Unraveling trastuzumab and lapatinib inefficiency in gastric cancer: Future steps (Review)

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Abstract. The newly developed concept of oncogene addiction provides a rationale for the use of targeted therapies. In sharp contrast to the field of breast cancer treatment, attempts to target human epidermal growth factor receptor 2 (HER2) among gastric cancer (GC) patients have been unsatisfactory. The ToGA trial reported only a modest prolongation of progression-free survival (PFS) with trastuzumab and the subsequent TYTAN and LOGiC trials failed to demonstrate any survival advantage with lapatinib. These results suggest that a response to the molecular-targeted therapies is achieved in only a fraction of the patients; in addition, even responders may experience secondary resistance, with the efficacy of the treatment being decreased or abrogated over a short period of time. Considering the increased recognition of primary or acquired resistance, recent investigations on targeted therapies have been primarily focused on determining in advance the mechanisms that may mediate resistance to treatment and the methods through which such obstacles may be circumvented. The proposed molecules or mechanisms that may be responsible for the development of resistance to single HER2-targeted therapy include a dimerization partner or crosstalk with HER2, such as HER3 and MET, as well as any subsequent activation of their downstream pathways, which exhibit a partial overlap with those of HER2. Furthermore, genetic alterations that stimulate the aberrant activation of the pathways downstream of HER2 may be the underlying mechanisms that restore prosurvival signaling. These mechanisms generate a complex signaling network with a significant potential for signal amplification and diversification. Although in the early stages of description, several compounds have been suggested as next generation treatments for GC, with expectations for their delineating the function of such receptors or molecules, with subsequent contributions of specific survival signaling blockades. This review focuses on the current achievements of anti-HER2 therapies in GC and the plausible mechanisms of resistance to these therapies. Elucidating these mechanisms of resistance may provide valuable information pertinent to the design of future strategies to improve molecular-targeted therapies.

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1. Introduction

Gastric cancer (GC) is the fourth most commonly diagnosed cancer and the second leading cause of cancer-related mortality (1). GC is usually diagnosed at an advanced stage and patients with GC exhibit a poor prognosis. Overall 5-year survival rates are ~30% (2) in the USA and are <45% for stage III GC, even in Japan (3). In patients with advanced-stage disease, the median overall survival (OS) and median progression-free survival (PFS) were 9-13 and 6-7 months, respectively, even with the implementation of doublet (4) or triplet (5) therapeutic regimens. These results suggested that even the most widely accepted regimens, such as fluoropyrimidine and/or platinum-based chemotherapy regimens, have not been able to achieve satisfactory survival times. Thus, there is an urgent need to develop more efficient therapies.

Furthermore, conventional chemotherapy agents, which are predominantly effective on proliferating cells, often lack an effective chemotherapeutic window, as they are unable to discriminate between rapidly dividing normal cells (e.g., bone marrow and gastrointestinal tract cells) and tumor cells, leading to undesirable toxicities. Drug development in oncology aims for specificity in eliminating malignant cells with minimal side effects, thus creating a niche for targeted-driven approaches that are aimed at key molecules, which are overexpressed on tumors and are crucial for the development and spread of the disease.

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This conceptual shift focusing on key molecules has prompted interest in the development of molecular-targeted therapy. This type of therapy is based on the concept of oncogene addiction, i.e., certain cancers are apparently dependent on one or more genes for the maintenance of the malignant phenotype (6). This concept has led to the development of novel treatment strategies, where the removal or suppression of such a gene or genes or their coded molecules may specifically inhibit the growth of cancer cells, with resultant improved patient survival. Candidates for targeted molecules in the field of cancer research are members of membrane-bound human epidermal growth factor receptors (HERs), which consist of a ligand-binding domain on the extracellular surface, a single transmembrane segment and a cytoplasmic portion harboring the protein kinase activity. Among HER family members, such as human epidermal growth factor receptor (EGFR, also referred to as HER1), HER2, HER3 and HER4, HER2 has become the focus of investigations in GC.

