannaH trial

results demonstrating non-inferiority, subcutaneous trastuzumab has recently received approval by the European Medicines Agency.

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, the FDA has recently issued a directive outlining the potential for accelerated approval using neoadjuvant trials [41]. 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 (to be used together with trastuzumab and chemotherapy). Using pCR as a surrogate end point 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 just started. 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 will randomize non-pCR patients to T-DM1 (see Chapter 1) versus continuation of trastuzumab for the remainder of the year.

Large adjuvant trials based on the promising data from neoadjuvant concepts such as ALTTO (NCT00490139), which evaluates the best schedule of lapatinib together with trastuzumab, or APHINITY (NCT00490139), which evaluates dual blockade of trastuzumab and pertuzumab, have already been fully recruited and are now collecting follow-up information. First results from these studies are not expected before 2014.

References

- 1 Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al; for the Herceptin Adjuvant (HERA) Trial Study Team. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med*. 2005;353:1659-1672.
- 2 Romond EH, Perez EA, Bryant J, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med*. 2005;353:1673-1684.

- 3 Yin W, Jiang Y, Shen Z, Shao Z, Lu J. Trastuzumab in the adjuvant treatment of HER2-positive early breast cancer patients: a meta-analysis of published randomized controlled trials. *PLoS One*. 2011;6:e21030.
- 4 Metzger-Filho O, Procter M, de Azambuja E, et al. Magnitude of trastuzumab benefit in patients with HER2-positive, invasive lobular breast carcinoma: results from the HERA trial. *J Clin Oncol*. 2013;31:1954-1960.
- 5 Hammond ME, Hayes DF, Wolff AC. Clinical Notice for American Society of Clinical Oncology-College of American Pathologists guideline recommendations on ER/PgR and HER2 testing in breast cancer. *J Clin Oncol*. 2011;29:e458.
- 6 Perez EA, Dueck AC, McCullough AE, et al. Predictability of adjuvant trastuzumab benefit in N9831 patients using the ASCO/CAP HER2-positivity criteria. *J Natl Cancer Inst*. 2012;104:159-162.
- 7 Dowsett M, Procter M, McCaskill-Stevens W, et al. Disease-free survival according to degree of HER2 amplification for patients treated with adjuvant chemotherapy with or without 1 year of trastuzumab: the HERA Trial. *J Clin Oncol*. 2009;27:2962-2969.
- 8 Leyland-Jones B. Dose scheduling--Herceptin. *Oncology*. 2001;61 (suppl 2):31-36.
- 9 Gianni L, Dafni U, Gelber RD, et al; for the Herceptin Adjuvant (HERA) Trial Study Team. Treatment with trastuzumab for 1 year after adjuvant chemotherapy in patients with HER2-positive early breast cancer: a 4-year follow-up of a randomised controlled trial. *Lancet Oncol*. 2011;12:236-244.
- 10 Perez EA, Romond EH, Suman VJ, et al. Four-year follow-up of trastuzumab plus adjuvant chemotherapy for operable human epidermal growth factor receptor 2-positive breast cancer: joint analysis of data from NCCTG N9831 and NSABP B-31. *J Clin Oncol*. 2011;29:3366-3373.
- 11 Slamon D, Eiermann W, Robert N, et al; for the Breast Cancer International Research Group. Adjuvant trastuzumab in HER2-positive breast cancer. *N Engl J Med*. 2011;365:1273-1283.
- 12 Perez EA, Suman VJ, Davidson NE, et al. Sequential versus concurrent trastuzumab in adjuvant chemotherapy for breast cancer. *J Clin Oncol*. 2011;29:4491-4497.
- 13 Gianni L, Eiermann W, Semiglazov V, et al. Neoadjuvant chemotherapy with trastuzumab followed by adjuvant trastuzumab vs. neoadjuvant chemotherapy alone, in patients with HER2-positive locally advanced breast cancer (the NOAH trial): a randomised controlled superiority trial with a parallel HER2-negative cohort. *Lancet*. 2010;375:377-384.
- 14 Untch M, Rezai M, Loibl S, et al. Neoadjuvant treatment with trastuzumab in HER2-positive breast cancer: results from the GeparQuattro study. *J Clin Oncol*. 2010;28:2024-2031.
- 15 Buzdar A, Suman VJ, Meric-Bernstam F, et al; for the Alliance for Clinical Trials in Oncology. ACOSOG Z1041 (Alliance): Definitive analysis of randomized neoadjuvant trial comparing FEC followed by paclitaxel plus trastuzumab (FEC + P+T) with paclitaxel plus trastuzumab followed by FEC plus trastuzumab (P+T + FEC+T) in HER2+ operable breast cancer. *J Clin Oncol*. 2013 (suppl; abstr 502).
- 16 Spielmann M, Roché H, Delozier T, et al. Trastuzumab for patients with axillary-node-positive breast cancer: results of the FNCLCC-PACS 04 trial. *J Clin Oncol*. 2009;27:6129-6134.
- 17 Arbeitsgemeinschaft Gynäkologische Onkologie (AGO). Diagnosis and Treatment of Patients with Primary and Metastatic Breast Cancer. AGO Kommission Mamma. 2012. www.ago-online.de/en/guidelines-mamma. Accessed November 11, 2013.
- 18 National Comprehensive Cancer Network (NCCN). Practice Guidelines in Oncology. Breast Cancer. NCCN Website. 2013. www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Accessed November 19, 2013.
- 19 Belkacémi Y, Gligorov J, Ozsahin M, et al. Concurrent trastuzumab with adjuvant radiotherapy in HER2-positive breast cancer patients: acute toxicity analyses from the French multicentric study. *Ann Oncol*. 2008;19:1110-1116.
- 20 Goldhirsch A, Gelber RD, Piccart-Gebhart MJ, et al; for the Herceptin Adjuvant (HERA) Trial Study Team. 2 years versus 1 year of adjuvant trastuzumab for HER2-positive breast cancer (HERA): an open-label, randomised controlled trial. *Lancet*. 2013; [Epub ahead of print].

- 21 Joensuu H, Bono P, Kataja V, et al. Fluorouracil, epirubicin, and cyclophosphamide with either docetaxel or vinorelbine, with or without trastuzumab, as adjuvant treatments of breast cancer: final results of the FinHer Trial. *J Clin Oncol*. 2009;27:5685-5692.
- 22 Pivot X, Romieu G, Debled M, Pet al; for the PHARE trial investigators. 6 months versus 12 months of adjuvant trastuzumab for patients with HER2-positive early breast cancer (PHARE): a randomised phase 3 trial. *Lancet Oncol*. 2013;14:741-748.
- 23 Untch M, Gelber RD, Jackisch C, et al; for the HERA Study Team. Estimating the magnitude of trastuzumab effects within patient subgroups in the HERA trial. *Ann Oncol*. 2008;19:1090-1096.
- 24 Kelly CM, Pritchard KI, Trudeau M, Andreopoulou E, Hess K, Pusztai L. Coping with uncertainty: T1a,bN0M0 HER2-positive breast cancer, do we have a treatment threshold? *Ann Oncol*. 2011;22:2387-2393.
- 25 Gonzalez-Angulo AM, Litton JK, Broglio KR, et al. High risk of recurrence for patients with breast cancer who have human epidermal growth factor receptor 2-positive, node-negative tumors 1 cm or smaller. *J Clin Oncol*. 2009;27:5700-5706.
- 26 Rodrigues MJ, Albiges-Sauvin L, Wassermann J, Cottu PH. Evaluating the risk-benefit ratio of adjuvant trastuzumab-based therapy for T1a,bN0M0 HER2-positive breast carcinomas. *Ann Oncol*. 2011;22:2530.
- 27 Horio A, Fujita T, Hayashi H, et al. High recurrence risk and use of adjuvant trastuzumab in patients with small, HER2-positive, node-negative breast cancers. *Int J Clin Oncol*. 2012;17:131-136.
- 28 McArthur HL, Mahoney KM, Morris PG, et al. Adjuvant trastuzumab with chemotherapy is effective in women with small, node-negative, HER2-positive breast cancer. *Cancer*. 2011;117:5461-5468.
- 29 Mackey JR, Clemons M, Côté MA, et al. Cardiac management during adjuvant trastuzumab therapy: recommendations of the Canadian Trastuzumab Working Group. *Curr Oncol*. 2008;15:24-35.
- 30 Romond EH, Jeong JH, Rastogi P, et al. Seven-year follow-up assessment of cardiac function in NSABP B-31, a randomized trial comparing doxorubicin and cyclophosphamide followed by paclitaxel (ACP) with ACP plus trastuzumab as adjuvant therapy for patients with node-positive, human epidermal growth factor receptor 2-positive breast cancer. *J Clin Oncol*. 2012;30:3792-3799.
- 31 Procter M, Suter TM, de Azavedo E, et al. Longer-term assessment of trastuzumab-related cardiac adverse events in the Herceptin Adjuvant (HERA) trial. *J Clin Oncol*. 2010;28:3422-3428.
- 32 Burstein HJ, Piccart-Gebhart MJ, Perez EA, et al. Choosing the best trastuzumab-based adjuvant chemotherapy regimen: should we abandon anthracyclines? *J Clin Oncol*. 2012;30:2179-2182.
- 33 Goss PE, Smith IE, O'Shaughnessy J, et al; for the TEACH investigators. Adjuvant lapatinib for women with early-stage HER2-positive breast cancer: a randomised, controlled, phase 3 trial. *Lancet Oncol*. 2013;14:88-96.
- 34 Untch M, Fasching PA, Konecny GE, et al. Pathologic complete response after neoadjuvant chemotherapy plus trastuzumab predicts favorable survival in human epidermal growth factor receptor 2-overexpressing breast cancer: results from the TECHNO trial of the AGO and GBG study groups. *J Clin Oncol*. 2011;29:3351-3357.
- 35 von Minckwitz G, Untch M, Nüesch E, et al. Impact of treatment characteristics on response of different breast cancer phenotypes: pooled analysis of the German neo-adjuvant chemotherapy trials. *Breast Cancer Res Treat*. 2011;125:145-156.
- 36 Untch M, Loibl S, Bischoff J, et al; for the German Breast Group (GBG); Arbeitsgemeinschaft Gynäkologische Onkologie-Breast (AGO-B) Study Group. Lapatinib versus trastuzumab in combination with neoadjuvant anthracycline-taxane-based chemotherapy (GeparQuinto, GBG 44): a randomised phase 3 trial. *Lancet Oncol*. 2012;13:135-144.

- 37 Baselga J, Bradbury I, Eidtmann H, et al; for the NeoALTTO Study Team. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. *Lancet*. 2012;379:633-640.
- 38 Gianni L, Pienkowski T, Im YH, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2012;13:25-32.
- 39 Schneeweiss A, Chia S, Hickish T, et al. Pertuzumab plus trastuzumab in combination with standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2-positive early breast cancer: a randomized phase II cardiac safety study (TRYPHAENA). *Ann Oncol*. 2013; [Epub ahead of print].
- 40 Ismael G, Hegg R, Muehlbauer S, et al. Subcutaneous versus intravenous administration of (neo)adjuvant trastuzumab in patients with HER2-positive, clinical stage I-III breast cancer (Hannah study): a phase 3, open-label, multicentre, randomised trial. *Lancet Oncol*. 2012;13:869-878.
- 41 Food and Drug Administration (FDA). Guidance for Industry Pathologic Complete Response in Neoadjuvant Treatment of High-Risk Early-Stage Breast Cancer: Use as an Endpoint to Support Accelerated Approval. May 2012. FDA website. www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM305501.pdf. Accessed November 19, 2013.

HER2-positive metastatic breast cancer: first-line treatment

Leticia De Mattos-Arruda, Javier Cortés

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. Over the last decade, trastuzumab-based therapy has become the standard first-line treatment option for patients with HER2+ metastatic breast cancer [1–5]. Lapatinib in combination with capecitabine is also an approved regimen after progression on a trastuzumab-containing chemotherapy [6]. However, other anti-HER2-targeted therapies, such as pertuzumab and trastuzumab emtansine (T-DM1) 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 [7,8] either alone or in combination with selected chemotherapeutic agents [1,8]. In randomized clinical trials, trastuzumab has also been proven to be beneficial when combined with taxanes (with or without platinum compounds), vinorelbine, and capecitabine [1–4,9–11]. These combination regimens are recommended in the current National Comprehensive Cancer Network (NCCN) guidelines as options for first-line treatment [12].

