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Review

EGFR and HER-2 Antagonists in Breast Cancer

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Abstract. *Both HER-2 and EGFR are expressed in breast cancer and are implicated in its development and progression. The discovery of the association between HER-2 gene amplification and poor prognosis in breast cancer led to the development of HER-2 targeted therapies. Trastuzumab, a monoclonal antibody to HER-2, has significantly improved the prognosis for HER-2-positive breast cancer patients. It is now approved for the treatment of both HER-2-positive metastatic breast cancer and early stage HER-2-positive breast cancer. Recent results from trials of the dual HER-2 and EGFR tyrosine kinase inhibitor, lapatinib, also show very promising results in HER-2-positive breast cancer. A number of EGFR inhibitors have been tested in breast cancer clinical trials, but with limited effect. This may be due to difficulty in selecting the appropriate patient population, caused by the lack of definitive predictive markers for response to EGFR inhibition.*

The human epidermal growth factor receptor (EGFR/HER) family of tyrosine kinases has been implicated in the development and progression of human cancer. Expression of all four members of the EGFR family (EGFR/HER-1, HER-2, HER-3, HER-4) have been reported in breast cancer (1). Ligands for EGFR, HER-3 and HER-4 are also detected frequently in breast cancer (2, 3). HER-2 does not bind any known ligands but is activated by heterodimerisation with other members of the EGFR family.

Studies in transgenic mouse models have demonstrated a role for EGFR in normal mammary epithelial development and in tumorigenesis (1). Frequent amplification and overexpression of HER-2 in breast cancer suggests a significant role in the development of breast tumours. Direct evidence of the involvement of HER-2 in mammary tumorigenesis was obtained using transgenic mice expressing an activated form of the *HER-2/neu* oncogene in the mammary epithelium, which resulted in rapid induction of mammary tumours in 100% of female transgenic carriers (4).

Recent microarray analysis of breast tumours has identified four distinct sub-types of tumours (5, 6). The sub-types are classified as (i) luminal (further sub-divided into A and B) (ii) HER-2-positive/estrogen receptor (ER) - negative, (iii) normal-like and (iv) basal-like. The basal-like sub-type lacks expression of ER, progesterone receptor (PR) and HER-2, but expression of EGFR is more frequently observed in this sub-type. A number of studies have shown that the HER-2-positive and the basal-like sub-types have a poorer prognosis than either luminal or normal-like tumours (5-7).

While significant progress has been made on targeting HER-2 in breast cancer, targeting EGFR has had limited success to date. The progress to date on targeting HER-2 and EGFR, individually, and in combination, in breast cancer will be reviewed.

HER-2 in Breast Cancer

The first association between *HER-2* amplification and prognosis in breast cancer was reported by Slamon *et al.* in 1987 (8). The *HER-2/neu* oncogene was amplified (2-20 fold) in 30% of breast tumors examined (n=189) and was a significant predictor of both overall survival and time to relapse. *HER-2/neu* amplification also had greater prognostic significance than traditional prognostic factors, in lymph node-positive disease. Subsequent studies also showed that HER-2 protein is a significant independent predictor of both the disease-free and the overall survival in node-positive breast cancer (9-11).

HER-2 Targeted Therapies

Trastuzumab. Trastuzumab (Herceptin™, Genentech), a recombinant humanised monoclonal antibody against HER-2, was developed from the murine antibody 4D5 which

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inhibited the growth of tumour cells overexpressing HER-2. Trastuzumab demonstrated an *in vivo* antiproliferative effect in HER-2 overexpressing tumours and also demonstrated synergy with several chemotherapeutic drugs *in vitro* (12-15).

Early studies with trastuzumab as a single agent in HER-2-positive metastatic breast cancer achieved overall responses of 11.6 and 15% for patients who had progressed after chemotherapy (16-18). As a first-line treatment for metastatic breast cancer, trastuzumab showed response rates of 26% in HER-2-positive patients and responses of 35% in patients with 3+ HER-2 overexpression by immunohistochemistry and 34% in patients positive for *HER-2* gene amplification by fluorescence *in situ* hybridisation (FISH) (17). A pivotal phase III trial of trastuzumab in combination with chemotherapeutic agents demonstrated an overall response rate of approximately 50% (*versus* 32%), longer duration of response (time to progression; 7.4 *versus* 4.6 months), longer survival (overall survival: 25.1 *versus* 20.3 months) and a 20% reduction in risk of death compared to chemotherapy alone in HER-2 overexpressing metastatic breast cancer (19).

