Perspective

Anti-HER2 therapies: When more is more

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Human epidermal growth factor receptor 2 (HER2) is a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases (RTKs), which includes EGFR (HER1), c-erbB2 (HER2), c-erbB3 (HER3), and c-erbB4 (HER4) (1). These four receptors share an extracellular domain in N-terminal position corresponding to the ligand binding site, a transmembrane domain, and an intracellular domain in C-terminal position having tyrosine kinase (TK) activity, except for HER3, whose kinase domain is inactive. Apart for HER2, for which no ligand has been identified, for other members of the HER family, ligands bind to the receptor leading to homo- or hetero-dimerisation, and self-activation of tyrosine residues in the C terminal domain, and transduction of signal *via* the Ras/Raf/MEK/ERK and the PI3K/AKT/mTOR pathways (2,3) involved in cell survival, migration, apoptosis and proliferation.

Approximately 10-15% of early breast cancers overexpress HER2 and/or harbour *HER2* gene amplification (2,3). HER2 protein overexpression has been shown to be underpinned by *HER2* gene amplification in >90% of cases (4). Importantly, HER2 overexpression and gene amplification have been shown to constitute drivers of breast cancer in *in vitro* and *in vivo* models (5,6), and are independent prognostic factors (2). A number of therapeutic approaches have been developed against HER2, including Trastuzumab (Herceptin™, Roche-Genentech, CA, USA), Pertuzumab (Omnitarg™, Roche-Genentech, CA, USA) and Lapatinib (Tykerb™/Tyverb™, GlaxoSmithKline, UK), which have either been incorporated into clinical practice or are being tested in the context of clinical trials (*Tables 1*, *2*).

Trastuzumab is a humanised monoclonal antibody (mAb) that binds to the extracellular, juxtamembrane portion of the HER2 receptor and suppresses HER2 signalling activity, resulting in inhibition of downstream signalling pathways, cell cycle arrest and a reduction in angiogenesis. As a result of antibody binding to the HER2 extracellular domain, Trastuzumab also leads to antibody-dependent cell-mediated cytotoxicity (ADCC), and prevents HER2 receptor extracellular domain cleavage, leading to tumour cell stasis and/or death (7-9). In patients with advanced *HER2*-amplified breast cancer, Trastuzumab has shown antitumour activity (10,11) and improves overall survival (OS) when given in combination with chemotherapy in the first-line setting (12,13). It has, however, become clear that a subset of patients with HER2-positive disease fails to benefit from Trastuzumab treatment, either due to primary (also known as *de novo*) resistance, or the acquisition of resistance during the course of treatment (secondary resistance) (14).

Pertuzumab is a humanised monoclonal antibody that binds to a distinct epitope of HER2 (domain II), preventing HER2 from dimerisation with other ligand-activated HER2 receptors, mostly HER3 (15). As a single agent Pertuzumab was assessed in HER2-negative breast cancer advanced patients showing only modest activity (16).

Given that Pertuzumab and Trastuzumab bind to different HER2 epitopes and have complementary mechanisms of action (15), preclinical studies have shown a synergistic effect when these two agents were administered together (17,18). In a phase 2 study in the advanced setting a benefit was observed for the Trastuzumab-Pertuzumab

Table 1 Published clinical trials including targeted agents in development to reverse or prevent Trastuzumab resistance

Phase	Compound	Target	Treatment arms	Stage	n patients	Primary endpoint	Trial ID
II	Pertuzumab	HER2	Pertuzumab+trastuzumab	Advanced	66	ORR	NCT00301899
II	Pertuzumab	HER2	Trastuzumab+docetaxel *vs*. trastuzumab+ docetaxel+ pertuzumab *vs*. Trastuzumab+pertuzumab *vs*. docetaxel+pertuzumab	neoadjuvant	417	pCR	NCT00545688
III	Pertuzumab	HER2	Trastuzumab+docetaxel *vs*. Trastuzumab+docetaxel+ pertuzumab	advanced 1st line	808	PFS	NCT00567190
II	T-DM1	HER2	T-DM1	advanced	112	ORR	NCT00509769
II	Neratinib	HER2/ EGFR	Neratinib	advanced	136	PFS	NCT00300781
II	Afatinib	HER2/ EGFR	Afatinib	advanced	41	ORR	NCT00431067