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) [1–4,11]. 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 [2,13].

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 paclitaxel (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 [14]. 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; 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 [11].

Trastuzumab and taxane-based therapy as a first-line treatment strategy

Drug combination	n	Regimen (doses in mg/m ²)	Response (%)			Median OS (m)	Median TTP (m)	Reference
			ORR	SD	PD			
Taxanes with or without trastuzumab	92	P 175 mg/m ² q3wk + T 4 mg/kg loading, then 2 mg/kg/wk	41*	NR	NR	22.1	6.9*	[1]
	96	P 175 mg/m ² q3wk	17	NR	NR	18.4	3.0	
	92	D 100 mg/m ² q3wk + T 4 mg/kg loading, then 2 mg/kg/wk	61*	27	NR	31.2*	11.7*	[2]
	94	D 100 mg/m ² 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/m ² q3wk + X 950 mg/m ² b.i.d days 1–14 q3wk	70.5	25	3.6	0.75†	18.6*	[11]
	110	T 8 mg/kg loading then 6 mg/kg + D 100 mg/m ² q3wk	72.7	16.4	9.1	0.66†	13.6	
	132	D 75 mg/m ² 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/m ² 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/m ² 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.

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 [15,16].

Trastuzumab and vinorelbine-based first-line therapy

In preclinical studies, vinorelbine was demonstrated to act synergistically with trastuzumab [17]. Given the high response rates with manageable toxicity observed with vinorelbine and trastuzumab in Phase II trials [10,18], two Phase III randomized studies investigated the combination of trastuzumab with either taxanes or vinorelbine [10,19].

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 [19]. Subsequently, the HERNATA trial has confirmed a role for vinorelbine + trastuzumab as an alternative first-line therapy combination [10]. 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 [10].

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 [1,20]. Liposomal forms of doxorubicin have been shown to provide efficacy similar to that of conventional doxorubicin with greater cardiac safety [21–23]. Several trials have evaluated pegylated and non-pegylated liposomal doxorubicin (NPLD) in combination with trastuzumab with or without taxanes [24–29]. 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) [26]. 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 [29]. 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 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 [29].

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 [30]. Compared with trastuzumab, which binds to an epitope near the subdomain IV of HER2 [31], pertuzumab binds to HER2 at an epitope near the centre of domain II (Figure 4.1) [32]. By blocking HER2 from interaction with itself or HER1, HER3, or HER4, pertuzumab hampers the activation of multiple HER signaling pathways [32].

Therapeutic efficacy

Pertuzumab was shown to be well tolerated and clinically active in Phase I trials in patients with advanced solid malignancies [33–35]. In the context of HER2-positive breast cancer, a dual anti-HER2 combination regimen containing pertuzumab and trastuzumab was initially investigated [36–38]. 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 objective responses plus stable disease

Pertuzumab- and trastuzumab-based therapy in HER2-positive metastatic breast cancer

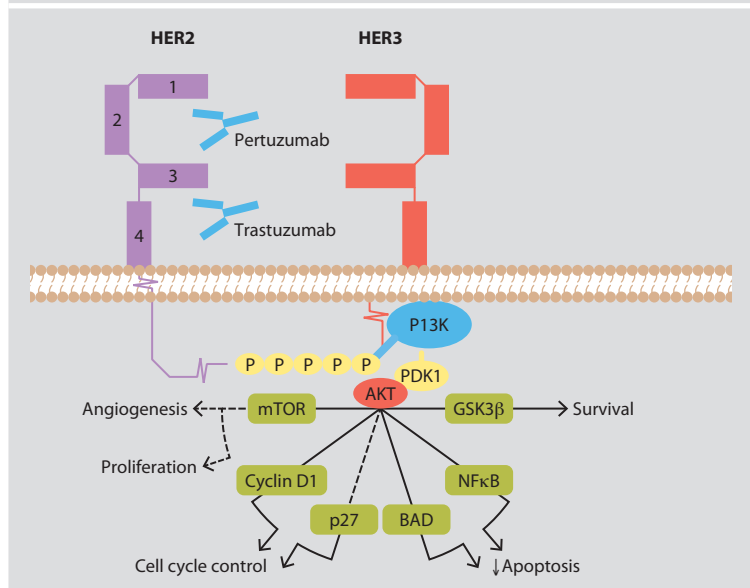


Figure 4.1 Pertuzumab- and trastuzumab-based therapy in HER2-positive metastatic breast cancer. Pertuzumab, a HER dimerization inhibitor, binds to an epitope on the subdomain II of HER2, preventing its ability to pair with other HER family members (HER1, HER3, and HER4) and with itself. By contrast, 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; GSK3 β , glycogen synthase kinase 3 beta; mTor, mammalian target of rapamycin; NF κ B, nuclear factor kappa beta; P13K, phosphatidylinositol 3-kinase; PDK1, phosphoinositide-dependent kinase 1.

for >6 months) of 50%; median PFS was 5.5 months [36]. 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%) [37].

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 [39].

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 end point, 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; $P<0.001$) [39]. In addition, the ORR was superior for the pertuzumab-containing arm (80.2% vs 69.3%; $P<0.001$). An interim analysis of OS favored the pertuzumab group, but the difference was not significant [39].

The confirmatory analysis of OS was reported after a median follow-up of 30 months [39]. In this second 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 not reached vs 37.6 months: HR 0.66; 95% CI 0.52–0.84; $P=0.0008$) [40]. Full efficacy results for CLEOPATRA can be found in Table 4.2 [39,40]

An exploratory post-hoc efficacy analysis was performed in elderly patients enrolled in the CLEOPATRA study (age ≥ 65 years). The

Pertuzumab-based therapy as a first-line treatment for HER2-positive breast cancer									
Study	n	Regimen (doses in mg/m ²)	Response (%)*					Median PFS (m)	Median OS (m)
			CR	PR	OR	SD	PD		
CLEOPATRA (NCT00567190) [39,40]	402	T 8 mg/kg loading then 6 mg/kg q3wk + D 75 mg/m ² q3wk + P 840 mg q3wk followed by 420 mg q3wk	5.5	74.6	80.2	14.6	3.8	18.5	NR
	406	T 8 mg/kg loading then 6 mg/kg q3wk + D 75 mg/m ² q3wk + placebo	4.2	65.2	69.3	20.8	8.3	12.4	37.6

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.

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]) [41].

Based on the results of the CLEOPATRA study, the combination of pertuzumab + trastuzumab + a taxane has been incorporated into clinical practice guidelines as a preferred first-line treatment for patients with HER2-positive metastatic breast cancer [12]. The efficacy and safety of an alternative option based on weekly paclitaxel in combination with trastuzumab and pertuzumab was confirmed in interim results from a single-arm, Phase II study [42].

Tolerability

In the BO17929 study, the administration of pertuzumab + trastuzumab was generally well tolerated [36,37]. 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%) [36]. 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%) [39]. 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) [39].

Treatment with pertuzumab combined with trastuzumab [36,37] or with trastuzumab and docetaxel [39] 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 (8.3% vs 4.4%) [39]. A specific analysis of safety in the elderly population (based on a cut-off of 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 [41]. 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 [43,44]. In the clinic, the combination of pertuzumab and trastuzumab appears to be more effective than pertuzumab monotherapy [37]. 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 represents a novel option for the first-line treatment of HER2-positive metastatic breast cancer [45].

Ongoing first-line studies

New anti-HER2 agents are being investigated as first-line strategies and might change the paradigm for the treatment of patients with HER2-positive breast cancer. There is a compelling rationale for simultaneously targeting multiple pathways, given the interactions between HER2 and other molecular pathways [9], and several studies are investigating pertuzumab in combination with other new anti-HER2-targeted therapies and chemotherapeutic agents in the first-line setting (Table 4.3) [46–62].

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 [63]. 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 [64]. Pertuzumab and T-DM1 may have synergistic and complementary mechanisms of action. An ongoing randomized Phase III study (MARIANNE) is investigating the role of pertuzumab in combination with T-DM1 as another first-line strategy for dual HER2 blockade [46]. In this multicenter trial, with a primary end point of PFS,

Clinical trials for the treatment of HER2-positive breast cancer in the first-line setting				
Clinical Trial / NCT ID	Phase of development	Regimen	Primary objective(s)	Estimated enrollment
Pertuzumab-based therapy				
MARIANNE (NCT01120184) [46]	III	T-DM1 + pertuzumab T-DM1 + placebo Trastuzumab + taxane	PFS	1092
PERUSE (pertuzumab global safety study) [47]	IIIb	Pertuzumab + trastuzumab + taxane	Safety and tolerability	1500
NCT01491737 [48]	II	Trastuzumab + AI ± pertuzumab + chemotherapy* Trastuzumab + AI ± chemotherapy*	PFS	250
EORTC (NCT01597414) (elderly patients) [49]	II	Pertuzumab + trastuzumab† Pertuzumab + trastuzumab + metronomic chemotherapy†	PFS	80
NCT01565083 [50]	II	Pertuzumab + trastuzumab + vinorelbine	ORR	210
NCT01276041 (0 or 1 prior treatment in the metastatic setting) [51]	II	Pertuzumab + trastuzumab + paclitaxel	6 months progression-free	69
NCT01730833 [52]	II	Pertuzumab + trastuzumab + albumin-stabilized nanoparticle formulation of paclitaxel	PFS and ORR	45
HELENA (NCT01777958) [53]	Observational	Pertuzumab + trastuzumab after adjuvant trastuzumab	PFS	478
Miscellaneous				
NEFERTT (NCT00915018) [54]	II	Trastuzumab + paclitaxel Neratinib + paclitaxel	PFS	480

Table 4.3 Clinical trials for the treatment of HER2-positive breast cancer in the first-line setting. (continues opposite).

Clinical trials for the treatment of HER2-positive breast cancer in the first-line setting (continued)

Clinical Trial / NCT ID	Phase of development	Regimen	Primary objective(s)	Estimated enrollment
Miscellaneous (continued)				
NCT01306942 [55]	I/II	Dasatinib + trastuzumab + paclitaxel	Phase I: maximum tolerated dose and recommended phase II dose Phase II: ORR	60
BOLERO-1 (NCT00876395) [56]	III	Everolimus + trastuzumab + paclitaxel Placebo + trastuzumab + paclitaxel	PFS	719
NCT00520975 [57]	III	Induction: trastuzumab + placebo + paclitaxel ± carboplatin Maintenance: trastuzumab + placebo Induction: trastuzumab + bevacizumab + paclitaxel ± carboplatin Maintenance: trastuzumab + bevacizumab	PFS	489
ICORG (NCT01526369) [58]	III	Paclitaxel + trastuzumab Paclitaxel + trastuzumab + lapatinib	PFS	600
NCT00496366 [59]	II	Lapatinib + capecitabine	ORR	11
NCT01835236 [60]	II	Pertuzumab + trastuzumab (1st line) → T-DM1 (2nd line) Pertuzumab + trastuzumab + paclitaxel (or vinorelbine) (1st line) → T-DM1 (2nd line)	OS at 24 months	208
NCT01269346 [61]	II	Eribulin + trastuzumab	ORR	52
NCT00033514 [62]	I/II	Erlotinib + trastuzumab	ORR	58

Table 4.3 Clinical trials for the treatment of HER2-positive breast cancer in the first-line setting.

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.

patients will be randomized to receive T-DM1 + pertuzumab, T-DM1 + placebo, or trastuzumab + a taxane [46].

The mammalian target of rapamycin (mTOR), an important protein kinase that regulates multiple signaling pathways, is involved in trastuzumab resistance [65,66]. Everolimus is an oral inhibitor of mTOR which has been investigated in trastuzumab-resistant HER2-positive metastatic breast cancer [66]. 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 [67]. The BOLERO-1 Phase III trial is evaluating the combination of everolimus with trastuzumab and paclitaxel (NCT00876395) in the first-line setting. The primary end point is PFS and the planned accrual is 719 patients [58].

