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Review

# Resistance to human epidermal growth factor receptor type 2-targeted therapies

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**Abstract** The overexpression of the human epidermal growth factor receptor type 2 (HER-2) is an independent prognostic factor of poor outcome in patients with breast cancer. Two compounds have been registered for HER-2-positive tumour treatment: trastuzumab, a humanised antibody directed against the HER-2 extracellular domain, and lapatinib, a small molecule acting as a dual EGF-R and HER-2 tyrosine kinase inhibitor. Although both drugs improve progression-free survival, many patients' tumours will exhibit primary resistance, or develop secondary resistance, to anti-HER-2 therapies. The recent significant improvement of survival gained with pertuzumab (an antibody disrupting dimerisation of the receptor) or trastuzumab emtansine (T-DM1, a cytotoxic drug vectored by trastuzumab binding) opened the way for new registrations.

This review describes the molecular mechanisms by which tumour cells may adapt to and evade HER-2 inhibition by HER-2-targeted therapies and discusses strategies to prevent and overcome resistance to trastuzumab and lapatinib. These strategies may include the establishment of predictive markers, exploration of combination therapies and modulation of nodal targets.


## 1. Introduction

The human epidermal growth factor receptor type 2 (HER-2) is overexpressed in about 20% of invasive breast carcinomas and its gene amplification is associated with an increased metastatic potential and decreased overall survival [1]. As others HER family members (EGFR, HER-3 and HER-4), HER-2's ectodomain consists of two IGF-like ligand-binding domains (I–III) and two cysteine-rich domains (II–IV) involved in the dimerisation process (Fig. 1). Noteworthy, HER-2 naturally displays a ligand-independent

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open conformation favouring its homo- or heterodimerisation. Consequently, transphosphorylation at the intracytoplasmic tyrosine kinase domain initiates a signal transduction through the MAPK and phosphoinositide 3-kinase (PI3K) pathways, which regulates cell proliferation, apoptosis, differentiation and migration. HER-3 is lacking the tyrosine kinase domain but is strongly associated with the p85 regulatory subunit of PI3K, and together with HER-2 or EGFR forms some highly active complexes.

Until recently, two compounds were registered in the metastatic setting, trastuzumab (Genentech, Roche), a humanised monoclonal antibody directed against HER-2 extracellular domain (ECD) [2,3] and lapatinib (Glaxosmithkline), a dual intracellular tyrosine kinase inhibitor (TKI) that blocks HER-2 and EGFR activation [4]. However, despite the progresses brought by these drugs in the treatment of patients with HER-2-positive breast cancer, many cancers develop resistance. For instance, about 40% of HER-2-positive breast cancer patients may not respond to a first-line regimen including trastuzumab, and most of them will develop resistance within one year after initiation of trastuzumab treatment [2,3]. The recent FDA's approval of Pertuzumab (Genentech, Roche) and the survival benefit obtained with trastuzumab emtansine (t-DM1) [5] in trastuzumab-resistant metastatic breast cancer (MBC), reward long-term efforts in the understanding of molecular mechanisms of resistance to trastuzumab. This review outlines the molecular mechanisms by which tumour cells may adapt to and resist HER-2 inhibition. Based on identified mechanisms of resistance and the discovery of new predictive markers, new strategies may be developed to overcome it.

## 2. Mechanisms of resistance to anti-HER-2

Complex networks of intracellular signalling pathways are involved in HER-2 activation. Usually, resistance mechanisms are classified according to genetic or environmental alterations of receptors tyrosine kinase (RTKs) and their downstream effectors (*de novo* resistance) or the activation of alternative pathways, to bypass the HER-2 inhibition after anti-HER-2 exposure (acquired resistance) [6]. Main clinical studies and current trials in trastuzumab-resistant MBC patients are summarised in Table 1.