Several clinical trials of trastuzumab in combination with chemotherapy agents have been conducted and have shown response rates ranging from 24% to 84% (20). A fraction of patients treated with trastuzumab develop clinical heart failure with a decrease in cardiac contractile function. This cardiotoxicity was most notable in patients who concurrently received anthracyclines (19). On the basis of these findings, concurrent use of anthracyclines and trastuzumab is not recommended.

HER-2 is expressed in adult cardiac myocytes, along with HER-4, and transmits growth and survival signals in response to the ligand neuregulin-1. Neuregulin treatment protects cardiomyocytes from cell death induced by anthracyclines, therefore inhibition of HER-2 signalling by trastuzumab may enhance the cytotoxic effects of anthracyclines in cardiomyocytes, increasing the likelihood of cardiac dysfunction (21, 22).

Combinations of trastuzumab with taxanes, platinum compounds and vinorelbine are safe and show favourable efficacy. The combination of a taxane with trastuzumab is currently considered the best first-line option for HER-2 overexpressing metastatic breast cancer (20). Novel combinations of trastuzumab with gemcitabine and cisplatin are currently under investigation (23). HER-2 overexpression has also been associated with resistance to hormonal therapy in ER-positive tumour cells (24). Combinations of trastuzumab with hormonal therapies, such as tamoxifen and aromatase inhibitors are also currently under investigation (25). A Phase III trial of trastuzumab plus anastrozole compared to anastrozole alone in postmenopausal women (TAnDEM study) showed significant improvements in progression-free survival, clinical response and time to progression in the trastuzumab plus anastrozole arm (26).

The most exciting advancement in the treatment of HER-2-positive breast cancer came from the results of five randomised trials showing the benefits of trastuzumab treatment for early stage HER-2 overexpressing breast cancer (Table I). These studies demonstrated that addition of trastuzumab to adjuvant therapy resulted in 39-52% reduction in disease recurrence (27). A statistically significant survival benefit was also seen in the combined analysis of the NSAPB-B31 and NCCTG N9831 trials (28).

Analysis of the trastuzumab-treated patients in the NSAPB-B31 study suggests that tumours which have co-amplified *c-myc* and *HER-2* show the best response to trastuzumab. The pro-apoptotic function of dysregulated c-myc may be balanced by the anti-apoptotic effects of HER-2 in HER-2 overexpressing cells and treatment with trastuzumab may then result in c-myc-mediated apoptosis induction (29).

In the BCIRG006 study, the combination of trastuzumab, docetaxel and carboplatin, which was based on laboratory observations, was tested along with adriamycin/cyclophoshophamide followed by docetaxel alone or in combination with trastuzumab (28). There was no significant difference between the two trastuzumab containing arms of the study (30). However, analysis of *topoisomerase II alpha* (*TOP2A*) amplification was carried out and *TOP2A* was co-amplified in one-third of HER-2-positive patients (31). Several studies have suggested an association between amplification of *TOP2A* and response to anthracycline treatment (32, 33). The first interim analysis of the BCIRG006 study suggested that patients with co-amplification of *TOP2A* may respond better to anthracycline containing regimens. However, the second interim analysis showed no difference in disease-free progression between the anthracycline and non-anthracycline containing arms of the study (34).

Based on the dramatic results of these adjuvant studies, the U.S. Food and Drug Administration recently approved the use of trastuzumab, in combination with chemotherapy, for the treatment of HER-2-positive breast cancer after surgery.