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 [68,69], 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 co-express both HER2 and the ER, although its efficacy seems to be inferior 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%; $P=0.003$) [70].

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%; $P=0.026$) [71]. 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 [70,71].

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 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.

HER2-targeted therapy for HER2-positive metastatic breast cancer in the first-line setting		
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 <i>or</i> 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
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 <i>or</i> 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 <i>or</i> 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 <i>or</i> 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 <i>or</i> weekly
Paclitaxel + carboplatin + trastuzumab	Paclitaxel 175 mg/m ² IV D1 Carboplatin AUC6 IV D1 <i>or</i> Paclitaxel 80 mg/m ² IV D1, 8, 15 Carboplatin AUC2 IV D1, 8, 15	Every 3 weeks

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

HER2-targeted therapy for HER2-positive metastatic breast cancer in the first-line setting (continued)		
Regimen	Content	Frequency
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 8 mg/kg IV as a 90-minute infusion D1 followed by 6 mg/kg IV 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 a 90-minute infusion D1 followed by 2 mg/kg IV as a 30-minute infusion weekly	Weekly
Anti-HER2 combined with endocrine therapy		
Lapatinib + letrozole	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.

References

- Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med*. 2001;344:783-792.
- Marty M, Cogne F, Maraninchi D, et al. Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2–positive metastatic breast cancer administered as first-line treatment: the M77001 study group. *J Clin Oncol*. 2005;23:4265-4274.
- Robert N, Leyland-Jones B, Asmar L, et al. Randomized phase III study of trastuzumab, paclitaxel, and carboplatin compared with trastuzumab and paclitaxel in women with HER-2–overexpressing metastatic breast cancer. *J Clin Oncol*. 2006;24:2786-2792.
- Valero V, Forbes J, Pegram MD, et al. Multicenter phase III randomized trial comparing docetaxel and trastuzumab with docetaxel, carboplatin, and trastuzumab as first-line chemotherapy for patients with HER2-gene-amplified metastatic breast cancer (BCIRG 007 study): two highly active therapeutic regimens. *J Clin Oncol*. 2011;29:149-156.
- Jackisch C. HER-2–positive breast cancer: optimizing trastuzumab-based therapy. *Oncologist*. 2006;11(suppl):34-41.
- Geyer CE, Forster J, Lindquist D, et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *N Engl J Med*. 2006;355:2733-2743.
- Vogel CL, Cobleigh MA, Tripathy D, et al. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J Clin Oncol*. 2002;20:719-726.
- Baselga J, Carbonell X, Castañeda-Soto NJ, et al. Phase II study of efficacy, safety, and pharmacokinetics of trastuzumab monotherapy administered on a 3-weekly schedule. *J Clin Oncol*. 2005;23:2162-2171.
- De Mattos-Arruda L, Cortes J. Advances in first-line treatment for patients with HER-2+ metastatic breast cancer. *Oncologist*. 2012;17:631-644.
- Andersson M, Lidbrink E, Bjerre K, et al. Phase III randomized study comparing docetaxel plus trastuzumab with vinorelbine plus trastuzumab as first-line therapy of metastatic or locally advanced human epidermal growth factor receptor 2–positive breast cancer: the HERNATA study. *J Clin Oncol*. 2011;29:264-271.

- 11 Wardley AM, Pivot X, Morales-Vasquez F, et al. Randomized phase II trial of first-line trastuzumab plus docetaxel and capecitabine compared with trastuzumab plus docetaxel in HER2-positive metastatic breast cancer. *J Clin Oncol*. 2010;28:976-983.
- 12 National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology: Breast Cancer. Version 3.2013. NCCN website. www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Accessed November 19, 2013.
- 13 Marty M, Coggiotti F, Maraninchi D, et al. Superior long-term survival benefits of trastuzumab plus docetaxel compared to docetaxel alone in patients with HER2-positive metastatic breast cancer: patients surviving more than 4 years in the M77001 study. Presented at: 29th Annual San Antonio Breast Cancer Symposium; December 14-17, 2006; San Antonio, TX.
- 14 Perez EA, Suman VJ, Rowland KM, et al. Two concurrent phase II trials of paclitaxel/carboplatin/trastuzumab (weekly or every-3-week schedule) as first-line therapy in women with HER2-overexpressing metastatic breast cancer: NCCTG study 983252. *Clin Breast Cancer*. 2005;6:425-432.
- 15 Fountzilas G, Christodoulou C, Tsavdaridis D, et al. Paclitaxel and gemcitabine, as first-line chemotherapy, combined with trastuzumab in patients with advanced breast cancer: a phase II study conducted by the Hellenic Cooperative Oncology Group (HeCOG). *Cancer Invest*. 2004;22:655-662.
- 16 Yardley DA, Burris HA III, Simons L, et al. A phase II trial of gemcitabine/carboplatin with or without trastuzumab in the first-line treatment of patients with metastatic breast cancer. *Clin Breast Cancer*. 2008;8:425-431.
- 17 Pegram MD, Konecny GE, O'Callaghan C, Beryt M, Pietras R, Slamon DJ. Rational combinations of trastuzumab with chemotherapeutic drugs used in the treatment of breast cancer. *J Natl Cancer Inst*. 2004;96:739-749.
- 18 Nielsen DL, Andersson M, Kamby C. HER-2 targeted therapy in breast cancer. Monoclonal antibodies and tyrosine kinase inhibitors. *Cancer Treat Rev*. 2009;35:121-136.
- 19 Burstein HJ, Keshaviah A, Baron AD, et al. Trastuzumab plus vinorelbine or taxane chemotherapy for HER2-overexpressing metastatic breast cancer: the trastuzumab and vinorelbine or taxane study. *Cancer*. 2007;110:965-972.
- 20 Seidman A, Hudis C, Pierri MK, et al. Cardiac dysfunction in the trastuzumab clinical trials experience. *J Clin Oncol*. 2002;20:1215-1221.
- 21 Harris L, Batist G, Belt R, et al; for the TLC D-99 Study Group. Liposome-encapsulated doxorubicin compared with conventional doxorubicin in a randomized multicenter trial as first-line therapy of metastatic breast carcinoma. *Cancer*. 2002;94:25-36.
- 22 Batist G, Ramakrishnan G, Rao CS, et al. Reduced cardiotoxicity and preserved antitumor efficacy of liposome-encapsulated doxorubicin and cyclophosphamide compared with conventional doxorubicin and cyclophosphamide in a randomized, multicenter trial of metastatic breast cancer. *J Clin Oncol*. 2001;19:1444-1454.
- 23 O'Brien MER, Wigler N, Inbar M, et al; on behalf of the CAELYX Breast Cancer Study Group. Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYX™/Doxil®) versus conventional doxorubicin for first-line treatment of metastatic breast cancer. *Ann Oncol*. 2004;15:440-449.
- 24 Theodoulou M, Batist G, Campos S, Winer E, Welles L, Hudis C. Phase I study of nonpegylated liposomal doxorubicin plus trastuzumab in patients with HER2-positive breast cancer. *Clin Breast Cancer*. 2009;9:101-107.
- 25 Amadori D, Milandri C, Comella G, et al. A phase I/II trial of non-pegylated liposomal doxorubicin, docetaxel and trastuzumab as first-line treatment in HER-2-positive locally advanced or metastatic breast cancer. *Eur J Cancer*. 2011;47:2091-2098.
- 26 Cortes J, Di Cosimo S, Climent MA, et al; on behalf of the Spanish Breast Cancer Cooperative Group SOLTI. Nonpegylated liposomal doxorubicin (TLC-D99), paclitaxel, and trastuzumab in HER-2-overexpressing breast cancer: a multicenter phase I/II study. *Clin Cancer Res*. 2009;15:307-314.

- 27 Chia S, Clemons M, Martin L-A, et al. Pegylated liposomal doxorubicin and trastuzumab in HER-2 overexpressing metastatic breast cancer: a multicenter phase II trial. *J Clin Oncol*. 2006;24:2773-2778.
- 28 Wolff AC, Wang M, Li H, et al. Phase II trial of pegylated liposomal doxorubicin plus docetaxel with and without trastuzumab in metastatic breast cancer: Eastern Cooperative Oncology Group trial E3198. *Breast Cancer Res Treat*. 2010;121:111-120.
- 29 De La Pena L, Cortes J, Manikhas A, et al. Phase III trial of non-pegylated liposomal doxorubicin (M) in combination with trastuzumab (T) and paclitaxel (P) in HER2+ metastatic breast cancer (MBC). *J Clin Oncol*. 2013;31 (suppl). Abstract 517.
- 30 Capelan M, Pugliano L, De Azambuja E, et al. Pertuzumab: new hope for patients with HER2-positive breast cancer. *Ann Oncol*. 2013;24:273-282.
- 31 Ceran C, Cokol M, Cingoz S, Tasan I, Ozturk M, Yagci T. Novel anti-HER2 monoclonal antibodies: synergy and antagonism with tumor necrosis factor- α . *BMC Cancer*. 2012;12:450.
- 32 Franklin MC, Carey KD, Vajdos FF, Leahy DJ, de Vos AM, Sliwkowski MX. Insights into ErbB signaling from the structure of the ErbB2-pertuzumab complex. *Cancer Cell*. 2004;5:317-328.
- 33 Agus DB, Gordon MS, Taylor C, et al. Phase I clinical study of pertuzumab, a novel HER dimerization inhibitor, in patients with advanced cancer. *J Clin Oncol*. 2005;23:2534-2543.
- 34 Albanell J, Montagut C, Jones ET, et al. A phase I study of the safety and pharmacokinetics of the combination of pertuzumab (rhuMab 2C4) and capecitabine in patients with advanced solid tumors. *Clin Cancer Res*. 2008;14:2726-2731.
- 35 Yamamoto N, Yamada Y, Fujiwara Y, et al. Phase I and pharmacokinetic study of HER2-targeted rhuMab 2C4 (pertuzumab, RO4368451) in Japanese patients with solid tumors. *Jpn J Clin Oncol*. 2009;39:260-266.
- 36 Baselga J, Gelmon KA, Verma S, et al. Phase II trial of pertuzumab and trastuzumab in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer that progressed during prior trastuzumab therapy. *J Clin Oncol*. 2010;28:1138-1144.
- 37 Cortés J, Fumoleau P, Bianchi GV, et al. Pertuzumab monotherapy after trastuzumab-based treatment and subsequent reintroduction of trastuzumab: activity and tolerability in patients with advanced human epidermal growth factor receptor 2-positive breast cancer. *J Clin Oncol*. 2012;30:1594-1600.
- 38 Portera CC, Walshe JM, Rosing DR, et al. Cardiac toxicity and efficacy of trastuzumab combined with pertuzumab in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer. *Clin Cancer Res*. 2008;14:2710-2716.
- 39 Baselga J, Cortés J, Kim S-B, et al; for the CLEOPATRA Study Group. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *N Engl J Med*. 2012;366:109-119.
- 40 Swain SM, Kim S-B, Cortés J, et al. Pertuzumab, trastuzumab, and docetaxel for HER2-positive metastatic breast cancer (CLEOPATRA study): overall survival results from a randomised, double-blind, placebo-controlled, phase 3 study. *Lancet Oncol*. 2013;14:461-471.
- 41 Miles D, Baselga J, Amadori D, et al. Pertuzumab (P) in combination with trastuzumab (T) and docetaxel (D) in elderly patients with HER2-positive metastatic breast cancer in the CLEOPATRA study. Presented at: 35th Annual San Antonio Breast Cancer Symposium; December 4-8, 2012; San Antonio, TX. Abstract P5-18-01.
- 42 Datko F, D'Andrea G, Dickler M, et al. Phase II study of pertuzumab, trastuzumab, and weekly paclitaxel in patients with metastatic HER2-overexpressing metastatic breast cancer. Presented at: 35th Annual San Antonio Breast Cancer Symposium; December 4-8, 2012; San Antonio, TX. Abstract P5-18-20.
- 43 Nahta R, Hung M-C, Esteva FJ. The HER-2-targeting antibodies trastuzumab and pertuzumab synergistically inhibit the survival of breast cancer cells. *Cancer Res*. 2004;64:2343-2346.
- 44 Scheuer W, Friess T, Burtscher H, Bossenmaier B, Endl J, Hasmann M. Strongly enhanced antitumor activity of trastuzumab and pertuzumab combination treatment on HER2-positive human xenograft tumor models. *Cancer Res*. 2009;69:9330-9336.
- 45 De Mattos-Arruda L, Cortes J. Use of pertuzumab for the treatment of HER2-positive metastatic breast cancer. *Adv Ther*. 2013;30:645-658.