Abbreviations: ORR, Overall Response Rate; pCR, pathological complete response; PFS, progression-free survival

combination in patients who had failed Trastuzumab (19). In early breast cancer, the results of the Neoadjuvant Study of Pertuzumab and Herceptin in an Early Regimen Evaluation (NEOSPHERE; NCT00545688) have shown a markedly higher pathological complete response rate with the triple therapy of docetaxel, Trastuzumab and Pertuzumab compared to docetaxel with either one of the anti-HER2 therapies alone (20). However, the true clinical significance of pathological complete response, or lack of, in oestrogen receptor-positive HER2-positive breast cancers remains to be fully established.

The recently published Clinical Evaluation of Pertuzumab and Trastuzumab study (CLEOPATRA; NCT00567190) (21) has provided direct evidence to demonstrate that the addition of Pertuzumab to Trastuzumab plus docetaxel improved progression-free survival (PFS) by 6.1 months (18.5 *vs*. 12.4 months for Pertuzumab arm *vs*. control, respectively; HR, 0.62; 95% CI, 0.51-0.75; P<0.001). This benefit was observed in all pre-defined subgroups based on previous neoadjuvant or adjuvant treatment (chemotherapy+/-Trastuzumab), geographic region, age, race, visceral involvement, hormone receptor status and if HER2-positivity had been determined by immunohistochemistry or fluorescence *in situ* hybridisation. There was a non-significant trend in benefit in overall survival (P=0.05), although the analysis was performed after only 43% of the pre-specified total number of events requested for the final analysis. These results are therefore consistent with those observed in the previously described phase II studies. The fact that the benefit was observed in both Trastuzumab pre-treated and Trastuzumab-naive patients suggests that Trastuzumab-resistant HER2-positive breast cancers may still depend on HER2 signalling. The observed clinical benefit of Pertuzumab is likely to stem from the different mechanisms of action of Trastuzumab and Pertuzumab. Unlike Trastuzumab, Pertuzumab-mediated blockade of the epitope II, involved in heterodimer formation, prevents the formation of HER2-HER3 heterodimers, which are the most potent in activating the PI3K pathway (22). This mechanisms of action would add to the previously described mechanism of Trastuzumab benefit, which are not solely linked to a direct HER2 inhibition (9). Importantly, no significant increase in toxicity, especially in terms of cardiac events, was observed with the combination of the two monoclonal antibodies compared to Trastuzumab alone and no significant differences in treatment exposure to docetaxel occurred between the two treatment groups. This is of crucial importance, given that one of the main challenges in combinatorial therapies where one of the agents is a targeted agent, is the fact that full dose chemotherapy treatment cannot be delivered due to compounded toxicity [e.g., Sunitinib in combination to docetaxel (23)].

In addition to the direct inhibition of HER2, alternative approaches for targeting the overexpression of this transmembrane receptor have emerged. Trastuzumab-maytansine (DM1) (T-DM1, Roche-Genentech, CA, USA) is an immunoconjugate agent that combines Trastuzumab with DM1, an antimicrotubule cytotoxic agent. A phase II study (NCT00509769) has now shown significant

Table 2 Unpublished or still recruiting clinical trials with novel anti-HER2 therapies or targeted agents to reverse or prevent Trastuzumab resistance