A number of studies have evaluated the addition of trastuzumab to chemotherapy in the neo-adjuvant setting and have achieved pathological complete response (pCR) rates of 12-65% and clinical complete response rates of 30-86% (20). A randomised trial to compare the trastuzumab plus chemotherapy to chemotherapy alone in the neo-adjuvant setting was performed in 42 patients. The trial was stopped early by the Data Monitoring Committee because of the superior results observed for the trastuzumab plus chemotherapy group (pCR 66.7% *versus* 25%) (35). Other trials of trastuzumab alone and in various combinations, for neo-adjuvant therapy are ongoing.

Table I. *Adjuvant trastuzumab clinical trials.*

Trial	Treatment	Hazard ratio for disease-free survival (95% CI) *P*-value	Ref.
NSAPB	1. AC x 4 (q3w) → P x 4 (q3w)		
	2. AC x 4 (q3w) → P x 4 (q3w) + T (qw) → T 1 yr	0.48 (0.39-0.59)	
		<0.0001	(28)
N9831	1. AC x 4 (q3w)→ P x 12 (qw)		
	2. AC x 4 (q3w)→ P x 12 (qw) + T (qw) → T 1 yr		
	3. AC x 4 (q3w)→ P x 12 (qw) → T 1 yr		
HERA	Any adjuvant chemotherapy followed by:		
	1. Observation		
	2. T (q3w) 1 yr	0.54 (0.43-0.67)	
	3. T (q3w) 2 yrs	<0.0001	(86)
BCIRG006	1. AC x 4 (q3w) → D x 4 (q3w)		
	2. AC x 4 (q3w) → D x 4 (q3w) + T (qw) → T 1 yr	0.49 (0.37-0.65)	
		<0.0001	(30)
	3. Carboplatin + D x 6 (q3w) + T (qw) → T 1 yr	0.61 (0.47-0.79)	
		0.0002	
FinnHER	1. D x 3 (q3w) → CEF x 3 (q3w) (+ Tam for ER or PR +ve)		
	→ HER-2 +ve: +/– D x 9 (qw) +/– T x 9 (qw)	0.43	
	2. V x 8 (qw) → CEF x 3 (q3w) (+ Tam for ER or PR +ve)	0.0078	(87)
	→ HER-2 +ve: +/– V x 9 (qw) +/– T x9 (qw)		

A: adriamycin; C: cyclophosphamide; P: paclitaxel; T: trastuzumab; D: docetaxel; Tam: tamoxifen; E: epirubicin; F: 5-fluorouracil; V: vinorelbine.

Pertuzumab. Pertuzumab (Omnitarg™, Genentech) is also a humanised monoclonal antibody against HER-2 but its mechanism of action is different to trastuzumab. Pertuzumab binds to domain II of HER-2, sterically blocking a binding pocket necessary for receptor dimerisation and signalling (36). Thus, pertuzumab inhibits ligand-induced heterodimerisation of HER-2 and is therefore more effective than trastuzumab, in cells which express low levels of HER-2 (37). *In vitro* studies have shown that pertuzumab combined with trastuzumab is synergistic in HER-2 overexpressing cell lines (38). A phase I study in patients with advanced cancer showed that pertuzumab is well tolerated and clinically active (39). However, a phase II study in patients with metastatic breast cancer with low expression of HER-2, reported that pertuzumab was safe but showed limited activity as a single agent in this population (40). Pertuzumab also disrupted heterodimerisation between HER-2 and the insulin-like growth factor receptor (IGF-IR) in trastuzumab-resistant cells (41). Therefore, pertuzumab may play a role in overcoming resistance to trastuzumab treatment. A clinical trial of pertuzumab combined with trastuzumab, in patients who did not respond to trastuzumab, is currently ongoing (protocol id: NCI-06-C-0035).