- 46 Ellis PA, Barrios CH, Im YH, et al. MARIANNE: a phase III, randomized, multicenter study of trastuzumab-DM1 (T-DM1) with or without pertuzumab compared with trastuzumab (H) plus taxane for first-line treatment of HER2-positive, progressive or recurrent locally advanced or metastatic breast cancer. *J Clin Oncol*. 2011;29 (suppl). Abstract TPS102.
- 47 Bachelot T, Ciruelos E, Peretz-Yablonski T, et al. A single-arm phase IIIb study of pertuzumab and trastuzumab with a taxane as first-line therapy for patients with HER2-positive advanced breast cancer (PERUSE). *Cancer Res*. 2012;74(24 suppl 3). Abstract OT1-1-02.
- 48 A study of pertuzumab in combination with trastuzumab plus an aromatase inhibitor in patients with hormone receptor-positive, metastatic HER2-positive breast cancer. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01491737. Accessed November 19, 2013.
- 49 Elderly metastatic breast cancer: pertuzumab-Herceptin vs pertuzumab-Herceptin-metronomic chemotherapy, followed by T-DM1. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01597414. Accessed November 19, 2013.
- 50 A study of pertuzumab in combination with Herceptin (trastuzumab) and vinorelbine in first line in patients with metastatic or locally advanced HER2-positive breast cancer. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01565083. Accessed November 19, 2013.
- 51 Paclitaxel, trastuzumab, and pertuzumab in the treatment of metastatic HER-2-positive breast cancer. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01276041. Accessed November 19, 2013.
- 52 Pertuzumab, trastuzumab, and paclitaxel albumin-stabilized nanoparticle formulation in treating patients with HER2-positive metastatic breast cancer. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01730833. Accessed November 19, 2013.
- 53 HELENA study: an observational study of Perjeta (pertuzumab) in first-line treatment in patients with Her2-positive advanced breast cancer after adjuvant Herceptin therapy. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01777958. Accessed August 23, 2013.
- 54 Study evaluating neratinib plus paclitaxel vs trastuzumab plus paclitaxel in ErbB-2 positive advanced breast cancer (NEFERTT). ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT00915018. Accessed November 19, 2013.
- 55 Dasatinib in combination with trastuzumab and paclitaxel in the first line treatment of Her2-positive metastatic breast cancer (MBC) patients. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01306942. Accessed November 19, 2013.
- 56 Hurvitz SA, Andre F, Burris HA, et al. BOLERO-1: a randomized, phase III, double-blind, placebo-controlled multicenter trial of everolimus in combination with trastuzumab and paclitaxel as first-line therapy in women with HER2-positive (HER2+), locally advanced or metastatic breast cancer (BC). *J Clin Oncol*. 2012;30(suppl). Abstract TPS648.
- 57 First-line chemotherapy and trastuzumab with or without bevacizumab in treating patients with metastatic breast cancer that overexpresses HER-2/NEU. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT00520975. Accessed November 19, 2013.
- 58 A randomized study of TH versus THL in first line treatment of HER2-positive breast cancer (TH v THL). ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01526369. Accessed November 19, 2013.
- 59 Capecitabine (Xeloda) and lapatinib (Tykerb) as first-line therapy in HER2/Neu-positive breast cancer. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT00496366. Accessed November 19, 2013.
- 60 Trastuzumab & pertuzumab followed by T-DM1 in MBC. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01835236. Accessed November 19, 2013.
- 61 Eribulin with trastuzumab as first-line therapy for locally recurrent or metastatic HER2 positive breast cancer. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01269346. Accessed November 19, 2013.

- 62 Trastuzumab and erlotinib as first-line therapy in treating women with metastatic breast cancer associated with HER2/Neu overexpression. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT00033514. Accessed November 19, 2013.
- 63 Lewis Phillips GD, Li G, Dugger DL, et al. Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody–cytotoxic drug conjugate. *Cancer Res.* 2008;68:9280–9290.
- 64 Hurvitz SA, Dirix L, Kocsis J, et al. Phase II randomized study of trastuzumab emtansine versus trastuzumab plus docetaxel in patients with human epidermal growth factor receptor 2–positive metastatic breast cancer. *J Clin Oncol.* 2013;31:1157–1163.
- 65 Shaw RJ, Cantley LC. Ras, PI(3)K and mTOR signalling controls tumour cell growth. *Nature.* 2006;441:424–430.
- 66 Nahta R, O'Regan RM. Evolving strategies for overcoming resistance to HER2-directed therapy: targeting the PI3K/Akt/mTOR pathway. *Clin Breast Cancer.* 2010;10(suppl 3):S72–S78.
- 67 Dalenc F, Campone M, Hupperets P, et al. Everolimus in combination with weekly paclitaxel and trastuzumab in patients (pts) with HER2-overexpressing metastatic breast cancer (MBC) with prior resistance to trastuzumab and taxanes: a multicenter phase II clinical trial. *J Clin Oncol.* 2010;28(15 suppl). Abstract 1013.
- 68 Osborne CK, Shou J, Massarweh S, Schiff R. Crosstalk between estrogen receptor and growth factor receptor pathways as a cause for endocrine therapy resistance in breast cancer. *Clin Cancer Res.* 2005;11(suppl):865s–870s.
- 69 Arpino G, Wiechmann L, Osborne CK, Schiff R. Crosstalk between the estrogen receptor and the HER tyrosine kinase receptor family: molecular mechanism and clinical implications for endocrine therapy resistance. *Endocr Rev.* 2008;29:217–233.
- 70 Johnston S, Pippin J Jr, Pivot X, et al. Lapatinib combined with letrozole versus letrozole and placebo as first-line therapy for postmenopausal hormone receptor–positive metastatic breast cancer. *J Clin Oncol.* 2009;27:5538–5546.
- 71 Kaufman B, Mackey JR, Clemens MR, et al. Trastuzumab plus anastrozole versus anastrozole alone for the treatment of postmenopausal women with human epidermal growth factor receptor 2–positive, hormone receptor–positive metastatic breast cancer: results from the randomized phase III TAnDEM study. *J Clin Oncol.* 2009; 27:5529–5537.

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.

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 [3,4]. 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 [4,5]. 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 [6,7].

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.

Trastuzumab emtansine

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 (derivate of maytansine)[7]. 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 [8–10]. The development of an effective ADC includes three major challenges [11]:

- 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 [12,13]. 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, $P=0.046$) [3]. 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 [15,16]; prevention of HER2 ectodomain cleavage [17]; inhibition of angiogenesis [18]; antibody-dependent cell-mediated cytotoxicity [19,20]; and induction of apoptosis [21].

Cytotoxic drug: derivate 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 [19–21]. However, they were inefficient in the clinical setting because of their inability to achieve therapeutic levels of the cytotoxic agent within the tumor cells [25]. 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 [26]. 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 [27–29].

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 [30–32].

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 [29]. 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 [30,33]. 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)[34,35].

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

Structure and mechanism of action of trastuzumab emtansine

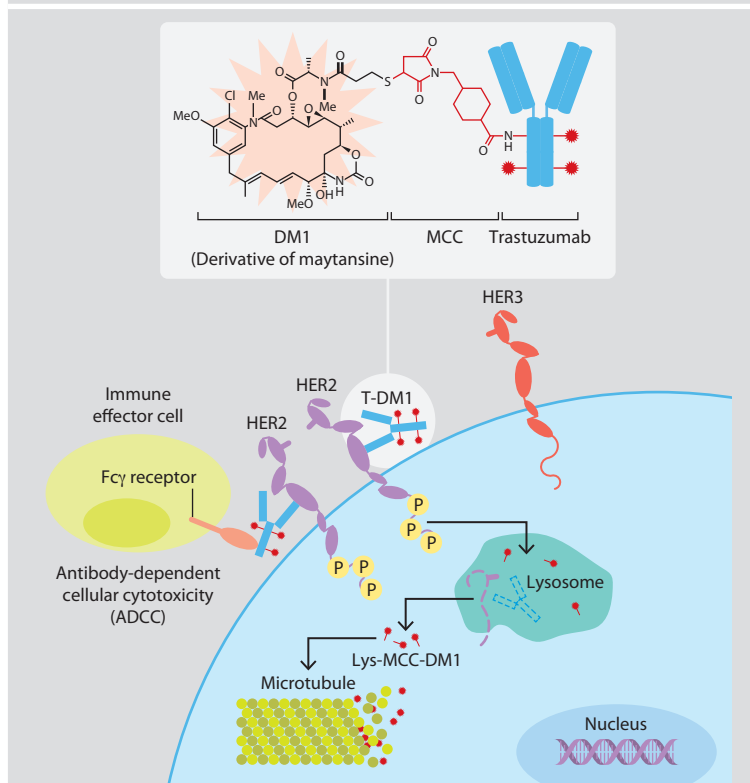


Figure 5.1 Structure and mechanism of action of trastuzumab emtansine. HER2, human epidermal growth factor receptor 2; T-DM1, trastuzumab emtansine.

through intracellular proteolytic degradation, in turn releasing the active maytansinoid metabolite (lysine-N-MCC-DM1) [35].

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 [36]. 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 thrombocytopenia, elevated transaminases, fatigue, nausea, and anemia [36]. 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 metastatic breast cancer. The first trial was a single-arm study of 112 patients who had progressed on prior trastuzumab and chemotherapy [37]. 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 [37]. 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 was no dose-limiting cardiotoxicity [37].

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 metastatic breast cancer who had previously been treated with anthracycline, trastuzumab, taxane, capecitabine, and lapatinib therapy [38].

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%)[38].

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 [6]. 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

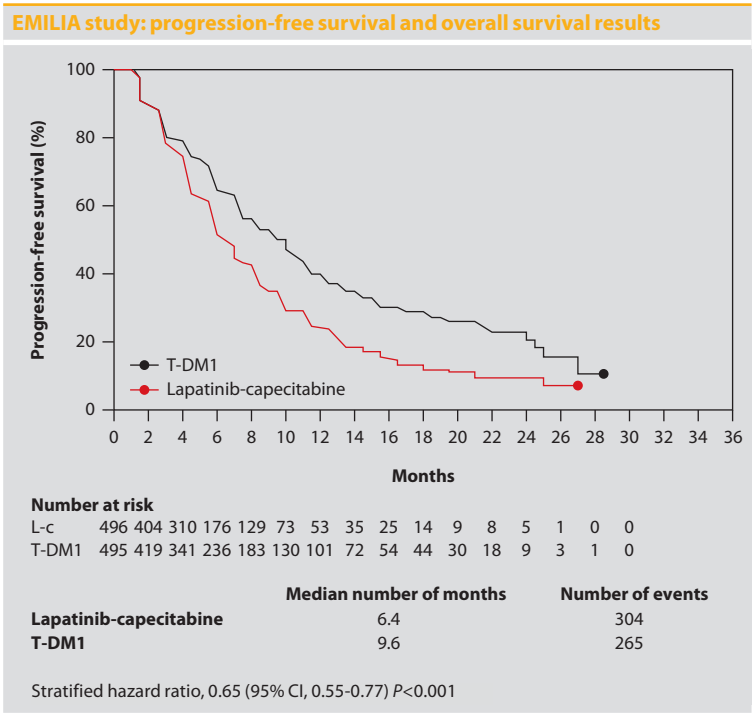


Figure 5.2A EMILIA study. (A) Progression-free survival.