The reported HER2 amplification in ~13-23% of GC cases (7) has prompted an investigation into the efficacy of targeting this molecule and the subsequent inhibition of the cell growth signaling cascade. HER2 overexpression is more frequent in intestinal compared with that in diffuse type GC and in cancers of the gastroesophageal junction compared with that in cancers located elsewhere in the stomach (7). As the overexpression of HER2 enables the constitutive activation of growth signaling pathways and thereby serves as an oncogenic driver, direct targeting of HER2 and inhibition of the HER2-activated signal transduction is likely to provide therapeutic possibilities for HER2-positive GC patients. Therefore, HER2 has become an attractive target in clinical trials; however, its efficacy was shown to be modest, or less satisfactory than what would be expected (8-10). These unsatisfactory results may be attributed to primary (*de novo*) or secondary (acquired) resistance to the HER2-targeted therapy. Therefore, the focus of future investigations should be the development of treatment strategies to overcome this resistance, on the basis of a better understanding of the molecular, structural and biological properties of HER2 and/or other HER family members. The aim of this review was to summarise the current achievements in targeting HER2 and the possible mechanisms underlying the limited efficacy of the HER2 axis-targeting therapies in GC. Furthermore, we aimed to provide insights for the development of strategies that may ultimately overcome resistance to or limited efficacy of anti-HER2 axis treatments for GC.

2. Current achievements with the clinical use of trastuzumab

Trastuzumab is a humanized monoclonal anti-HER2 antibody directed against the HER2 ectodomain. Although no specific ligands for HER2 have been identified (11), several extracellular and intracellular antitumor mechanisms of trastuzumab have been determined. These mechanisms include internalization and degradation of HER2 (12), downregulation of HER2 expression (13), inhibition of ligand-independent HER2 signaling (14), antibody-dependent cytotoxic activity (15), inhibition of p95HER2 formation, which is a constitutively active form of HER2 (16) and a predictor of unfavorable outcome (17), suppression of tumor angiogenesis (18) and inhibition of DNA damage repair (14).

Trastuzumab has been approved for the treatment of HER2-positive GC and a recent randomized ToGA trial (8) demonstrated that a combination of trastuzumab and chemotherapy achieved a clinical benefit compared to chemotherapy alone in terms of tumor response, PFS and OS. However, the absolute increase in the response rate with the addition of trastuzumab was only 12%, with an overall response rate of <50%. Furthermore, the GC patients who achieved an initial therapeutic response to trastuzumab eventually exhibited disease progression within 7 months. Those findings suggested that a significant proportion of patients with HER2-positive GC either do not satisfactorily respond to trastuzumab or develop an acquired resistance to this antibody. Indeed, the tumor response to trastuzumab treatment in HER2-positive breast cancer patients was limited and the majority of those who initially responded to trastuzumab ultimately became resistant during prolonged treatment (19,20). Those findings suggested that there may be a weakness in molecular-targeted therapies against single receptors, such as HER2, due to the potential activation of compensatory or overlapping survival pathways. Considering the increasing incidence in cancers of the gastroesophageal junction (21), in which HER2-overexpression is characteristic, the development of alternative strategies to overcome trastuzumab resistance is critical.

3. Lapatinib is currently unsatisfactory

Another molecule used for targeting HER2 is lapatinib. Lapatinib is a dual potent tyrosine kinase inhibitor that simultaneously curbs the phosphorylation of HER2 and EGFR, thus interrupting the EGFR/HER2-associated downstream signaling cascades. Theoretically, lapatinib and trastuzumab act synergistically, which was confirmed by the significantly improved PFS and OS in trastuzumab-refractory breast cancer patients undergoing lapatinib-trastuzumab combination treatment (22). Furthermore, lapatinib was also found to be effective against cancer cells expressing an aberrant form of HER2 (p95HER2), which may be one of the factors conferring trastuzumab resistance, as it lacks the extracellular domain of HER2 that is responsible for the impaired access of trastuzumab to the HER2 protein (23). A recent meta-analysis of randomized phase III trials clearly demonstrated the clinical benefits of lapatinib in the treatment of HER2-positive breast cancer patients (24).