### 2.1. De novo resistance

#### 2.1.1. Alteration of receptor access

Cleavage of HER-2 leaves after the release of its ectodomain (ECD), a 95–100 kDa constitutively active membrane bound form (648-CTF p95HER-2). This process, mediated by the sheddase ADAM10 [7], might be a source of resistance to trastuzumab [8] but not to lapatinib [9], since the constitutive intrinsic tyrosine kinase activity would still be inhibited by lapatinib. ADAM inhibitors such as INCB7839 demonstrated *in vivo*

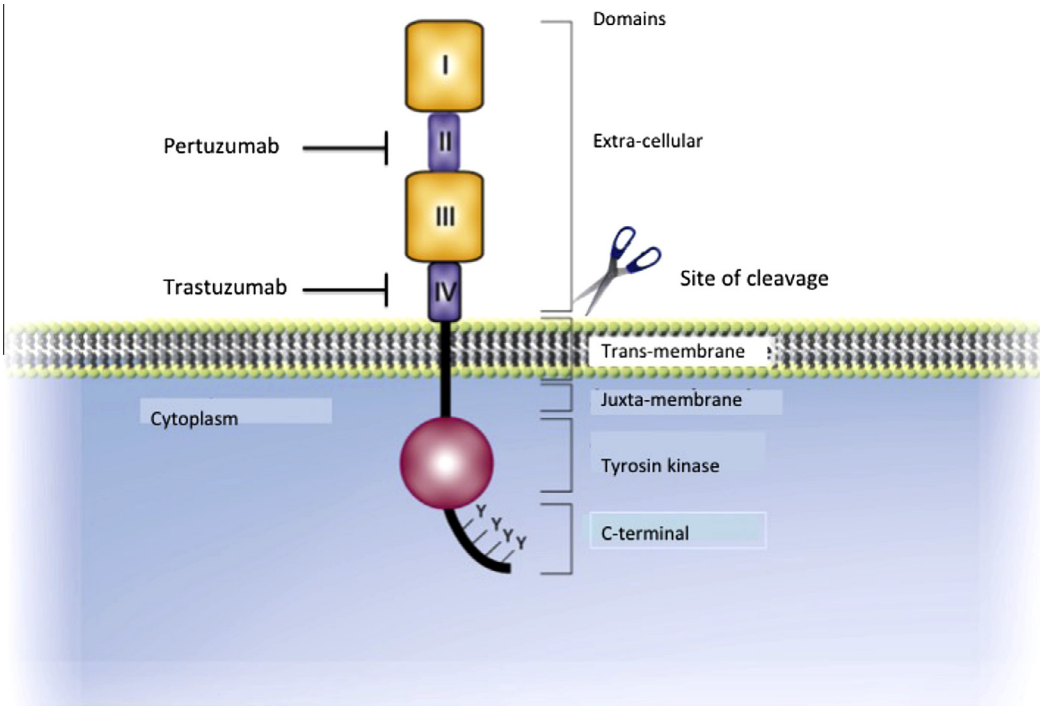


Fig. 1. HER-2 structure (with the courtesy and adapted from Penault-Llorca F).

Table 1
Main clinical studies of single agent or combination in HER-2 positive metastatic breast cancer.

| Strategy | Agent(s) | Phase | *N* | Main end-point | Ref. |
|---|---|---|---|---|---|
| Combine anti-HER-2 and chemotherapy | Trastuzumab | III | 469 | OS 25.1 versus 20.3 months ($P = 0.46$) | [3] |
| | Lapatinib | III | 399 | TTP 8.4 versus 4.4 months ($P < .001$) | [66] |
| Combine anti-HER-2 | T + L | III | 296 | PFS 12.0 versus 8.1 weeks ($P = .008$) | [72] |
| Target HER2/HER3 | Pertuzumab | III | 808 | PFS 18.5 versus 12.4 months ($P < .001$) | [40] |
| | MM-111 | I | 21 | Recruiting | NCT01097460 |
| Irreversible dual HER1/2 inhibitor | Neratinib | II | 136 | PFS prior/naïve T 22.3 versus 39.6 weeks | [67] |
| | | II | 480 | Ongoing | NCT00915018 |
| | Afatinib | II | 41 | ORR 12% ($n = 4/34$ evaluable) | [68] |
| HER-2 targeting immunotoxin | T-DM1 | II | 112 | ORR 25.9% (95% CI, 18.4–34.4%) | [81] |
| | | III | 1092 | Recruiting | NCT01120184 |
| | | III | 980 | Recruiting | NCT00829166 |
| *Combine multiple pathways targeting* | | | | | |
| PI3K/Akt mTOR inhibitor | Everolimus | I | 33 | ORR 44% | [23] |
| | GDC-0941 | Ib | 60 | Recruiting | NCT00928330 |
| | BEZ235 | I/II | 140 | Not yet recruiting | NCT01471847 |
| | INK-128 | I | 95 | Recruiting | NCT01351350 |
| ER pathway | Letrozole | III | 219 | PFS 8.2 versus 3.0 months ($P = .019$) | [54] |
| Anti-angiogenic | Bevacizumab | II | 50 | ORR 48% | [57] |
| IgF-1-R | Cixutumumab | II | 154 | Recruiting | NCT00684983 |
| Inhibit anti-HER-2 degradation | Tanespimycin | I/II | 31 | ORR 22% | [82] |
| | Retaspimycin | II | 29 | Ongoing | NCT00817362 |