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; $P<0.001$) (Figure 5.2) [6]. 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

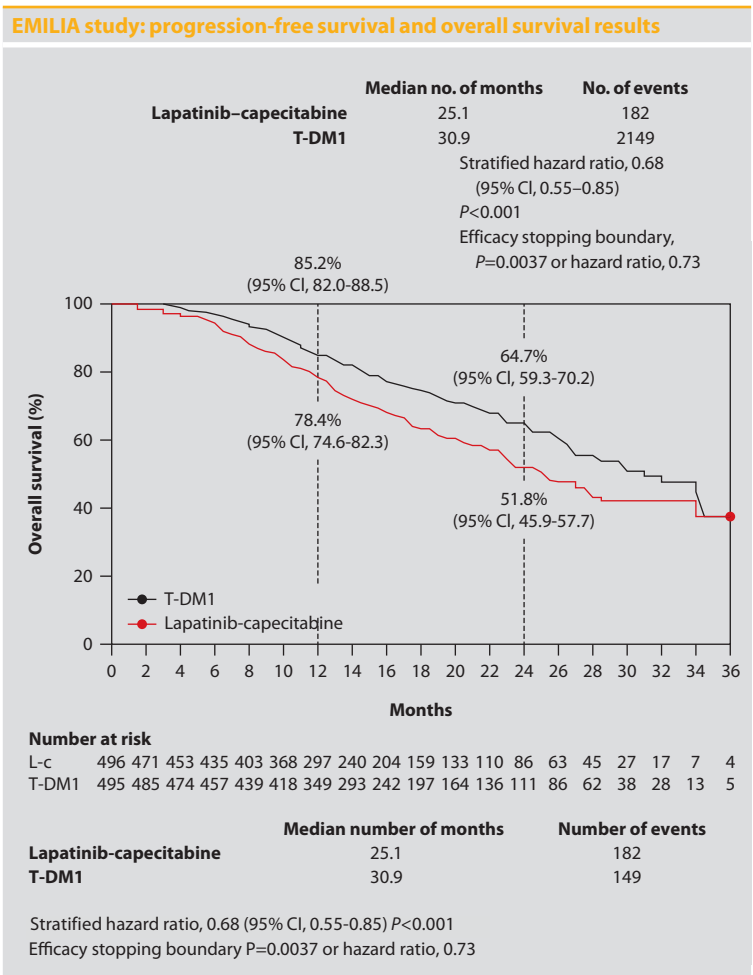


Figure 5.2B EMILIA study. (B) overall survival results for lapatinib + capecitabine versus T-DM1. CI, confidence interval; T-DM1, trastuzumab emtansine.

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 erythrodyesthesia than capecitabine + lapatinib [6].

The results of EMILIA study provide solid evidence for T-DM1 as the treatment of choice in the second-line setting for HER2-positive metastatic breast cancer and were used as the basis for T-DM1's approval [7]. MARIANNE (NCT01120184), another Phase III randomized trial is currently investigating T-DM1 in the first-line setting. MARIANNE will compare the efficacy of T-DM1 + pertuzumab, T-DM1 + placebo, and the combination of trastuzumab + a taxane. A third Phase III study, TH3RESA (NCT01419197), recently evaluated T-DM1 as a third-line or later therapy, with results expected in 2015. In this study, 606 patients with HER2-positive metastatic breast cancer who previously received trastuzumab, lapatinib, and a taxane, were randomly selected to receive either T-DM1 or physician's choice of therapy [40]. T-DM1 resulted in significant prolongation of PFS compared to treatment with physician's choice (median 6.2 months vs 3.3 months, respectively) and reduced the risk of disease progression or death by 47 percent (hazard ratio [HR]=0.528; $P<0.0001$). In the planned interim analysis, OS favored T-DM1, but the survival data are not yet mature [41].

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 [42,43] and can lead to apoptosis [36] in human tumor cells overexpressing HER2 [44].

ErbB receptors form hetero- or homodimers after ligand binding, causing autophosphorylation of specific tyrosine residues within the conserved catalytic kinase domains of these receptors [45,46]. 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 [47]. 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 [36]. 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) [47]. The most common drug-related AEs were diarrhea (42%) and rash (31%). No grade 4 toxicities were reported. Four patients with trastuzumab-resistant metastatic breast cancer had partial responses and 24 patients experienced stable disease [47].

The activity of lapatinib monotherapy in patients with advanced HER2-positive or metastatic breast cancer that progressed on a first- or second-line trastuzumab-containing regimen was evaluated in a multicenter Phase II study (n=78) [48]. 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 [48]. 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 [48].

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 metastatic breast cancer that had progressed after prior treatment with anthracyclines, taxanes, and trastuzumab [49]. Patients received lapatinib at 1250 mg/day continuously plus capecitabine 2000 mg/m² per day on Days 1–14 of a 21-day cycle or capecitabine 2500 mg/m² per day on Days 1–14 of a 21-day cycle. The primary end point was the time to tumor progression (TTP)[49]. 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 [49]. 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; $P<0.001$) [50]. 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; $P=0.210$ [51]. However, the study design did not allow for sufficient power to detect a survival benefit [51].

Investigations of 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 [52–55]. 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 [56]. 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 cross-over. 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 [56]. 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 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 for patients with HER2-positive breast cancer in development (see Chapter 6).

References

- 1 King CR, Kraus MH, Aaronson SA. Amplification of a novel v-erbB-related gene in a human mammary carcinoma. *Science*. 1985;229:974-976.
- 2 Nahta R, Esteva FJ. Trastuzumab: triumphs and tribulations. *Oncogene*. 2007;26:3637-3643.
- 3 Tykerb [prescribing information]. Research Triangle Park, NC: GlaxoSmithKline; 2013.
- 4 Baselga J, Cortés J, Kim S-B, et al; for the CLEOPATRA Study Group. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *N Engl J Med*. 2012;366:109-119.
- 5 Perjeta [prescribing information]. South San Francisco, CA: Genentech, Inc.; 2012.
- 6 Verma S, Miles D, Gianni L, et al; for the EMILIA Study Group. Trastuzumab emtansine for HER2-positive advanced breast cancer. *N Engl J Med*. 2012;367:1783-1791.
- 7 Kadcyla [prescribing information]. South San Francisco, CA: Genentech, Inc.; 2013.
- 8 Carter PJ. Potent antibody therapeutics by design. *Nat Rev Immunol*. 2006;6:343-357.
- 9 Easton DF, Pooley KA, Dunning AM, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature*. 2007;447:1087-1093.
- 10 Wu AM, Senter PD. Arming antibodies: prospects and challenges for immunoconjugates. *Nat Biotechnol*. 2005;23:1137-1146.
- 11 Smith I, Procter M, Gelber RD, et al; for the HERA study team. 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. *Lancet*. 2007;369:29-36.
- 12 Li J, Glencer A, Lugo HS. T-DM1: a novel and effective immunoconjugate for the treatment of HER2+ breast cancer. *The International Journal of Targeted Therapies in Cancer* website. www.targetedonc.com/publications/targeted-therapies-cancer/2012/november-2012/T-DM1-A-Novel-and-Effective-Immunoconjugate-for-the-Treatment-of-HER2-Breast-Cancer. Accessed November 19, 2013.

- 13 Romond EH, Perez EA, Bryant J, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med*. 2005;353:1673-1684.
- 14 Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med*. 2001;344:783-792.
- 15 Junttila TT, Akita RW, Parsons K, et al. Ligand-independent HER2/HER3/PI3K complex is disrupted by trastuzumab and is effectively inhibited by the PI3K inhibitor GDC-0941. *Cancer Cell*. 2009;15:429-440.
- 16 Albanell J, Codony J, Rovira A, Mellado B, Gascon P. Mechanism of action of anti-HER2 monoclonal antibodies: scientific update on trastuzumab and 2C4. In: Llombart-Bosch A, Felipe V, eds. *New Trends in Cancer for the 21st Century*. New York, NY: Springer Science+Business Media; 2003:253-268.
- 17 Molina MA, Codony-Servat J, Albanell J, Rojo F, Arribas J, Baselga J. Trastuzumab (Herceptin), a humanized anti-Her2 receptor monoclonal antibody, inhibits basal and activated Her2 ectodomain cleavage in breast cancer cells. *Cancer Res*. 2001;61:4744-4749.
- 18 Izumi Y, Xu L, di Tomaso E, Fukumura D, Jain RK. Tumour biology: Herceptin acts as an anti-angiogenic cocktail. *Nature*. 2002;416:279-280.
- 19 Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. *Nat Med*. 2000;6:443-446.
- 20 Cooley S, Burns LJ, Repka T, Miller JS. Natural killer cell cytotoxicity of breast cancer targets is enhanced by two distinct mechanisms of antibody-dependent cellular cytotoxicity against LFA-3 and HER2/neu. *Exp Hematol*. 1999;27:1533-1541.
- 21 Mohsin SK, Weiss HL, Gutierrez MC, et al. Neoadjuvant trastuzumab induces apoptosis in primary breast cancers. *J Clin Oncol*. 2005;23:2460-2468.
- 22 Dillman RO, Johnson DE, Shawler DL, Koziol JA. Superiority of an acid-labile daunorubicin-monoclonal antibody immunoconjugate compared to free drug. *Cancer Res*. 1988;48:6097-6102.
- 23 Johnson DA, Laguzza BC. Antitumor xenograft activity with a conjugate of a *Vinca* derivative and the squamous carcinoma-reactive monoclonal antibody PF1/D. *Cancer Res*. 1987;47:3118-3122.
- 24 Shen W-C, Ballou B, Ryser HJ-P, Hakala TR. Targeting, internalization, and cytotoxicity of methotrexate-monoclonal anti-stage-specific embryonic antigen-1 antibody conjugates in cultured F-9 teratocarcinoma cells. *Cancer Res*. 1986;46:3912-3916.
- 25 Allen TM. Ligand-targeted therapeutics in anticancer therapy. *Nat Rev Cancer*. 2002;2:750-763.
- 26 Cassady JM, Chan KK, Floss HG, Leistner E. Recent developments in the maytansinoid antitumor agents. *Chem Pharm Bull (Tokyo)*. 2004;52:1-26.
- 27 Blum RH, Kahlert T. Maytansine: a phase I study of an ansa macrolide with antitumor activity. *Cancer Treat Rep*. 1978;62:435-438.
- 28 Cabanillas F, Rodriguez V, Hall SW, Burgess MA, Bodey GP, Freireich EJ. Phase I study of maytansine using a 3-day schedule. *Cancer Treat Rep*. 1978;62:425-428.
- 29 Chabner BA, Levine AS, Johnson BL, Young RC. Initial clinical trials of maytansine, an antitumor plant alkaloid. *Cancer Treat Rep*. 1978;62:429-433.
- 30 Chari RVJ. Targeted delivery of chemotherapeutics: tumor-activated prodrug therapy. *Adv Drug Deliv Rev*. 1998;31:89-104.
- 31 Chari RVJ, Martell BA, Gross JL, et al. Immunoconjugates containing novel maytansinoids: promising anticancer drugs. *Cancer Res*. 1992;52:127-131.
- 32 Burris HA III. Trastuzumab emtansine (T-DM1): hitching a ride on a therapeutic antibody. ASCO 2012 Educational Book. ASCO website. www.meetinglibrary.asco.org/sites/meetinglibrary.asco.org/files/EducationalBook/PDFFiles/2012/zds00112000159.pdf. Accessed November 19, 2013.