Lapatinib. Lapatinib (Tykerb™, GlaxoSmithKline) is an orally-administered small molecule tyrosine kinase inhibitor of both HER-2 and EGFR. Lapatinib binds reversibly to the ATP-binding site of both receptors and blocks receptor phosphorylation and activation (42). Unlike trastuzumab, which binds to the extracellular domain of HER-2, lapatinib can also inhibit the p95 truncated form of HER-2 which lacks the extracellular domain (43). Lapatinib inhibits activation of EGFR, HER-2, Akt and Erk 1/2 both *in vitro* and in tumour xenografts (44). Lapatinib inhibited proliferation in breast cancer cell lines that overexpress either EGFR or HER-2 (45). Lapatinib has a concentration-dependent effect on proliferation in breast cancer cell lines and response correlates with its ability to inhibit HER-2, Raf, Akt and Erk phosphorylation. Furthermore, synergistic inhibition of proliferation was observed for combined lapatinib and trastuzumab treatment in four HER-2 overexpressing cell lines (46). Lapatinib in combination with tamoxifen also effectively inhibited the growth of tamoxifen-resistant tumour cells in a xenograft model of HER-2-overexpressing tamoxifen-resistant breast cancer (47). Lapatinib has been shown to induce apoptosis in tumour cells *in vivo* (48) and the combination of lapatinib and trastuzumab enhanced apoptosis-induction in HER-2 overexpressing breast cancer cells *in vitro* (49).

A phase I study in heavily pre-treated patients with metastatic carcinomas established that lapatinib is well tolerated when administered at doses of 550 to 1600 mg daily. Clinical activity was observed in patients with EGFR-positive and/or HER-2 overexpressing metastatic cancers, including four partial responses in patients with trastuzumab-resistant breast cancers (50). No evidence of

lapatinib-related cardiac dysfunction was observed in the study. Two phase II trials of single-agent lapatinib have been conducted in refractory metastatic breast cancer, one in HER-2 overexpressing metastatic breast cancer, with progressive disease on prior trastuzumab-containing regimens, and the second in metastatic breast cancer patients who developed progressive disease following prior treatment with anthracyclines, taxanes and capecitabine (including HER-2 overexpressing trastuzumab-refractory and HER-2 non-overexpressing metastatic breast cancer in two separate arms).The overall response rates were 22% and 14% respectively for these two studies (51). Combined biomarker analysis for the two studies suggested that metastatic breast cancer patients were more likely to respond if their tumours were ER- and PR-negative, and HER-2 overexpressing. A decrease in serum levels of the extracellular domain of HER-2 also correlated with clinical response (52).

A phase III trial of lapatinib and capecitabine *versus* capecitabine alone was conducted in patients with HER-2-positive refractory advanced or metastatic breast cancer who were previously treated with anthracycline, taxane and trastuzumab (53). The study was stopped at the interim analysis because lapatinib combined with capecitbine demonstrated a clinically meaningful and statistically significant improvement in median time to progression *versus* capecitabine alone (8.5 months *versus* 4.5 months). In the combination arm, four patients (n=160) experienced treatment-related cardiac events and all fully recovered. In addition, fewer patients in the combination arm developed brain metastases compared to the single-agent capecitabine group. This difference may be due to the ability of lapatinib to cross the blood brain barrier. Trastuzumab cannot cross the blood brain barrier and therefore is not effective for treatment of brain metastases (54). A phase II trial of lapatinib in HER-2 overexpressing breast cancer patients with new or progressing brain metastases showed some evidence of clinical activity (55).

Interim results of a phase II trial of lapatinib as a first-line treatment in locally advanced or metastatic breast cancer with HER-2 amplification have shown a 35% partial response (56). Several trials of lapatinib in combination with trastuzumab, chemotherapy and endocrine therapies are ongoing in breast cancer. A phase II trial of lapatinib after adjuvant chemotherapy has recently been initiated in early stage breast cancer (42).

EGFR in Breast Cancer

Studies in the late 1980s and early 1990s showed that EGFR overexpression in breast cancer is an indicator of poor prognosis. In a study of 135 primary breast cancers, the presence of EGFR was the most important variable for predicting relapse-free and overall survival (57). Relapse-free survival was significantly worse for EGFR-positive patients than EGFR-negative patients, particularly in node-positive patients (58). Patients that were positive for both HER-2 and EGFR had a particularly high risk for relapse. In node-negative patients, EGFR levels were superior to ER in predicting relapse and survival (59). EGFR overexpression was also associated with failure to respond to endocrine therapy in ER-positive breast cancer (60, 61).