*Abbreviations:* T = trastuzumab; L = lapatinib; OS = overall survival; PFS = progression-free survival; ORR = overall response rate; TTP = time to progression.

ability to reduce ECD HER-2 level and could potentially enhance response to trastuzumab, particularly in p95+ patients [10]. Alternative translation from internal AUG codons also leads to truncated forms (611 and 678-CTF) corresponding to 100–115 kDa and 90–95 kDa p95HER-2 fragments respectively. All these truncated fragments display disparate levels of activity, with a remarkable oncogenicity for the 611-CTF form illustrated by transgenic mice models and the ability to regulate a different subset of genes not involved in the classical p185HER-2 pathway [11]. Despite a lot of interest for this biomarker, it appears that the various forms embedded in the term of p95HER-2 and corollary the equivocality of available tools to detect them [12–14] resulted in conflicting results, premature for clinical practice. For instance, the poor outcomes observed under trastuzumab in a cohort of metastatic HER-2-positive patients with high levels of p95HER-2, as assessed by the VeraTag assay on the primary samples [14], were balanced by the results from the neo-adjuvant GeparQuattro study, which unexpectedly showed a higher response to trastuzumab in patients with tumours overexpressing p95/611-CTF forms at baseline [15]. Interestingly, a recent hypothesis argued that, a decrease of the antigen steric hindrance may facilitate the binding of trastuzumab to its epitope (on p185HER-2) in the presence of a p95HER-2 contingent [16]. Similarly, the preclinical model of higher sensitivity to lapatinib for p95+ patients, in which overexpression of p95HER-2 induced *in vitro* an increase of EGFR and its downstream signalling, and constitutive activation of Erk-1/2 kinases [8], was disappointing in various retrospective clinical studies [9,17]. Thus, p95 clearly awaits technical standardisation and clinical validation.

### 2.1.2. FcγRIIIa polymorphisms

Antibodies such as trastuzumab or pertuzumab, through their constant fragment (Fc) can activate antibody-dependent cytotoxicity (ADCC), involving lymphokine-activated killer cells (LAK), and complement-dependent cytotoxicity [18]. Such an immune mechanism might barely contribute to their therapeutic effect, as illustrated by the enrichment of NK cells and the presence of Granzyme B in the tumour infiltrates after trastuzumab exposure in the neo-adjuvant setting [19]. Polymorphisms on the FcγRIIIa are constitutively expressed on LAK [20], in particular the 131 H/R and 158 V/F polymorphisms which appear to exert a strong effect on IgG1 affinity for FcγR, and consequently on the ADCC-related activity [21]. In metastatic breast cancer, the FcγRIIIa-158 V/V and 131 H/H genotypes were significantly correlated with better objective response rates (ORRs) and progression-free survival (PFS) in patients treated with trastuzumab [22]. However, a large genotyping analysis failed to confirm these results [23].

#### 2.1.3. Changes in signal transmission

Changes in downstream signalling may lead to constitutive activation of the pathways. In patients treated by trastuzumab, loss or inactivating mutations of phosphatase and tensin homologue on chromosome 10 (PTEN), or the oncogenic mutations of phosphatidylinositol 3 kinase catalytic subunit (PI3KCA) were associated with poor response and survival [24,25]. Consequently, everolimus an oral mTOR inhibitor was tested in combination with trastuzumab and chemotherapy [26,27]. Nonetheless, in the N9831 trial published recently [28] no interaction between PTEN status and disease-free survival was reported. Other strategies like PI3K inhibitors (GDC-0941) [29], dual PI3K/mTOR inhibitors (BEZ235) [30] or a novel dual mTORC1/2 INK-128 [31] in combination with anti-HER-2 therapies are being investigated. Such strategies might be expected to fail with lapatinib, considering that sensitivity to lapatinib was not dependent on PTEN expression or PI3K pathway activation [32,33], however these results are unclear in regard to some specific PI3KCA mutations [34,35]. Moreover, the anti-HER-2 effect of lapatinib appears to be mediated preferentially through the MAPK pathway, and resistance to lapatinib might be overcome by a MEK inhibitor in the context of Ras overexpression or mutation [36]. Finally, the co-amplification of *CCNE1* coding for Cyclin E, which occurs in ~20% of HER-2-amplified breast cancers and is associated with poorer outcome under trastuzumab [37], might be implicated in trastuzumab resistance and lead to the potential effect of CDK2 inhibitors in this situation [38].