- 33 Singh R, Erickson HK. Antibody–cytotoxic agent conjugates: preparation and execution. In: Dimitrov AS, ed. *Therapeutic Antibodies: Methods and Protocols*. New York, NY: Humana Press (Springer Science+Business Media); 2009:445–467.
- 34 Erickson HK, Park PU, Widdison WC, et al. Antibody–maytansinoid conjugates are activated in targeted cancer cells by lysosomal degradation and linker-dependent intracellular processing. *Cancer Res*. 2006;66:4426–4433.
- 35 Lewis Phillips GD, Li G, Dugger DL, et al. Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody–cytotoxic drug conjugate. *Cancer Res*. 2008;68:9280–9290.
- 36 Krop IE, Beeram P, Modi S, et al. Phase I study of trastuzumab-DM1, an HER2 antibody–drug conjugate, given every 3 weeks to patients with HER2-positive metastatic breast cancer. *J Clin Oncol*. 2010;28:2698–2704.
- 37 Burris HA III, Rugo HS, Vukelja SJ, et al. Phase II study of the antibody drug conjugate trastuzumab-DM1 for the treatment of human epidermal growth factor receptor 2 (HER2)-positive breast cancer after prior HER2-directed therapy. *J Clin Oncol*. 2011;29:398–405.
- 38 Krop IE, LoRusso P, Miller KD, et al. A phase II study of trastuzumab emtansine in patients with human epidermal growth factor receptor 2–positive metastatic breast cancer who were previously treated with trastuzumab, lapatinib, an anthracycline, a taxane, and capecitabine. *J Clin Oncol*. 2012;30:3234–3241.
- 39 A study of trastuzumab emtansine (T-DM1) plus pertuzumab/pertuzumab placebo versus trastuzumab (Herceptin) plus a taxane in patients with metastatic breast cancer (MARIANNE). ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/NCT01120184. Accessed November 19, 2013.
- 40 A study of trastuzumab emtansine in comparison with treatment of physician's choice in patients with HER2-positive breast cancer who have received at least two prior regimens of HER2-directed therapy (TH3RESA). ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/NCT01419197. Accessed November 19, 2013.
- 41 Wildiers H, Kim SB, Gonzalez-Martin A, et al. Late Breaking Abstract: T-DM1 for HER2-positive metastatic breast cancer (MBC): Primary results from TH3RESA, a phase 3 study of T-DM1 vs treatment of physician's choice. Presented at: European Cancer Congress (ECCO); September 27– Oct 1, 2013; Amsterdam, Netherlands. Abstract 15.
- 42 Spector NL, Xia W, Burris HL III, et al. Study of the biologic effects of lapatinib, a reversible inhibitor of ErbB1 and ErbB2 tyrosine kinases, on tumor growth and survival pathways in patients with advanced malignancies. *J Clin Oncol*. 2005;23:2502–2512.
- 43 Rusnak DW, Lackey K, Affleck K, et al. The effects of the novel, reversible epidermal growth factor receptor/ErbB-2 tyrosine kinase inhibitor, GW2016, on the growth of human normal and tumor-derived cell lines *in vitro* and *in vivo*. *Mol Cancer Ther*. 2001;1:85–94.
- 44 Xia W, Mullin RJ, Keith BR, et al. Anti-tumor activity of GW572016: a dual tyrosine kinase inhibitor blocks EGF activation of EGFR/erbB2 and downstream Erk1/2 and AKT pathways. *Oncogene*. 2002;21:6255–6263.
- 45 Konecny GE, Pegram MD, Venkatesan N, et al. Activity of the dual kinase inhibitor lapatinib (GW572016) against HER-2–overexpressing and trastuzumab-treated breast cancer cells. *Cancer Res*. 2006;66:1630–1639.
- 46 Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol*. 2001;2:127–137.
- 47 Olayioye MA, Graus-Porta D, Beerli RR, Rohrer J, Gay B, Hynes NE. ErbB-1 and ErbB-2 acquire distinct signaling properties dependent on their dimerization partner. *Mol Cell Biol*. 1998;18:5042–5051.
- 48 Burris HA III, Hurlwitz HJ, Dees EC, et al. Phase I safety, pharmacokinetics, and clinical activity study of lapatinib (GW572016), a reversible dual inhibitor of epidermal growth factor receptor tyrosine kinases, in heavily pretreated patients with metastatic carcinomas. *J Clin Oncol*. 2005;23:5305–5313.

- 49 Blackwell KL, Pegram MD, Tan-Chiu E, et al. Single-agent lapatinib for HER2-overexpressing advanced or metastatic breast cancer that progressed on first- or second-line trastuzumab-containing regimens. *Ann Oncol*. 2009;20:1026-1031.
- 50 Geyer CE, Forster J, Lindquist D, et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *N Engl J Med*. 2006;355:2733-2743.
- 51 Cameron D, Casey M, Press M, et al. A phase III randomized comparison of lapatinib plus capecitabine versus capecitabine alone in women with advanced breast cancer that has progressed on trastuzumab: updated efficacy and biomarker analyses. *Breast Cancer Res Treat*. 2008;112:533-543.
- 52 Cameron D, Casey M, Oliva C, Newstat B, Imwalle B, Geyer CE. Lapatinib plus capecitabine in women with HER-2-positive advanced breast cancer: final survival analysis of a phase III randomized trial. *Oncologist*. 2010;15:924-934.
- 53 Zardavas D, Cameron D, Krop I, Piccart M. Beyond trastuzumab and lapatinib: new options for HER2-positive breast cancer. ASCO 2013 Educational Book. ASCO website. meetinglibrary.asco.org/sites/meetinglibrary.asco.org/files/Educational%20Book/PDF%20Files/2013/EdBookAM201333e2.pdf. Accessed November 19, 2013.
- 54 Scaltriti M, Verma C, Guzman M, et al. Lapatinib, a HER2 tyrosine kinase inhibitor, induces stabilization and accumulation of HER2 and potentiates trastuzumab-dependent cell cytotoxicity. *Oncogene*. 2009;28:803-814.
- 55 Xia W, Gerard CM, Liu L, Baudson NM, Ory TL, Spector NL. Combining lapatinib (GW572016), a small molecule inhibitor of ErbB1 and ErbB2 tyrosine kinases, with therapeutic anti-ErbB2 antibodies enhances apoptosis of ErbB2-overexpressing breast cancer cells. *Oncogene*. 2005;24:6213-6221.
- 56 Blackwell KL, Burstein HJ, Storniolo AM, et al. Overall survival benefit with lapatinib in combination with trastuzumab for patients with human epidermal growth factor receptor 2-positive metastatic breast cancer: final results from the EGF104900 study. *J Clin Oncol*. 2012;30:2585-2592.

Emerging targeted agents for HER2-positive breast cancer

Dimitrios Zardavas , Martine Piccart

Challenges and unmet needs

Advances in understanding the biology of human epidermal growth factor receptor (HER)2-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 [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 emtasine (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 [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 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.

Mechanisms of trastuzumab resistance in HER2-positive breast cancer		
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 end point 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]. Currently, there are several ongoing trials evaluating neratinib in HER2-positive breast cancer (Table 6.2) [16–18]. Notably, a Phase III study investigating neratinib + capecitabine compared with lapatinib + capecitabine in patients with HER2-positive metastatic breast cancer who have received two or more prior HER2-targeted regimens has recently been initiated [18].

Selected ongoing clinical trials with neratinib					
Trial	Phase	Description	Patients (n)	Primary end point(s)	Secondary end point(s)
NCT01494662 [16]	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) [17]	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) [18]	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.

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) [19]. 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 [19].

In a randomized, open-label, neoadjuvant Phase II study, afatinib was compared with trastuzumab and lapatinib 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 [20]. There are several ongoing trials evaluating afatinib in HER2-positive breast cancer

Selected ongoing clinical trials with afatinib					
Trial	Phase	Description	Patients (n)	Primary end point(s)	Secondary end point(s)
LUX-Breast 1 (NCT01125566) [21]	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) [22]	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) [23]	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) [24]	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.

(Table 6.3) [21–24]. Unfortunately, a Phase III randomized study (LUX-Breast 1, NCT01125566) comparing afatinib plus vinorelbine to trastuzumab plus vinorelbine as first- or second-line treatment in the metastatic setting was preliminary terminated.

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 [25], primarily:

- overexpression of PI3K-activating receptor 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 [26]. 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 [26].

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) [27]. 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%) [27]. A different Phase Ib study evaluated the combination of everolimus, vinorelbine, and trastuzumab in 50 heavily pretreated patients with HER2-positive metastatic breast cancer [28]. 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, 28–44.9 weeks). In terms of toxicity, neutropenia (92%) and stomatitis (70%) of any grade were the two most frequently reported AEs [28]. 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 [29].

BOLERO-3 (NCT01007942) 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 or placebo in combination with trastuzumab and vinorelbine (Figure 6.1) [30]. Final PFS results showed that the addition of everolimus to trastuzumab and vinorelbine reduced the risk of disease progression by 22% (hazard ratio=0.78 [95% confidence interval (CI): 0.65 to 0.95]; $P<0.01$), with median time to progression being 7.0 months in the everolimus arm and 5.8 months in the placebo arm. Findings about OS were not yet mature at the time of the presentation. The results of an international collaborative Phase III trials assessing everolimus in the setting of HER2-positive metastatic breast cancer are being awaited with great interest. BOLERO-1 (NCT00876395) is evaluating the triple combination of everolimus, trastuzumab, and paclitaxel as first-line treatment (Figure 6.1) [31].

Other PI3K/Akt/mTOR-blocking agents

mTOR inhibitors block the mTORC1 complex of the PI3K/Akt/mTOR signaling pathway, while the mTORC2 complex remains uninhibited [32]. 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:

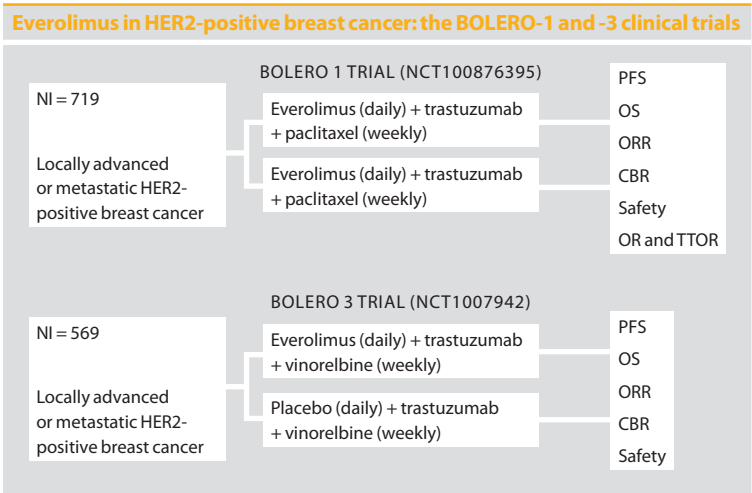


Figure 6.1 Everolimus in HER2-positive breast cancer: the BOLERO-1 and -3 clinical trials. CBR, clinical benefit rate; OS, overall survival; ORR, overall response rate; PFS, progression-free survival; PRO, patient-reported outcome; TTOR, time to objective response.

- dual PI3K/mTOR inhibitors;
- pan-PI3K inhibitors;
- isoform-selective PI3K inhibitors (p110 α -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) [30,31,33–39]. 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 [40]. 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 [41].

Selected ongoing clinical trials with everolimus and other PI3K/Akt/mTOR blocking agents					
Trial	Phase	Description	Patients (n)	Primary end point(s)	Secondary end point(s)
Everolimus					
BOLERO-1 (NCT00876395) [30]	III	Efficacy of everolimus in combination with trastuzumab + paclitaxel vs placebo in combination with the same agents	Trastuzumab-naïve (719)	PFS	OS, ORR, CBR
BOLERO-3 (NCT01007942) [31]	III	Efficacy of everolimus in combination with trastuzumab + vinorelbine vs placebo in combination with the same agents	Trastuzumab-pretreated (569)	PFS	OS, RR, CBR, safety, HRQoL, PK
NCT01305941 [33]	II	Efficacy and tolerability of everolimus in combination with vinorelbine + trastuzumab	Progressive HER2-positive breast cancer and brain metastases (35)	Intracranial ORR	Extracranial ORR, safety, TTP, PFS, OS, HRQoL
Other PI3K/Akt/mTOR-blocking agents					
NeoPHOEBE (NCT01816594) [34]	II	Efficacy of buparsilib (BKM120) or placebo in combination with trastuzumab + paclitaxel	Neoadjuvant (220)	pCR	OCRR, safety, rate of BCS, number of patients with node-negative disease at definitive surgery
PIKHER2 (NCT01589861) [35]	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 [36]	I/II	Dose escalation of XL147 in combination with trastuzumab or trastuzumab + paclitaxel	Trastuzumab-pretreated (42)	MTD, objective tumor response	PFS, PK, PD

Table 6.4 Selected ongoing clinical trials with everolimus and other PI3K/Akt/mTOR blocking agents (continues overleaf).