With regard to GC, several preclinical studies demonstrated the antiproliferative effects of lapatinib (25), in addition to the synergic action of lapatinib with trastuzumab (26) or with other chemotherapeutic agents (25). Furthermore, lapatinib was shown to downregulate thymidylate synthase, which is a key determinant of fluoropyrimidine sensitivity, suggesting the potentiation of fluoropyrimidine efficacy (27). However, a phase II study on lapatinib as first-line treatment achieved only a 9% response rate, with a median OS of 4.8 months (28). Subsequently, a TYTAN study, comparing a combination of lapatinib and weekly paclitaxel to weekly paclitaxel alone as second-line treatment in HER2-positive GC, failed to demonstrate any OS and PFS benefit, although the median OS was prolonged by 2 months with lapatinib (9). Furthermore, the LOGiC phase III trial investigated the efficacy of lapatinib with capecitabine and

oxaliplatin in gastric, esophageal and esophagogastric adeno-carcinoma. The results of that trial were recently reported and demonstrated a non-significant prolongation (1.7 months) of OS with the addition of lapatinib (10).

The aforementioned limited efficacy of trastuzumab in the ToGA trial (8) and the unsatisfactory results of the TYTAN (9) and LOGiC trials (10) suggest the presence of drug resistance mechanisms or alternative pathways of escape from HER2-targeted therapy. To improve the treatment results of targeting HER2 in HER2-positive GC patients, there is an urgent need to elucidate the mechanisms underlying the alterations in tumor cell sensitivity to anti-HER2 therapy and to establish rational strategies to overcome this phenomenon. Therefore, the roles and functions of HER2 should be revisited and reconsidered.

4. HER2 is an orphan receptor

HER2 is a 185-kDa transmembrane receptor tyrosine kinase (29). Ligand-induced dimerization, with resultant auto-phosphorylation of their cytoplasmic domains, is a major mode of activation of receptor tyrosine kinases. In other HER family members, such as EGFR (HER1), HER3 and HER4, ligand binding to cognate HER includes conformational changes in the receptor, which subsequently promote homo- or heterodi-merization with other HER family members. By contrast, no ligands of HER2 have been identified (11). However, the over-expression of HER2 in cell lines leads to transformation in the absence of a ligand (30), suggesting that HER2 is an orphan receptor. Instead, HER2 is a preferred dimerization partner and is poised for interaction with other HER family members; thus, HER2 mainly functions as a shared co-receptor (31).

As the only member of the family lacking an exogenous ligand, HER2 is transactivated through heterodimerization with all the other HER family members. The HER2 extracellular domain is always in the open conformation, leading to autoactivation (32) in addition to the formation of heterodimers with other HER family members, even when monomeric (32,33), which may explain why HER2 is a preferred dimerization partner for other HER family members (31). Conversely, HER1 and HER3 cannot be transformed in the absence of a ligand (32). Notably, the HER2-containing heterodimers are the most potent complexes (34), leading to the activation of cascades in the downstream signaling transduction of the Ras/Raf/mitogen-activated protein kinase (MAPK) and phos-phatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathways (35). In HER2-positive GC cells, such pathways transmit signals promoting cell growth, differ-entiation, adhesion, migration and survival, contributing to tumor aggressiveness (35).

Among HER2-associated dimers, the HER2/HER3 heterodimer is considered to be the most active (29,36,37), since HER2 has no known ligand and the intrinsic tyrosine kinase of HER3 is defective. Indeed, the overexpression of HER3 has been associated with worse survival rates in various types of cancer (37). Furthermore, in GC, HER2 and/or HER3 overexpression were found to be significant predictors of poor survival (38-40). Therefore, inhibiting HER2/HER3 heterodimerization and targeting molecules involved in HER2-associated signal amplification and diversification may

be a viable strategy to potentiate the therapeutic activity of or overcome resistance to anti-HER2 therapy. There are several approaches to realize this potentiation, as described below.