Phase	Compound	Target	Treatment arms	Stage	Status trial	Trial ID
Ib/II	BEZ235	PI3K/mTORC	Trastuzumab+BEZ235	advanced	not open yet	NCT01471847
Ib/II	BKM120	PI3K	Trastuzumab+BKM120	advanced	recruiting	NCT01132664
Ib/II	XL147	PI3K	XL147+trastuzumab *vs*. paclitaxel+trastuzumab	advanced	recruiting	NCT01042925
Ib	GDC-0941	PI3K	GDC-0941+T-DM1 *vs*. GDC-0941+trastuzumab	advanced	recruiting	NCT00928330
III	T-DM1/ Pertuzumab	HER2	T-DM1+pertuzumab *vs*. pertuzumab+placebo *vs*. trastuzumab+taxane	advanced	recruiting	NCT01120184
III	T-DM1	HER2	T-DM1 *vs*. lapatininb+ capecitabine	advanced	recruiting	NCT00829166
Ib	T-DM1/ Pertuzumab	HER2	T-DM1+paclitaxel+/- pertuzumab	advanced	recruiting	NCT00951665
II	T-DM1	HER2	T-DM1 *vs*. trastuzumab+ docetaxel	advanced	recruitment completed	NCT00679341
II	Pertuzumab	HER2	Pertuzumab+trastuzumab	early or LABC	finished recruitment	NCT00545688
II	Pertuzumab	HER2	Pertuzumab+rastuzumab+ aromatase inhibitor	advanced 1st line	not open yet	NCT01491737
II	Pertuzumab	HER2	Trastuzumab+capecitabine +/- pertuzumab	advanced	recruiting	NCT01026142
II	Pertuzumab	HER2	Pertuzumab+trastuzumab+ chemotherapy	early or LABC	recruiting	NCT00976989
I/II	Temsirolimus/ Neratinib	mTOR/HER2/ EGFR	Temsirolimus+neratinib	advanced	recruiting	NCT01111825
I	Neratinib	HER2/EGFR	Paclitaxel+neratinib+ trastuzumab	advanced	recruiting	NCT01423123
II	Neratinib	HER2/EGFR	Neratinib+paclitaxel *vs*. trasuzumab+paclitaxel	advanced	recruiting	NCT00915018
I/II	Neratinib	HER2/EGFR	Neratinib+paclitaxel	advanced	finished recruitment	NCT00445458
I/II	Neratinib	HER2/EGFR	Neratinib+trastuzumab	advanced	finished recruitment	NCT00398567
I/II	Neratinib	HER2/EGFR	Neratinib+vinorelbine	advanced	finished recruitment	NCT00706030
I/II	Neratinib	HER2/EGFR	Neratinib+capecitabine	advanced	finished recruitment	NCT00741260
III	Everolimus	mTOR	Trastuzumab+paclitaxel+/- everolimus	advanced 1st line	finished recruitment	NCT01007942
III	Everolimus	mTOR	Trastuzumab+vinorelbine+/- everolimus	advanced	finished recruitment	NCT00876395
III	Afatinib	HER2/EGFR	Vinorelbine+afatinib *vs*. vinorelbine+trastuzumab	advanced	recruiting	NCT01125566
II	Afatinib	HER2/EGFR	Afatinib *vs*. afatinib+ paclitaxel *vs*. afatinib+ vinorelbine	advanced	recruiting	NCT01271725

Abbreviations: LABC, locally-advanced breast cancer

antitumour activity in patients with HER2-positive metastatic breast cancer who had progressed on anti-HER2 plus chemotherapy (24). Several clinical trials are currently testing T-DM1 in the metastatic setting at several stages of the disease: after progression on Trastuzumab in a head-to-head comparison with Lapatinib plus capecitabine (EMILIA, NCT00829166) or with Pertuzumab and paclitaxel (NCT00951665). A phase II clinical trial comparing PFS of T-DM1 *vs*. Trastuzumab plus docetaxel in first line metastatic HER2-positive has completed recruitment (NCT00679341). The impact of this trial on clinical practice will be affected to some extent by the publication of the CLEOPATRA study (21), since Trastuzumab plus docetaxel can no longer be considered as standard first line therapy.