Selected ongoing clinical trials with everolimus and PI3K/Akt/mTOR blocking agents (continued)					
Trial	Phase	Description	Patients (n)	Primary end point(s)	Secondary end point(s)
Other PI3K/Akt/mTOR-blocking agents (contd)					
NCT01132664 [37]	Ib/II	Safety and efficacy of buparsilib in combination with trastuzumab	Trastuzumab-pretreated (88)	AEs, DLT	ORR
NCT01285466 [38]	Ib	Dose escalation of BEZ235 and buparsilib in combination with paclitaxel or paclitaxel + trastuzumab	Metastatic or locally advanced HER2-positive breast cancer (110)	DLTs	AEs, ORR, PK, impact of treatment on biomarkers of PI3K pathway
NCT00928330 [39]	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 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.

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 [42]. 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 [43].

Other signaling pathways

HER3 inhibition

HER3, the third member of the ErbB type I transmembrane receptor tyrosine kinases, 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 [44]. 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 [45]. 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 [46].

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 [47]. Based on this evidence, early-phase clinical trials are currently ongoing with anti-HER3 monoclonal antibodies (eg, U3-1287/AMG 888) (Table 6.5) [48–51].

Src inhibition

The Src family of tyrosine kinases fuels malignant progression through induction of cellular proliferation, metastatic dissemination, and angiogenesis [52,53]. Src has been shown to be an important mediator of intracellular transduction of HER2 signaling [54]. 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

Selected ongoing clinical trials with other emerging targeted agents in HER2-positive breast cancer					
Trial	Phase	Description	Patients (n)	Primary end point(s)	Secondary end point(s)
HER3-blocking agents					
NCT01512199 [48]	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 [49]	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 [50]	II	Safety and efficacy of capecitabine and lapatinib ± cixutumumab	Trastuzumab-pretreated (154)	PFS	OS, TTTF, CTR, DR, AEs
Met-blocking agents					
NCT01138384 [51]	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.

lines [55]. Early-phase clinical trials assessing the efficacy of anti-Src small-molecule inhibitors (eg, dasatinib) are currently recruiting patients in the setting of HER2-positive breast cancer (Table 6.5).

Insulin growth factor inhibition

The insulin growth factor (IGF) signaling pathway represents an intracellular transduction system commonly deregulated in breast cancer [56], 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 [57].

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 (IGF-1R) and HER3 expression by short-hairpin RNA [9]. An interesting finding is that IGF-mediated trastuzumab resistance is associated with a decrease of p27Kip1 protein levels through heightened ubiquitination involving the PI3K pathway [58].

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%; $P=0.001$) [59]. 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 [60]. 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 [61]. 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) [62–64]. Moreover, adaptive immune responses involving CD8+ cytotoxic lymphocytes and myeloid differentiation have been implicated in trastuzumab-mediated antitumor activity [65,66].

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 [67]. 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 [68].

Another promising strategy to boost immunologic response to HER2 blockade is the co-administration of agents that impede the programmed cell 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 [69,70]. 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 [66].

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 [71], with multiple HER2-targeting vaccines under clinical development (Table 6.6) [72–75]. 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 [76]. A Phase I study also assessed a HER2 multi-peptide 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 [77]. Whether such strategies will result in objective tumor responses in the metastatic setting is yet to be documented.

Selected ongoing clinical trials with HER2 vaccines in HER2-positive breast cancer					
Trial	Phase	Description	Patients (n)	Primary end point(s)	Secondary end point(s)
NCT01355393 [72]	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 [73]	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 [74]	I	Safety and immunogenicity of a multi-epitope HER2 peptide vaccine	Optimally treated, Stage II-III (24)	Safety, IR	DFS
NCT01526473 [75]	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 [78], 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 [79]. 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 [80]. 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 ($P < 0.001$) [81] and the second a trial of 107 patients ($P < 0.01$) [82].

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 [83]. 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%) [84].

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 [85]. 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 [86]. 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%) [87]. 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 [88]. The study did not meet its primary end point, 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 [88]. 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 [88]. 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.

References

- 1 Arteaga CL, Sliwkowski MX, Osborne CK, Perez EA, Puglisi F, Gianni L. Treatment of HER2-positive breast cancer: current status and future perspectives. *Nat Rev Clin Oncol*. 2012;9:16-32.
- 2 Tykerb [prescribing information]. Research Triangle Park, NC: GlaxoSmithKline; 2013.
- 3 Perjeta [prescribing information]. South San Francisco, CA: Genentech, Inc.; 2012.
- 4 Kadcyla [prescribing information]. South San Francisco, CA: Genentech, Inc.; 2013.
- 5 Stern HM. Improving treatment of HER2-positive cancers: opportunities and challenges. *Sci Transl Med*. 2012;4:127rv2.
- 6 Leyland-Jones B, Smith BR. Serum HER2 testing in patients with HER2-positive breast cancer: the death knell tolls. *Lancet Oncol*. 2011;12:286-295.
- 7 Molina MA, Codony-Servat J, Albanell J, Rojo F, Arribas J, Baselga J. Trastuzumab (Herceptin), a humanized anti-HER2 receptor monoclonal antibody, inhibits basal and activated HER2 ectodomain cleavage in breast cancer cells. *Cancer Res*. 2001;61:4744-4749.
- 8 Fiszman GL, Jasnis MA. Molecular mechanisms of trastuzumab resistance in HER2 overexpressing breast cancer. *Int J Breast Cancer*. 2011;2011:352182.
- 9 Huang X, Gao L, Wang S, et al. Heterotrimerization of the growth factor receptors erbB2, erbB3 and insulin-like growth factor-I receptor in breast cancer cells resistant to Herceptin. *Cancer Res*. 2010;70:1204-1214.
- 10 Shattuck DL, Miller JK, Carraway KL III, Sweeney C. Met receptor contributes to trastuzumab resistance of Her2-overexpressing breast cancer cells. *Cancer Res*. 2008;68:1471-1477.
- 11 Zhang S, Huang W-C, Li P, et al. Combating trastuzumab resistance by targeting SRC, a common node downstream of multiple resistance pathways. *Nat Med*. 2011;17:461-469.
- 12 Burstein HJ, Sun Y, Dirix LY, et al. Neratinib, an irreversible ErbB receptor tyrosine kinase inhibitor, in patients with advanced ErbB2-positive breast cancer. *J Clin Oncol*. 2010;28:1301-1307.
- 13 Swaby R, Blackwell KL, Jiang Z, et al. Neratinib in combination with trastuzumab for the treatment of advanced breast cancer: a phase I/II study. *J Clin Oncol*. 2009;27(suppl). Abstract 1004.
- 14 Jankowitz RC, Abraham J, Tan AR, et al. A phase I dose-escalation study evaluating weekly paclitaxel with neratinib and trastuzumab in women with metastatic HER2-positive breast cancer. NSABP FB-8. *J Clin Oncol*. 2012;30(suppl). Abstract 611.
- 15 Martin M, Bonnetterre J, Geyer CE Jr, et al. A phase two randomised trial of neratinib monotherapy vs lapatinib plus capecitabine combination therapy in patients with HER2+ advanced breast cancer. *Eur J Cancer*. 2013; [Epub ahead of print].
- 16 HKI-272 for HER2-positive breast cancer and brain metastases. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01494662. Accessed August 26, 2013.
- 17 Study evaluating neratinib plus paclitaxel vs trastuzumab plus paclitaxel in ErbB-2 positive advanced breast cancer (NEFERTT). ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT00915018. Accessed November 19, 2013.

- 18 A study of neratinib plus capecitabine versus lapatinib plus capecitabine in patients with HER2+ metastatic breast cancer who have received two or more prior HER2 directed regimens in the metastatic setting (NALA). ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/NCT01808573. Accessed November 19, 2013.
- 19 Lin NU, Winer EP, Wheatley D, et al. A phase II study of afatinib (BIBW 2992), an irreversible ErbB family blocker, in patients with HER2-positive metastatic breast cancer progressing after trastuzumab. *Breast Cancer Res Treat*. 2012;133:1057-1065.
- 20 Rimawi MF, Aleixo SB, Rozas AA, et al. A neoadjuvant, randomized, open-label phase II trial of afatinib (A) versus trastuzumab (T) versus lapatinib (L) in patients (pts) with locally advanced HER2-positive breast cancer (BC). *J Clin Oncol*. 2012;30(suppl). Abstract 606.
- 21 LUX-Breast 1: BIBW 2992 (afatinib) in HER2-positive metastatic breast cancer patients after one prior Herceptin treatment. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01125566. Accessed November 19, 2013.
- 22 LUX-Breast 2: afatinib in HER2 (human epidermal growth factor receptor)-treatment failures. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01271725. Accessed November 19, 2013.
- 23 LUX-Breast 3: afatinib alone or in combination with vinorelbine in patients with human epidermal growth factor receptor 2 (HER2) positive breast cancer suffering from brain metastases. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01441596. Accessed November 19, 2013.
- 24 Dual blockage with afatinib and trastuzumab as neoadjuvant treatment for patients with locally advanced or operable breast cancer receiving taxane-anthracycline containing chemotherapy (DAFNE). ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01594177. Accessed November 19, 2013.
- 25 Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov*. 2009;8:627-644.
- 26 Berns K, Horlings HM, Hennessy BT, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell*. 2007;12:395-402.
- 27 Andre F, Campone M, O'Regan R, et al. Phase I study of everolimus plus weekly paclitaxel and trastuzumab in patients with metastatic breast cancer pretreated with trastuzumab. *J Clin Oncol*. 2010;28:5110-5115.
- 28 Jerusalem G, Fasolo A, Dieras V, et al. Phase I trial of oral mTOR inhibitor everolimus in combination with trastuzumab and vinorelbine in pre-treated patients with HER2-overexpressing metastatic breast cancer. *Breast Cancer Res Treat*. 2011;125:447-455.
- 29 Morrow PK, Wulf GM, Ensor J, et al. Phase I/II study of trastuzumab in combination with everolimus (RAD001) in patients with HER2-overexpressing metastatic breast cancer who progressed on trastuzumab-based therapy. *J Clin Oncol*. 2011;29:3126-3132.
- 30 O'Regan R, Ozguroglu M, Andre F, et al. Phase III, randomized, double-blind, placebo-controlled multicenter trial of daily everolimus with weekly trastuzumab and vinorelbine in trastuzumab-resistant, advanced breast cancer (BOLERO-3). *J Clin Oncol*. 2013;31(suppl). Abstract 505.
- 31 Hurvitz SA, Andre F, Burris HA, et al. BOLERO-1: a randomized, phase III, double-blind, placebo-controlled multicenter trial of everolimus in combination with trastuzumab and paclitaxel as first-line therapy in women with HER2-positive (HER2+), locally advanced or metastatic breast cancer (BC). *J Clin Oncol*. 2012;30(suppl). Abstract TPS648.
- 32 Choo AY, Blenis J. Not all substrates are treated equally: implications for mTOR, rapamycin-resistance and cancer therapy. *Cell Cycle*. 2009;8:567-572.
- 33 A study of everolimus, trastuzumab and vinorelbine in HER2-positive breast cancer brain metastases. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01305941. Accessed November 19, 2013.

