Cancer Treatment Reviews xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Cancer Treatment Reviews

journal homepage: www.elsevierhealth.com/journals/ctrv

Anti-Tumour Treatment

Molecular determinants of trastuzumab efficacy: What is their clinical relevance?

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ARTICLE INFO

Article history:
Received 27 February 2013
Accepted 28 February 2013
Available online xxxx

Keywords:
Biomarker
Breast cancer
HER2
Trastuzumab

ABSTRACT

Trastuzumab-containing therapy is a standard of care for human epidermal growth factor receptor-2 (HER2)-positive breast cancer. In pre-clinical models, a wide range of molecular mechanisms have been associated with reduced sensitivity to trastuzumab *in vitro*. These include expression of the truncated HER2 receptor fragment $p95^{HER2}$, activating mutation of the gene encoding the class 1A catalytic subunit of phosphatidylinositol 3-kinase (*PIK3CA*), loss of phosphatase and tensin homolog (PTEN), activation of other downstream signal transducers, prevention of cell cycle arrest, increased signaling through alternative (HER or non-HER) tyrosine kinase receptors, and resistance to antibody-dependent cellular cytotoxicity. However, the clinical significance of these mechanisms as determinants of trastuzumab efficacy *in vivo* has been unclear. Here, we review clinical studies of potential predictive biomarkers of trastuzumab efficacy in HER2-positive breast cancer and consider whether evaluation of such markers might inform patient selection for therapy. We find that clinical evidence relating to potential predictive biomarkers is mostly limited to small, retrospective studies, many of which have yielded conflicting findings. Some trends are evident in the retrospective data and in biomarker analyses from randomized clinical trials, particularly relating to activation of the phosphatidylinositol 3-kinase pathway, but none is sufficiently strong to form a basis for patient selection. This may be explained by the fact that multiple mechanisms of action determine the clinical efficacy of trastuzumab. In the absence of novel, validated biomarkers of efficacy, trastuzumab eligibility should continue to be based on evaluation of HER2 status according to standard methods.

Introduction

The advent of human epidermal growth factor receptor-2 (HER2)-targeted therapy has significantly improved the outlook for patients with HER2-positive breast cancer.[1] Two main classes of HER2 inhibitors are currently approved or in development: humanized monoclonal antibodies (mAbs) against the HER2 extracellular domain (ECD) and small-molecule tyrosine kinase inhibitors (TKIs) that compete with ATP for its intracellular binding site. Trastuzumab, a mAb that binds to subdomain IV of the HER2 ECD, is currently the only adjuvant treatment approved by the US Food and Drug Administration (FDA) specifically for patients with HER2-positive early breast cancer (EBC). Combined with chemotherapy, trastuzumab has been demonstrated to significantly extend both disease-free survival (DFS) and overall survival (OS) in pivotal clinical trials.[2–4] Trastuzumab is also widely used for treatment of HER2-positive metastatic breast cancer (MBC), either as monotherapy or combined with chemotherapy.[5,6] A second HER2-targeted mAb, pertuzumab, which binds to a different region of the HER2 ECD (subdomain II) compared with trastuzumab and possesses a distinct mechanism of action,[7,8] has recently been approved for use in combination with trastuzumab plus docetaxel in first-line MBC.[9] Currently, the only other FDA-approved anti-HER2 treatment is lapatinib, a TKI with demonstrated efficacy in HER2-positive MBC that has progressed after therapy including trastuzumab, anthracyclines and taxanes.[10]

Trastuzumab-containing therapy is now an established standard of care across all disease stages.[11] However, a proportion of patients do not respond to initial trastuzumab-containing therapy and those who do respond may subsequently relapse during treatment.[5,6,12] In MBC trials, overall response rates (ORRs) with single-agent trastuzumab are reported in the range of 15–26%[5,13]; however, substantially higher response rates have been achieved in combination with standard chemotherapy.[6,12] These observations have challenged researchers and clinicians alike towards an improved understanding of the fundamental mechanisms of trastuzumab action and molecular determinants of response. Although preclinical research has characterized a wide range of potential molecular mechanisms of trastuzumab resistance, their clinical relevance has been unclear. In this review, we examine

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http://dx.doi.org/10.1016/j.ctrv.2013.02.006

Please cite this article in press as: De P et al. Molecular determinants of trastuzumab efficacy: What is their clinical relevance? *Cancer Treat Rev* (2013), http://dx.doi.org/10.1016/j.ctrv.2013.02.006

the evidence linking specific molecular resistance mechanisms to the efficacy of trastuzumab in patients with HER2-positive breast cancer, with a specific focus on whether resistance-related biomarkers can be incorporated into patient eligibility criteria.

Role of HER2 in breast tumorigenesis

HER2 belongs to a family of receptor tyrosine kinases regulating diverse biologic processes, including growth and proliferation.[14] Although HER2 lacks ligand-binding activity, it performs a key function as the universal coreceptor for other HER family members.[15] In HER2-positive breast tumors, overabundance of HER2 at the cell membrane stimulates constitutive HER2 phosphorylation and ongoing activation of downstream signaling, which drives tumor growth.[14] Although there is a possible role for HER2 homodimers, *in vitro* and *in vivo* studies suggest the HER2–HER3 heterodimer as the critical oncogenic signaling unit.[16] A further ligand-independent mechanism of HER2 activation in HER2-positive tumors is provided by metalloprotease-mediated shedding of the HER2 ECD, which generates a membrane-anchored, catalytically active 95-kDa C-terminal fragment (CTF) known as $p95^{HER2}$.[17,18]