### 2.2. Acquired resistance

#### 2.2.1. Activation of HER-3 and HER-1 pathways

Overexpressions of other RTKs or their ligands are also involved in trastuzumab and lapatinib resistance. Junttila et al. [39] showed that the overexpression of HER-3 along with HER-2 leads to the formation of HER-2/HER-3 heterodimers, through the PI3K/Akt pathway. Similarly, as demonstrated *in vitro* [40] and in xenograft models [41], HER-1 overexpression or the presence of its ligands might be also responsible for acquired resistance to trastuzumab. Interestingly, trastuzumab resistance in SKBR3 and BT474 cell lines is mediated by an inability to decrease HER2 phosphorylation, through a mechanism involving a dimerisation with others RTKs consecutively to their ligands release [42]. Under trastuzumab, the protein kinase B (PKB) phosphorylation is decreased, as a result of a decrease of HER3 phosphorylation. Pointing to a negative feedback loop from PKB to ADAM17, and the role of this protease in the ligand release, this study hypothesised that trastuzumab itself induces an ADAM17-mediated up-regulation of HER ligands, and thus resistance. The dramatic antiproliferative effect of trastuzumab combined to TAPI-1, an ADAM17 inhibitor, or to a panHER inhibitor, reinforces this model and displays promising development. Similarly, the increased expression of TGFα observed in trastuzumab-resistant tumour cell lines may be involved in extending the stimulation of HER-1 homo/heterodimers [43] and may induce resistance to lapatinib [44].

These considerations of acquiring resistance through the heterodimerisation of HER-2 drove the CLEOPATRA phase III study, in which the combination of pertuzumab (which acts by disrupting heterodimerisation) with docetaxel plus trastuzumab regimen led to an increase of 6.1 months in progression free survival (HR = 0.62, 95% confidence interval (CI) [0.51–0.75], $p < 0.0001$) and a strong trend in overall survival in favour of the combination arm which supported the FDA approval [45]. In the neo-adjuvant setting, pertuzumab plus trastuzumab with docetaxel yielded a significant improvement of pathological complete response (pCR) compared to the control group (45.8% versus 29%, $p = 0.0141$). Noteworthy, the chemo-free regimen gave interesting rates of pCR (16.8%, 95% CI [10.3–25.3]) [46]. Similarly, data from *in vitro* and xenograft models with a bispecific antibody targeting the HER2/HER3/Heregulin complex (MM-111), displayed promising activity, especially in combination with trastuzumab or lapatinib [47].

#### 2.2.2. IGF-1-receptor pathway

The Insulin Growth Factor-1 receptor (IGF-1R) plays an important role in cell proliferation and survival via the PI3K pathway. By-passing the negative effect of trastuzumab on p27 degradation, IGF-1R can degrade it through the ubiquitin ligase Skp2, resulting in the abolition of cdk2 inhibition and cell cycle blockage on the G1/G0 phase [48,49]. Thus, cross-talk between IGF-1R and HER-2 appears as a major potential mechanism for acquired resistance to trastuzumab. A strong interaction between IGF-1R, HER-2 and HER-3 leading to heterotrimeric complex formation was reported only in trastuzumab-resistant breast cancer cells, and their disruption by a specific knockdown of HER-3 or IGF-1R abrogated trastuzumab resistance [50]. *In vitro*, the phenolic compound nordihydroguaiaretic acid (NDGA), a dual cytotoxic IGF-1R/HER-2 inhibitor, exhibited major antitumour activity and enhanced-trastuzumab activity against trastuzumab-naïve and resistant breast cancer cells by reducing signal transduction via the PI3K/Akt pathway [51]. Noteworthy, lapatinib was shown to induce apoptosis in trastuzumab-resistant cells by blocking Akt activation, increasing p27 expression and inhibiting IGF-1R-mediated signal transduction, independently of IGF1 [52]. No change in sensitivity or resistance to lapatinib was detected in the presence of the anti-IGF-1R αIR3 antibody, probably due to

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the direct inhibition of survivin independently of Akt inhibition.