5. Possible strategies to overcome *de novo* and acquired resistance to targeting HER2

Pertuzumab. In sharp contrast to trastuzumab, which binds to domain IV of HER2, pertuzumab is a monoclonal antibody that binds to domain II of HER2, the interface of dimer formation. As the HER2 domain IV is a region not involved in receptor dimerization (32), this may explain why HER ligands are able to induce the formation of HER2-containing heterodimers even in the presence of the trastuzumab, and why pertuzumab successfully inhibits the dimerization of HER2 with other HER family proteins (including HER3) and prevents ligand-dependent HER signaling (36). Previous *in vitro* studies have suggested that pertuzumab exhibits antitumor activity through HER2/HER3 signaling inhibition in non-small-cell lung (41) and breast cancer (42). A recent phase III trial (CLEOPATRA) demonstrated prolonged PFS (43) and OS (44) in metastatic breast cancer patients with the addition of pertuzumab to trastuzumab plus docetaxel, compared to trastuzumab plus docetaxel alone. A subsequent review demonstrated the clinical benefits of pertuzumab in several types of cancer (36).

The number of available studies on pertuzumab in GC, even in preclinical models, is limited. A HER2-positive GC xenograft model consistently demonstrated enhanced antitumor activity with the combination of pertuzumab and trastuzumab (45).

Targeting HER3. HER3 cannot form homodimers and only forms heterodimers that are required for activating signaling pathways (46). HER3 has been disregarded as a cancer target due to the absence of intrinsic kinase activity in its intracellular domain. However, HER3 is currently recognized as one of the key molecules associated with resistance to HER2-targeted therapies. HER3 was shown to potently activate the PI3K/Akt signaling pathway, despite its lack of intrinsic kinase activity, through its six docking sites for the p85 subunit of PI3K (47,48), which allow HER3 to funnel its signals as a scaffold protein and, consequently, to evade the actions of trastuzumab or HER-associated tyrosine kinase inhibitors. Therefore, the upregulated activity of HER3 may be a means of escape from therapeutic suppression by anti-HER2 agents, rendering currently available anti-HER2 therapies consider-ably less effective. Therefore, the HER2/HER3 heterodimer is considered to be a functional HER signaling unit. For example, upregulation of HER3 was observed in trastuzumab-resistant breast cancer cells following long-term exposure to trastu-zumab (49). Furthermore, preferential phosphorylation of HER3 was observed in HER2-amplified breast cancer tissues (42). Furthermore, the loss of HER3 compromised the viability of HER2-overexpressing breast cancer cells (50). An *in vitro* study on HER2-positive breast cancer cells demon-strated a PI3K/Akt-dependent upregulation of HER3 mRNA following inhibition of HER2 tyrosine kinase with lapatinib, suggesting a HER3-mediated compensation pathway in the presence of HER2 inhibition (51). Indeed, EGFR and HER2

Table I. Ongoing clinical trials investigating molecules that may overcome *de novo* or acquired resistance to trastuzumab in gastric cancer.

Molecules	Trial number	Conditions	Combined agents	Phase	Refs.
Afatinib[a]	NCT01649271	HER2-positive gastric or breast cancer	Trastuzumab	Phase I	(53,78)
	NCT01522768	HER2-positive, trastuzumab-refractory esophagogastric cancer	None	Phase II	
LJM716[b]	NCT01602406	HER2-positive, trastuzumab-refractory gastric or breast cancer	Trastuzumab	Phase I	(79)
MK-2206[c]	NCT01705340	HER2-positive breast, gastric, or gastroesophageal cancer	Trastuzumab, lapatinib	Phase I	(80)
MM-111[d]	NCT01774851	HER2-positive distal esophageal, gastric, or gastroesophageal cancer	Trastuzumab, paclitaxel	Randomized phase II	(81)
Pertuzumab[e]	NCT01461057	HER2-positive gastric or gastroesophageal cancer	Trastuzumab, cisplatin, capecitabine	Phase II	(36)
	NCT01774786	HER2-positive gastric or gastroesophageal cancer	Trastuzumab, cisplatin, capecitabine (or 5-fluorouracil)	Phase III	
Poziotinib[f]	NCT01746771	HER2-positive gastric or gastroesophageal cancer	Trastuzumab, paclitaxel	Phase I/II	(82)

[a]Inhibitor of all HER family receptor tyrosine kinases. [b]Anti-HER3 antibody. [c]Akt inhibitor. [d]Bispecific antibody that targets HER2 and HER3. [e]Anti-HER2 domain II. [f]Pan-HER inhibitor. HER, human epidermal growth factor receptor.

targeting was succeeded by a feedback upregulation of HER3 (52). Those findings suggested that the HER3-PI3K axis may be essential for the survival of HER2-dependent cells and the ligand-less HER2 and kinase-dead HER3 may form a formidable molecule when dimerized. Since HER3 may weaken the action of anti-HER2 therapy, the combined targeting of HER2 and HER3 may act synergistically on HER2 and HER3 downstream signaling pathways and may be of therapeutic value. In GC, this hypothesis was confirmed in a preclinical study, in which a pan-HER inhibitor was shown to act synergistically with trastuzumab (53).