Mechanisms of action of trastuzumab

Current evidence supports three direct effects mediated by the trastuzumab–HER2 interaction (Fig. 1). First, trastuzumab disrupts the ligand-independent heterodimerization of HER2 and HER3 that occurs under conditions of HER2 overexpression.[8] In contrast, trastuzumab has little impact on the ligand-inducible association of these receptors. A second and complementary direct effect of trastuzumab is to prevent proteolytic cleavage of the HER2 ECD, and thereby inhibit formation of the active $p95^{HER2}$ fragment.[18]

Along with its effects on signaling, trastuzumab directs antibody-dependent cellular cytotoxicity (ADCC) toward HER2-positive tumors by engaging with Fc receptors (FcRs) on immune effector cells. *In vitro*, trastuzumab stimulates the lysis of HER2-overexpressing cells by interleukin-2-activated natural killer (NK) cells,[19] and its activity against HER2-positive xenograft tumors is substantially reduced in transgenic mice lacking activating FcRs.[20] Clinical support for this mechanism is provided by the observation of tumoral leukocyte accumulation in patients receiving trastuzumab-containing neoadjuvant therapy for HER2-positive EBC,[21,22] and correlations between baseline measures of ADCC potency and trastuzumab efficacy.[23,24] Taken together, these data provide strong evidence for a role of ADCC in the clinical efficacy of trastuzumab.

These three direct actions of trastuzumab should be distinguished from secondary effects arising as downstream consequences of the primary modes of action, such as reduction in PI3K/Akt and mitogen-activated protein kinase (MAPK) signaling, enhanced nuclear import and stabilization of the cyclin-dependent kinase (CDK) inhibitor $p27^{Kip1}$, reduced secretion of angiogenic factors, and impaired DNA damage response.[14]

Molecular determinants of trastuzumab efficacy: mechanisms and clinical evidence

A range of putative mechanisms allowing tumor growth in the presence of trastuzumab have been described *in vitro* (Fig. 2),

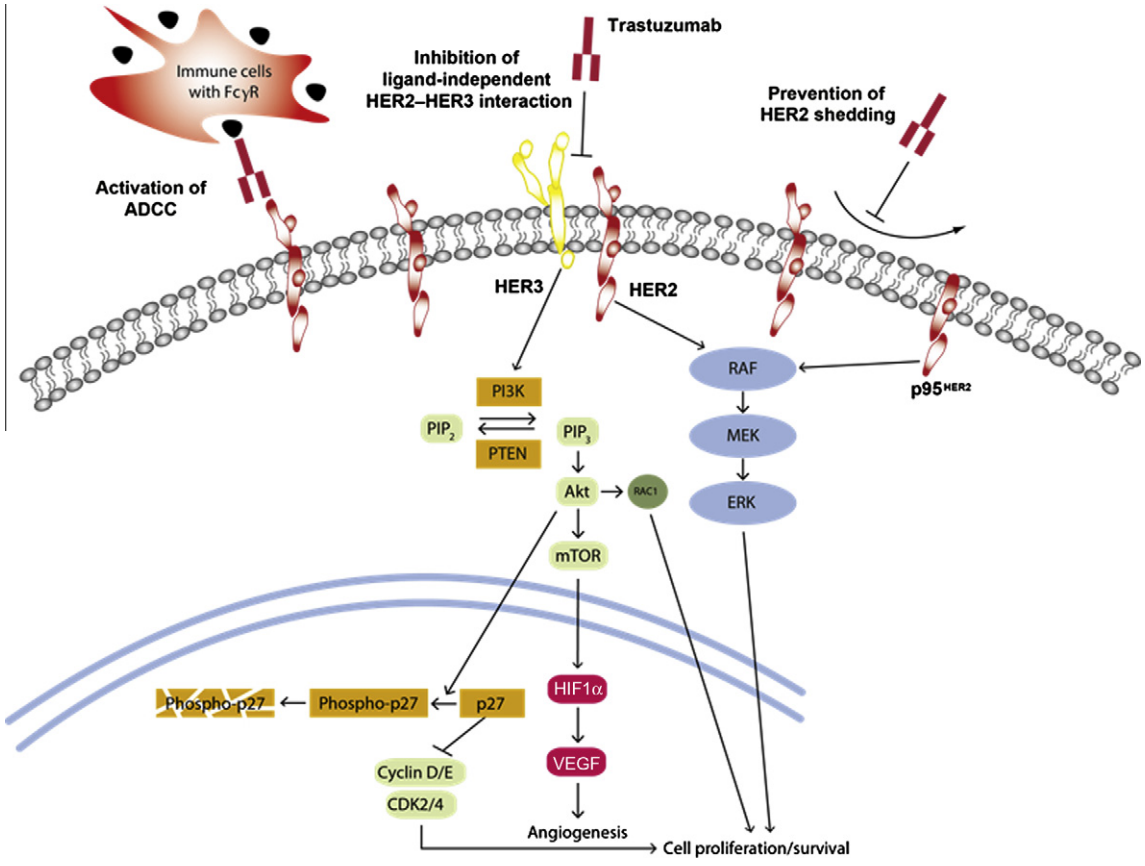

Fig. 1. Summary of mechanisms of trastuzumab action. ADCC, antibody-dependent cellular cytotoxicity; CDK, cyclin-dependent kinase; ERK, extracellular signal-regulated kinase; HER, human epidermal growth factor receptor; HIF, hypoxia inducible factor; MEK, mitogen-activated protein kinase/ERK kinase; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PIP_2, phosphatidylinositol (4,5)-bisphosphate; PIP_3, phosphatidylinositol (3,4,5)-triphosphate; PTEN, phosphatase and tensin homolog; VEGF, vascular endothelial growth factor.

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