There is clear interest in developing other strategies to deal with progressive HER2-positive metastatic breast cancer. As discussed above Lapatinib, an oral, small-molecule tyrosine kinase inhibitor that inhibits the kinase activity of both HER1 (EGFR) and HER2, has been approved for clinical use in combination with capecitabine after progression on Trastuzumab (25). Furthermore, several oral small-molecule, tyrosine kinase inhibitors are also in development. Afatinib (BIBW 2992, Boehringer Ingelheim, Germany) is an oral, irreversible HER family inhibitor that targets EGFR, HER2 and HER4. Initial reports confirmed antitumour activity of this compound alone or in combination with chemotherapy in patients who have progressed on anti-HER2 therapy (26). Neratinib (Pfizer, NY, USA) is an irreversible EGFR/HER2 kinase inhibitor that covalently binds the target kinase as part of its mechanism of action. A phase I dose - escalation study (NCT00146172) reported a response rate of 24% and clinical benefit rate of 38% in patients with *HER2*-amplified metastatic breast cancer, all of whom had progressed on prior anthracycline, taxane and Trastuzumab therapy (27). In a large phase II clinical trial in patients with *HER2*-amplified breast cancer (NCT00300781), Neratinib therapy resulted in a response rate of 24% (95% CI: 14-36%) for patients with prior Trastuzumab and a response rate of 56% (95% CI: 43-69%) for patients with no prior Trastuzumab (28). Several phase I/II trials of Neratinib combinations are in ongoing (*Table 2*) and preliminary safety and efficacy data have been presented [reviewed in (29)]. Phase III clinical trials utilising this agent in combination with other regimens have been planned for patients with metastatic or locally-advanced breast cancer.

In addition to targeting HER2 itself, alternative therapeutic approaches targeting pathways downstream of HER2 are currently being tested to overcome primary and/or acquired resistance to anti-HER2 agents in HER2-positive breast cancers (*Table 2*). For instance, one of the mechanisms of resistance to anti-HER2 agents is activation of the PI3K pathway through activating *PIK3CA* mutations and PTEN loss of function (29,30). An ongoing phase I study is currently investigating the activity of a PI3K inhibitor (GDC-0941; Roche-Genentech) in combination with either T-DM1 or Trastuzumab in an attempt to reverse Trastuzumab resistance (NCT00928330). Given the potential cumulative toxicity and cost implications of these various combinations, the identification of robust biomarkers to guide for the best approach is imperative.

Studying HER2-positive breast cancers has shifted the paradigm of how subgroups of breast cancer patients can be treated effectively. The trials performed so far have objectively demonstrated that contrary to the maxim sometimes more is more: dual blockade is better than Trastuzumab only treatment and this should need to be initiated at early stages of the disease. Furthermore, from a conceptual standpoint, the results of the clinical trials discussed in this editorial lend further credence to the notion that HER2 is a *bona fide* driver of a subset of breast cancers and to the concept of 'oncogene addiction' (31) (i.e. that despite the multiple genetic aberrations found in HER2-positive cancers, these cancers are dependent on the activity of this oncogenes for the maintenance of their malignant phenotype).

From a clinical standpoint, despite the changes in clinical practice, CLEOPATRA and the other trials discussed above raise numerous questions: (I) what are the best therapeutic agents for patients with HER2-positive disease: dual or multiple HER2 inhibition? (II) is dual inhibition required in all HER2-positive breast cancers? (III) at what stage of disease progression should Pertuzumab, T-DM1, Lapatinib and other novel anti-HER2 agents be introduced? (IV) does the order of the agents affects the likelihood of sustained responses? (V) how many different anti-HER2 and targeted agents should be administered in combination? (VI) should they be used concurrently or sequentially? With the panoply of valid therapeutic approaches available, clearly, answers to most of the questions above will be required for the full realisation of the potentials of personalised medicine, maximising the chances of preventing, delaying or reversing resistance to HER2-blockade.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.3978/j.issn.2218-676X.2012.03.07). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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