Inhibition of MET. A functional crosstalk between MET and HER family members has been reported in the context of the acquisition of invasive and progressive phenotypes (54-56). The receptor tyrosine kinase MET stimulates cell proliferation and invasion via the activation of the MAPK and the PI3K/Akt axis, which are also involved in the downstream signaling cascade of the HER family (57). For example, gefitinib-sensitive lung cancer cell lines reportedly developed resistance to gefitinib as a result of the amplification of the MET gene and the subsequent HER3-dependent PI3K activation (58). Similarly, MET contributed to evoking resistance to anti-EGFR therapy in colorectal cancer (59) and to anti-HER2 therapy in breast cancer (60). Those findings suggested that MET may be involved in the compensation for HER family inhibition.

With regard to GC, amplification of the MET gene and overexpression of the MET protein were observed in 10-20 and 50% of tumors, respectively (61-63). The hepatocyte growth factor-mediated activation of MET may also mediate resistance to lapatinib in HER2-amplified GC cell lines by restimulating downstream signaling (64). It was demonstrated that MET and HER family members form an extensive network of downstream signaling in the MKN-45 GC cell line (65). Furthermore, the MET-independent reactivation of the MAPK or PI3K pathways may alleviate the growth-inhibiting effects of MET kinase inhibition (66). Those findings suggested that the MET and HER families may cooperate, in the sense that inhibition of one receptor may drive cells to activate alernative oncogenic signaling pathways. These mechanisms provide a rationale for the use of MET inhibitors to overcome resistance to HER2 axis inhibition. However, one should bear in mind that HER3 may also play a significant role in the acquisition of resistance to MET inhibition. GC cells resistant to MET inhibitors were shown to overexpress HER3 and HER3 silencing led to a reversion of the resistance to MET inhibitors, suggesting that the overexpression of HER3 may contribute to the development of resistance to MET inhibitors (67).

Targeting molecules downstream of HER2. Since the PI3K/Akt/mTOR cascade is a pathway downstream of HER2 and phosphatase and tensin homolog (PTEN) antagonizes the function of PI3K, the constitutive activation of PI3K occurs via two mechanisms: loss of PTEN function or activation of mutations in the gene encoding the catalytic PI3K subunit p110α (PIK3CA). In breast cancer, the decreased expression of PTEN was found to be significantly correlated with a lower response to trastuzumab (68), whereas the introduction of antisense PTEN was associated with decreased sensitivity to trastuzumab (69), suggesting that PTEN deficiency may confer resistance to trastuzumab. Furthermore, PI3K inhibitors were shown to overcome trastuzumab resistance in PTEN-deficient breast cancer cells (68). PIK3CA activates Akt signaling, induces cell transformation (70), attenuates apoptosis and facilitates tumor invasion (71). Since gain-of-function muta-

tions in PIK3CA may be involved in modulating the efficacy of HER2-directed therapies, the inhibition of Akt signaling effectively suppresses the growth of cancer cells that are dependent on this pathway (72). Indeed, PIK3CA mutations have been associated with shorter PFS after trastuzumab therapy (73). Those results suggested that trastuzumab resistance due to the aberrant activation of PI3K/Akt/mTOR signaling may be resolved by the inhibition of this pathway.

In GC, PIK3CA amplification was observed in 36% of GC tissues and 60% of GC cell lines and was associated with Akt activation (74). Since mutations and loss of heterozygosity of PTEN were observed in 10 and 17% of GC, respectively (75,76), inhibitors of the PI3K/Akt/mTOR pathway activated by PTEN loss may resolve *de novo* and acquired non-responsiveness to trastuzumab. Notably, mutations of PTEN and PIK3CA are mutually exclusive (74). This may explain why PTEN loss or PIK3CA mutations were not individually sufficient to predict a diminished response to trastuzumab-based therapy, whereas their combinations were (77). Elucidating the genetically defined response mechanisms may indicate the optimal target therapy for each patient.