#### 2.2.3. Others alternatives pathways

Met receptor tyrosine kinase may also contribute to trastuzumab resistance, by revoking p27 induction [53]. Cross-talks between HER-2 and oestrogen receptor (ER) pathways may intervene in trastuzumab [54] and lapatinib [55] resistance. In the neoadjuvant setting, a lower pCR rate was reported in the ER+ subgroup under lapatinib, trastuzumab or their combination [56]. Conversely, an increase in ER signalling was observed in patients with HER-2-positive/ER-positive tumour treated with lapatinib monotherapy [55]. This increase may involve the activation of FOXO3A, as a result of inhibition of PI3K/Akt. Consistent with this, the sensitivity to lapatinib was restored by fulvestrant *in vitro*. Another mechanism involves AXL overexpression, silencing or inhibition of which by foretinib (a multikinase inhibitor of AXL, MET and VEGFR) or fulvestrant treatment reverse trastuzumab or lapatinib resistance *in vitro* [57]. These data suggest that positive hormonal receptor status might be a marker of lower sensitivity to anti-HER-2 therapies and may go some way to explain the successful combination of anti-HER-2 therapy with endocrine therapy in the clinic [58–60].

Previous data supported an up-regulation of the VEGF pathway in HER-2-overexpressed MBC [61], with a safe and promising first-line therapy combining bevacizumab with either trastuzumab or lapatinib [62,63]. The results of the BEVERLY 2 study combining bevacizumab, trastuzumab and chemotherapy in primary inflammatory HER-2 positive breast cancer confirmed the safety and the efficacy of the regimen, with high pCR rates (63.5%, 95% CI 49.4–77.5) [64]. The BETH study (NCT00625898) randomising the addition of bevacizumab to the current adjuvant setting in HER-2 positive early breast cancer may define a new standard of care.

## 3. Designing the best strategy

The knowledge of these mechanisms of resistance has driven the development of new drugs or drugs combinations (Fig. 2). Their best use will require definition of predictive factors of resistance and rational sequences of treatment. The search for targetable nodes, common to multiple resistance pathways, is also appealing.

### 3.1. A better prediction of anti-HER-2 efficacy

As discussed earlier, numerous markers have been proposed as predictive factors of *de novo* resistance, but still warrant clinical validation [65]. In the GeparQuattro study [66], multivariate analysis showed that ALDH1 and p4E-BP1 were significantly up-regulated in resistant patients, suggesting that respectively particular features of stem cells and activation of the mTOR pathway are involved in the mechanisms of resistance to trastuzumab. Conversely, phosphorylated HER-2 receptor [65], or co-amplification of c-Myc and HER-2 [67] may be markers of sensitivity to trastuzumab or lapatinib. Predictive value of some of these biomarkers (*c-Myc*, p95HER-2, PTEN) is being prospectively investigated in the ALTTO (NCT00490139) and NeoALTTO (NCT00553358) trials. Nonetheless, 20 years later, the only fully validated prognostic and predictive marker of anti-HER-2 therapies remains the over-expression and/or amplification of HER-2. Such observation brings one to consider that, contrary to colorectal or lung cancer, such a quest of *de novo* or acquired predictive factor of resistance might be in vain.

### 3.2. Logical sequences of drugs combination

Several studies validated the efficacy of keeping therapeutic pressure on HER-2 by maintaining trastuzumab in association with a new chemotherapy after disease progression following first line trastuzumab treatment [68–70]. Currently, ongoing randomised studies THOR (NCT00448279) and ML19944 (NCT00444587) are evaluating the value of continuing trastuzumab beyond progression.

Switching to another therapy was successful with the approval of lapatinib by FDA as second-line anti-HER-2 therapy in association with capecitabine, however without any benefit in overall survival [4,71].

In a phase II trial, neratinib (HKI-272, Pfizer), an irreversible ErbB receptor tyrosine kinase inhibitor, yielded promising ORRs with 56% in naive patients and 24% in previously trastuzumab-exposed amongst patients HER-2-positive breast cancer patients [72]. Currently, its efficacy in early and advanced HER-2-positive breast cancer is being evaluated in two clinical trials (ExteNET (NCT00878709) and NEFERTT (NCT00915018)). In another recent phase II study, afatinib (BIBW 2992), an irreversible dual erbB RTK inhibitor showed interesting clinical benefit (46%) in heavily pretreated and trastuzumab-resistant MBC patients. As with neratinib, the most frequent adverse events with afatinib were diarrhoea and rash [73].