- 34 NeoPHOEBE: neoadjuvant trastuzumab + BKM120 in combination with weekly paclitaxel in HER2-positive primary breast cancer. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01816594. Accessed November 19, 2013.
- 35 Safety and efficacy of BKM120 and lapatinib in HER2+/PI3K-activated, trastuzumab-resistant advanced breast cancer (PIKHER2). ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01589861. Accessed November 19, 2013.
- 36 Study of XL147 (SAR245408) in combination with trastuzumab or paclitaxel and trastuzumab in subjects with metastatic breast cancer who have progressed on a previous trastuzumab-based regimen. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01042925. Accessed November 19, 2013.
- 37 Safety and efficacy of BKM120 in combination with trastuzumab in patients with relapsing HER2 overexpressing breast cancer who have previously failed trastuzumab. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01132664. Accessed November 19, 2013.
- 38 A trial of oral BEZ235 and BKM120 in combination with paclitaxel with or without trastuzumab. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01285466. Accessed November 19, 2013.
- 39 Trastuzumab and trastuzumab-MCC-DM1 administered intravenously and GDC-0941 administered orally to patients with HER2-positive metastatic breast cancer who have progressed on previous trastuzumab-based therapy. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT00928330. Accessed November 19, 2013.
- 40 Junttila TT, Akita RW, Parsons K, et al. Ligand-independent HER2/HER3/PI3K complex is disrupted by trastuzumab and is effectively inhibited by the PI3K inhibitor GDC-0941. *Cancer Cell*. 2009;15:429-440.
- 41 Eichhorn PJA, Gili M, Scaltriti M, et al. Phosphatidylinositol 3-kinase hyperactivation results in lapatinib resistance that is reversed by the mTOR/phosphatidylinositol 3-kinase inhibitor NVP-BEZ235. *Cancer Res*. 2008;68:9221-9230.
- 42 Han HS, Swanton C, Janjigian YY, et al. A phase I study of the AKT inhibitor (MK-2206) with concurrent trastuzumab and lapatinib in patients with HER2-positive solid tumors. *J Clin Oncol*. 2011;29(suppl). Abstract 3028.
- 43 Krop IE, Saura C, Ahnert JR, et al. A phase I/IB dose-escalation study of BEZ235 in combination with trastuzumab in patients with PI3-kinase or PTEN altered HER2+ metastatic breast cancer. *J Clin Oncol*. 2012;30(suppl). Abstract 508.
- 44 Schoeberl B, Pace EA, Fitzgerald JB, et al. Therapeutically targeting ErbB3: a key node in ligand-induced activation of the ErbB receptor-PI3K axis. *Sci Signal*. 2009;2:ra31.
- 45 Lee-Hoeflich ST, Crocker L, Yao E, et al. A central role for HER3 in HER2-amplified breast cancer: implications for targeted therapy. *Cancer Res*. 2008;68:5878-5887.
- 46 Choi B-K, Fan X, Deng H, Zhang N, An Z. ERBB3 (HER3) is a key sensor in the regulation of ERBB-mediated signaling in both low and high ERBB2 (HER2) expressing cancer cells. *Cancer Med*. 2012;1:28-38.
- 47 Garrett JT, Sutton CR, Kuba MG, Cook RS, Arteaga CL. Dual blockade of HER2 in HER2-overexpressing tumor cells does not completely eliminate HER3 function. *Clin Cancer Res*. 2013;19:610-619.
- 48 Phase 1b/2 study of U3-1287 in combination with trastuzumab plus paclitaxel in newly diagnosed metastatic breast cancer (MBC). ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01512199. Accessed November 19, 2013.
- 49 Dasatinib in combination with trastuzumab and paclitaxel in the first line treatment of Her2-positive metastatic breast cancer (MBC) patients. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01306942. Accessed November 19, 2013.
- 50 Capecitabine and lapatinib with or without cixutumumab in treating patients with previously treated HER2-positive stage IIIB, stage IIIC, or stage IV breast cancer. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT00684983. Accessed November 19, 2013.

- 51 Study of foretinib in combination with lapatinib in patients with metastatic breast cancer. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01138384. Accessed November 19, 2013.
- 52 Parsons SJ, Parsons JT. Src family kinases, key regulators of signal transduction. *Oncogene*. 2004;23:7906-7909.
- 53 Laird AD, Li G, Moss KG, et al. Src family kinase activity is required for signal transducer and activation of transcription 3 and focal adhesion kinase phosphorylation and vascular endothelial growth factor signaling *in vivo* and for anchorage-dependent and -independent growth of human tumor cells. *Mol Cancer Ther*. 2003;2:461-469.
- 54 Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol*. 2001;2:127-137.
- 55 Boyer AP, Collier TS, Vidavsky I, Bose R. Quantitative proteomics with siRNA screening identifies novel mechanisms of trastuzumab resistance in HER2 amplified breast cancers. *Mol Cell Proteomics*. 2013;12:180-193.
- 56 Foekens JA, Portengen H, van Putten WLJ, et al. Prognostic value of receptors for insulin-like growth factor 1, somatostatin, and epidermal growth factor in human breast cancer. *Cancer Res*. 1989;49:7002-7009.
- 57 Nahta R, Yuan LXH, Zhang B, Kobayashi R, Esteva FJ. Insulin-like growth factor-I receptor/human epidermal growth factor receptor 2 heterodimerization contributes to trastuzumab resistance of breast cancer cells. *Cancer Res*. 2005;65:11118-11128.
- 58 Lu Y, Zi X, Pollak M. Molecular mechanisms underlying IGF-I-induced attenuation of the growth-inhibitory activity of trastuzumab (Herceptin) on SKBR3 breast cancer cells. *Int J Cancer*. 2004;108:334-341.
- 59 Harris LN, You F, Schnitt SJ, et al. Predictors of resistance to preoperative trastuzumab and vinorelbine for HER2-positive early breast cancer. *Clin Cancer Res*. 2007;13:1198-1207.
- 60 Edakuni G, Sasatomi E, Satoh T, Tokunaga O, Miyazaki K. Expression of the hepatocyte growth factor/c-Met pathway is increased at the cancer front in breast carcinoma. *Pathol Int*. 2001;51:172-178.
- 61 Liu L, Shi H, Liu Y, et al. Synergistic effects of foretinib with HER-targeted agents in MET and HER1- or HER2-coactivated tumor cells. *Mol Cancer Ther*. 2011;10:518-530.
- 62 Hudis CA. Trastuzumab—mechanism of action and use in clinical practice. *N Engl J Med*. 2007;357:39-51.
- 63 Varchetta S, Gibelli N, Oliviero B, et al. Elements related to heterogeneity of antibody-dependent cell cytotoxicity in patients under trastuzumab therapy for primary operable breast cancer overexpressing Her2. *Cancer Res*. 2007;67:11991-11999.
- 64 Musolino A, Naldi N, Bortesi B, et al. Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/*neu*-positive metastatic breast cancer. *J Clin Oncol*. 2008;26:1789-1796.
- 65 Park S, Jiang Z, Mortenson ED, et al. The therapeutic effect of anti-HER2/*neu* antibody depends on both innate and adaptive immunity. *Cancer Cell*. 2010;18:160-170.
- 66 Stagg J, Loi S, Divisekera U, et al. Anti-ErbB-2 mAb therapy requires type I and II interferons and synergizes with anti-PD-1 or anti-CD137 mAb therapy. *Proc Natl Acad Sci U.S.A.* 2011;108:7142-7147.
- 67 Junttila TT, Parsons K, Olsson C, et al. Superior *in vivo* efficacy of afucosylated trastuzumab in the treatment of HER2-amplified breast cancer. *Cancer Res*. 2010;70:4481-4489.
- 68 Suzuki E, Niwa R, Saji S, et al. A nonfucosylated anti-HER2 antibody augments antibody-dependent cellular cytotoxicity in breast cancer patients. *Clin Cancer Res*. 2007;13:1875-1882.
- 69 Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med*. 2002;8:793-800.
- 70 Zou W, Chen L. Inhibitory B7-family molecules in the tumour microenvironment. *Nat Rev Immunol*. 2008;8:467-477.

- 71 Cheever MA, Allison JP, Ferris AS, et al. The prioritization of cancer antigens: a National Cancer Institute pilot project for the acceleration of translational research. *Clin Cancer Res*. 2009;15:5232-5237.
- 72 Vaccine therapy in combination with rintatolimod and/or sargramostim in patients with stage II-IV HER2-positive breast cancer. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01355393. Accessed November 19, 2013.
- 73 Vaccine therapy in treating patients with stage IV breast cancer. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT00791037. Accessed November 19, 2013.
- 74 Vaccine therapy in treating patients with previously treated stage II-III HER2-positive breast cancer. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01632332. Accessed November 19, 2013.
- 75 A phase I study to evaluate the antitumor activity and safety of AVX901. ClinicalTrials.gov website. www.clinicaltrials.gov/ct2/show/study/NCT01526473. Accessed November 19, 2013.
- 76 Peoples GE, Holmes JP, Hueman MT, et al. Combined clinical trial results of a HER2/*neu* (E75) vaccine for the prevention of recurrence in high-risk breast cancer patients: U.S. Military Cancer Institute Clinical Trials Group Study I-01 and I-02. *Clin Cancer Res*. 2008;14:797-803.
- 77 Wiedermann U, Wiltschke C, Jasinska J, et al. A virosomal formulated Her-2/*neu* multi-peptide vaccine induces Her-2/*neu*-specific immune responses in patients with metastatic breast cancer: a phase I study. *Breast Cancer Res Treat*. 2010;119:673-683.
- 78 Yen L, You X-L, Al Moustafa A-E, et al. Heregulin selectively upregulates vascular endothelial growth factor secretion in cancer cells and stimulates angiogenesis. *Oncogene*. 2000;19:3460-3469.
- 79 Loureiro RMB, Maharaj ASR, Dankort D, Muller WJ, D'Amore PA. ErbB2 overexpression in mammary cells upregulates VEGF through the core promoter. *Biochem Biophys Res Commun*. 2005;326:455-465.
- 80 Klos KS, Wyzomierski SL, Sun M, et al. ErbB2 increases vascular endothelial growth factor protein synthesis via activation of mammalian target of rapamycin/p70S6K leading to increased angiogenesis and spontaneous metastasis of human breast cancer cells. *Cancer Res*. 2006;66:2028-2037.
- 81 Konecny GE, Meng YG, Untch M, et al. Association between HER-2/*neu* and vascular endothelial growth factor expression predicts clinical outcome in primary breast cancer patients. *Clin Cancer Res*. 2004;10:1706-1716.
- 82 Yang W, Klos K, Yang Y, Smith TL, Shi D, Yu D. ErbB2 overexpression correlates with increased expression of vascular endothelial growth factors A, C, and D in human breast carcinoma. *Cancer*. 2002;94:2855-2861.
- 83 Yardley DA, Raefsky E, Castillo R, et al. Phase II study of neoadjuvant weekly nab-paclitaxel and carboplatin, with bevacizumab and trastuzumab, as treatment for women with locally advanced HER2+ breast cancer. *Clin Breast Cancer*. 2011;11:297-305.
- 84 Pierga J-Y, Petit T, Delozier T, et al. Neoadjuvant bevacizumab, trastuzumab, and chemotherapy for primary inflammatory HER2-positive breast cancer (BEVERLY-2): an open-label, single-arm phase 2 study. *Lancet Oncol*. 2012;13:375-384.
- 85 Rugo HS, Chien AJ, Franco SX, et al. A phase II study of lapatinib and bevacizumab as treatment for HER2-overexpressing metastatic breast cancer. *Breast Cancer Res Treat*. 2012;134:13-20.
- 86 Hurvitz S, Pegram M, Lin L, et al. Final results of a Phase II trial evaluating trastuzumab and bevacizumab as first-line treatment of HER2-amplified advanced breast cancer. *Cancer Res*. 2009;69(24 suppl). Abstract 6094.
- 87 Martin M, Makhson A, Gligorov J, et al. Phase II study of bevacizumab in combination with trastuzumab and capecitabine as first-line treatment for HER-2-positive locally recurrent or metastatic breast cancer. *Oncologist*. 2012;17:469-475.
- 88 Gianni L, Romieu GH, Lichinitser M, et al. AVEREL: a randomized Phase III trial evaluating bevacizumab in combination with docetaxel and trastuzumab as first-line therapy for HER2-positive locally recurrent/metastatic breast cancer. *J Clin Oncol*. 2013;31:1719-1725.