6. Ongoing clinical trials

Numerous molecules designed to overcome resistance to anti-HER2 therapy were introduced over the last few years. Table I lists the ongoing clinical trials investigating novel molecules that may help overcome primary and acquired trastuzumab resistance in GC. These molecules include afatinib [irreversible inhibitor of all HER family receptor tyrosine kinases (53,78)], LJM716 [anti-HER3 antibody (79)], MK-2206 [Akt inhibitor (80)], MM-111 [bispecific antibody that targets HER2 and HER3 (81)], pertuzumab and poziotinib [pan-HER inhibitor (82)]. These molecules are administered alone or in combination with trastuzumab and/or lapatinib, with or without concurrent chemotherapy.

7. Future perspectives

Anti-HER2 therapy, such as trastuzumab and lapatinib, accrues clinical benefit in certain GC patients; however, a number of patients exhibit primary resistance and those who initially respond to treatment may eventually develop secondary resistance, suggesting that other factors that involve resistance or hinder response should be considered prior to treatment or at the time of resistance development. Two major categories of resistance mechanisms to anti-HER2 therapy have been proposed: *de novo* resistance, due to genetic alterations of HER2 and its downstream signaling targets; and acquired resistance, primarily due to alternative pathway activation to compensate for HER2 inhibition.

Although the precise mechanisms underlying therapeutic responsiveness have not been fully elucidated, even in oncogene-addicted tumors, it is essential to understand that the targeting of one receptor may be neutralized or attenuated by the parallel activation of other receptors, molecules and pathways. Such an orchestration bestows resistance on cancer cells, helping them escape from targeted therapies. For example, the signal elicited by a heterodimerized receptor is not simply the sum of the signaling properties of the individual dimerization partners. The complexity of these plausible resistance mechanisms underlines the potential limitations of monotherapy and should prompt a conceptual change from inhibiting only one molecule to a combined oncogenic pathway inhibition. Therefore, it is advisable to employ combinations of targeted therapies that may delineate more than single HER2-associated biological outcomes, thereby yielding maximal therapeutic benefit.

The key goals of future studies in the research field of GC treatment should include the development of novel therapeutics that overcome resistance to anti-HER2 therapy effectively and safely, with the identification of accurate predictors of response. For these purposes, certain compounds have already entered clinical development, for the targeting of other receptors or molecules synergizing with HER2. Accordingly, it was attempted to use these compounds in combination with current anti-HER2 therapy, rather than as single agents. Although clinical proof of the concept behind these agents has not yet been provided, this is anticipated in the immediate future and may further accelerate the inclusion of such inhibitors in combination treatments, as part of a repertoire of more effective anticancer agents. Parallel efforts should also be focused on identifying suitable biomarkers for the selection of the optimal combination agents and the patients who are most likely to benefit from such treatment. Ideally, the predictive biomarkers for targeted therapy should be identified by feasible and reliable assays, so that they are applicable to other targeted therapies.

Considering that oncogene addiction is commonly observed in several types of cancer, the mechanisms of abnormal interplay between molecules involved in survival cascades, as well as novel agents that effectively inhibit the processes identified in a specific type of cancer, may also be applied to other types of cancer. This approach may provide more flexibility in terms of targeting resistance mediators in one type of cancer to be applicable to other cancers that share common resistance mechanisms. Expanding the understanding of resistance mechanisms from 'each-organ based' to a 'trans-organ basis' may facilitate the establishment of more global treatment strategies against tumors addicted to a common oncogenic pathway, rather than against individual tumors. We are confident that such joint efforts, i.e., the continued exchange of information between the clinical fields of each cancer, or the translation of the findings from preclinical studies into the clinical realm, will uncover novel agents that underlie clinical responses and overcome resistance to molecular-targeted therapy. This may help establish effective treatment strategies rationally rather than empirically. These novel agents, once verified and validated, may become the next 'gold standard' among cancer therapies and, if combined with reliable biomarkers for resistance and response prediction, may ultimately introduce a new era of personalized medicine.

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