Different approaches focused on the reinforcement of the anti-HER-2 pressure, by counteracting HER-2 and its downstream effectors promoting their degradation. In preclinical studies *in vivo*, chronic administration of Hsp90 inhibitors led to a reduction in sustained expression of HER-2 and p95HER-2, downregulation of the PI3K/Akt pathway, and inhibition of cell proliferation by apoptosis induction [74]. Inhibitors such as tanespimycin (KOS-953) and retaspimycin (IPI-504) (NCT00817362) are currently under clinical develop-

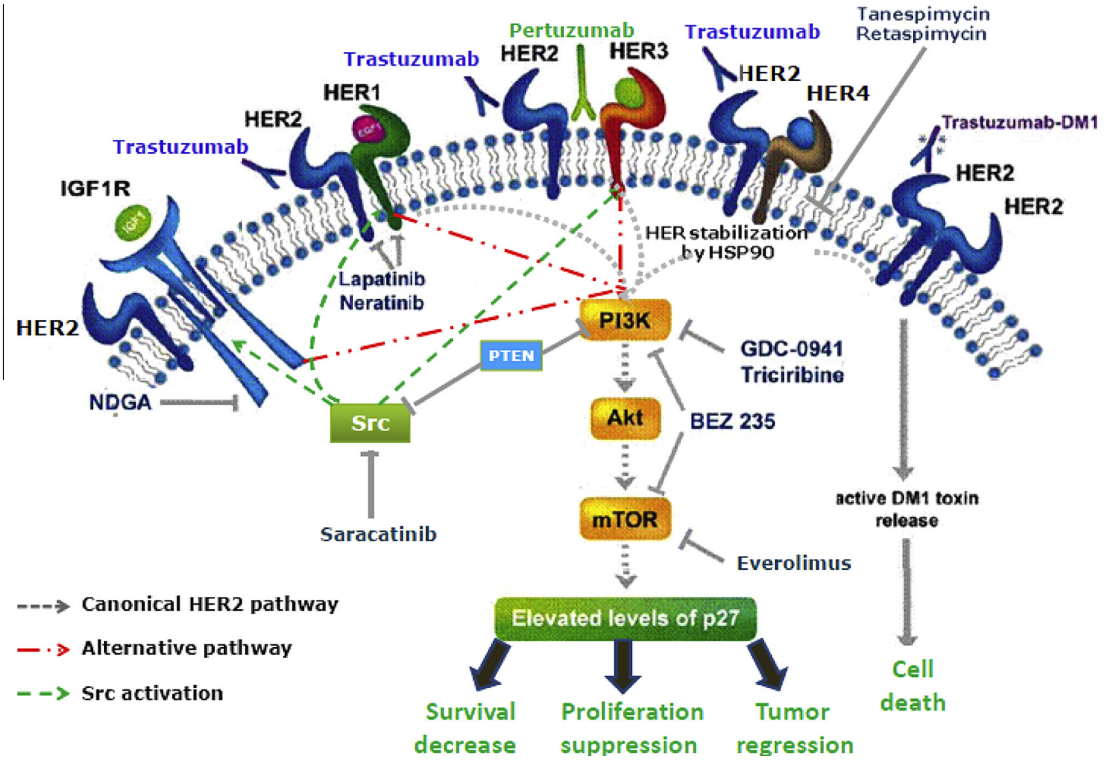


Fig. 2. Resistance pathways and corresponding drugs.

ment. Similarly, in a *in vitro* study conducted on lapatinib-resistant cell lines, the combination of lapatinib with bortezomib (Velcade®), a proteasome inhibitor, resulted in apoptosis and inhibition of transmission of HER-2 signal, in both lapatinib-resistant and sensitive cell lines [75].

Another approach is to combine multiple HER-2 targeted agents. Thus, the benefit of adding lapatanib to trastuzumab has emerged from preclinical models showing a synergistic effect [76]. This was confirmed in a randomised phase III study in patients with heavily pretreated trastuzumab-resistant HER-2-positive breast cancer, with an increased PFS [77]. This combination was also tested in a neoadjuvant strategy within the framework of the NeoALTTO study, yielding better pCR compared to each monotherapy associated with chemotherapy [56].

### 3.3. Delivering cytotoxicity through HER-2

The emtansine trastuzumab (T-DM1), a HER-2-targeting immunotoxin formed by conjugation of trastuzumab with maytansinoid cytotoxin DM1, a tubular polymerisation inhibitor, recently succeed in the use of trastuzumab as a vector for delivering cytotoxicity. T-DM1 is specifically internalised in HER-2-overexpressing cells and, after lysosomal degradation, releases DM1, leading to cell death [78]. Obviously, the conjugated antibody preserves the mechanism of action of trastuzumab [79]. The results of the EMILIA phase III study in a heavily pre-treated trastuzumab-refractory patients were strongly in favour of the T-DM1 compared to the conventional arm capecitabine-lapatinib, with a respectively 35% and 32% significant improvement of PFS and OS while grade 3/4 toxicities were less frequent [5]. Another phase III study (MARIANNE, NCT01120184) comparing T-DM1 plus placebo versus T-DM1 plus pertuzumab versus trastuzumab plus taxane, in first-line MBC is ongoing.

### 3.4. The 'drug sedimentation theory'

All these compounds might improve progression or relapse-free survival of HER-2 positive patients but must be delivered with the best strategy, based on host and tumour characteristics. Acquired resistance raise the hypothesis of predictable, or more modestly, understandable models of resistance. Campone et al. [80] tried to understand the natural history of HER-2 positive breast cancer under a *drug sedimentation theory*. In this model, the clones selected under anti-HER-2 therapy pressure will display several acquired mechanisms of resistance. As we reported earlier, acquired mechanisms of resistance involved other addictive pathways such as c-MET, IGF-1R, HER-1 and HER-3, or PI3K/Akt/mTOR. All these pathways are secondary 'addictive' pathways, needing to be targeted as well as the HER-2. Based on this theory, current and future designs will associate in second line chemotherapy, an anti-HER-2 and a targeted therapy focusing on this secondary

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'addictive' pathway such as an anti-IGF-1R, and so on for the further lines. The power of this theory should come from identification of markers of resistance. Unfortunately, as already discussed, no clear and robust markers rose from clinical studies. Nonetheless, in order to better address the additional targeted therapy, systematical biopsies at the time of relapse will be warranted. The evident weakness of this theory is that tumours are heterogeneous and may display concomitantly several resistance mechanisms.

### *3.5. Overcoming HER-2 resistance by targeting node common to multiple resistance pathways: the theory of the 'Gordian knot'*

As described in our review, resistance pathways are multiple, interconnected but autonomous. Choosing to address each resistance pathway by an additional drug as proposed in the sedimentation theory might be challenged by the safety and cost considerations. The Holy Grail is to identify a critical node amongst the downstream effectors of the RTKs, from which the targeting would counteract or prevent multiple acquired resistance pathways. Zhang et al. [6] may have identified c-Src as one of these nodes. This tyrosine kinase appeared hyper-activated in cell line models of acquired trastuzumab resistance, involving various alternative RTKs pathways, suggesting a common node status. Interestingly, c-Src activation might be a consequence of PTEN loss, since Src Tyr416 is a direct substrate for PTEN protein phosphatase activity. Thus, in this model, *de novo* resistance directed by loss of PTEN may lead to acquired resistance throughout c-Src activation. As a confirmation, resistance to trastuzumab was reversed *in vivo* and *in vitro* by exposure to the Src inhibitor saracatinib combined with trastuzumab. Clinical validation of this model is warranted and might address to different concomitant mechanisms of HER-2 resistance.

## 4. Take home message

*De novo* or acquired resistance to trastuzumab and lapatinib, for example those discovered in preclinical studies, may lead to therapeutic failure in the clinic. Currently, different strategies are being explored, such as maintaining the therapeutic pressure on the HER-2 pathway while targeting other key points of regulation (as exemplified by the drug sedimentation concept). Identification of node targets common to multiple resistance pathways should revolutionise our understanding and clinical approach. Finally, current studies, in particular neo-adjuvant trials, will help to better characterise predictive factors of resistance, in order to individualise HER-2-targeted strategy. Nonetheless, considering the prolific but conflicting literature concerning mechanisms of resistance to anti-HER-2 therapies and predictive factors of such resistance, it is possible that no dominant marker will emerge and become useful for clinical practice, nor even that the ADCC-mediated immune mechanism of these antibodies constitutes their core effect, making exciting research for the future.

## Conflict of interest statement

None declared.

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