In a recent study of a large cohort of breast cancer patients (n=807), EGFR expression was detected in 15% of tumours. Patients whose tumours demonstrated HER-2 phosphorylation (4.6%) or co-overexpression of HER-2 and EGFR (13.3%) had the shortest survival (62). Coexpression of EGFR and HER-2 also modulated response to trastuzumab treatment in HER-2 overexpressing breast cancer cell lines (63). Another potentially interesting sub-group for EGFR inhibition are triple negative or basal-like breast cancers which frequently overexpress EGFR (64).

Given the evidence for the role of EGFR in breast cancer and the development of several small molecule inhibitors and monoclonal antibodies to EGFR, there was great hope for the benefits of targeting EGFR in breast cancer. However, the results so far have been disappointing. This may be due to difficulties in selecting the patients who are most likely to respond to EGFR inhibition. In addition, unlike HER-2, *EGFR* is rarely amplified in breast cancer and overexpression of EGFR protein does not appear to predict response to EGFR inhibition.

EGFR Targeted Therapies in Breast Cancer

Gefitinib. Gefitinib (Iressa™, AstraZeneca) is a small molecule inhibitor of EGFR, which binds reversibly to the ATP binding site of EGFR. Phase II trials of gefitinib in refractory metastatic breast cancer yielded disappointing response rates of 2-13% (65). A phase II study of gefitinib in advanced breast cancer showed poor clinical activity and examination of biological profiles in the tumour tissues suggested that the lack of activity was not due to lack of receptor inhibition in the tumours but rather to lack of EGFR dependence in the tested population (66). A phase II study of gefitinib in combination with paclitaxel and carboplatin as first-line therapy for advanced breast cancer showed no benefit of addition of gefitinib based on previously reported response for the combination of paclitaxel and carboplatin alone (67).

Preclinical testing showed that gefitinib was active and restored sensitivity to docetaxel or paclitaxel in multidrug-resistant, taxane-resistant human breast cancer cells (68). Based on this data, a phase II trial of docetaxel in combination with gefitinib, as first-line treatment, was conducted in patients with metastatic breast cancer (69).

The overall response rate was 54% (22/41) with 5 complete responses and 17 partial responses observed. Six patients had stable disease and 13 patients progressed. The authors conclude that this is an active and generally well-tolerated regimen in women with metastatic breast cancer who have not been previously treated for metastatic disease and that the response rates observed are comparable to taxanes combined with anthracyclines. An interesting correlation was observed between ER status and response, with ER-positive patients showing a 70% response rate compared to 21% in ER-negative patients. Studies in the ER-positive cell line, MCF7, have shown that the development of tamoxifen resistance was associated with increased expression of both EGFR and HER-2 and tamoxifen-resistant cells show an increased dependence on EGFR signalling and increased sensitivity to gefitinib (60). Therefore, Ciardiello *et al.* (69) propose that the increased response in the ER-positive patients may have been due to the development of EGFR-dependence in these tumours following up to 5 years of tamoxifen treatment in the adjuvant setting.

The potential benefits of gefitinib treatment in overcoming tamoxifen resistance have also been investigated. A phase II trial of gefitinib was conducted in tamoxifen-resistant ER-positive breast cancer and ER-negative breast cancer (70). Of the three ER-positive evaluable patients, 1 patient had a partial response and 2 had stable disease whereas of the 16 evaluable with ER-negative tumours, only 1 patient had a partial response, 1 patient had stable disease and 14 patients had progressive disease. A pre-operative trial of gefitinib alone or in combination with the aromatase inhibitor, anastrozole was conducted in postmenopausal patients with ER-positive and EGFR-positive primary breast cancer (71). Tumour cell proliferation, assessed by measuring Ki67 levels, was decreased by 98% in the gefitinib plus anastrozole group *versus* 92% in the gefitinib alone group, suggesting a direct anti-proliferative effect of gefitinib. Tumour size was reduced by 30-99% (partial response) in 14 of 28 patients assigned gefitinib and anastrozole and in 12 of 22 assigned gefitinib, as assessed by ultrasonography.

There is also evidence that within the ER-positive group of patients those that are PR-negative may respond better to gefitinib treatment. BCIRG103 studied gefitinib treatment in the pre-operative setting and assessed biological markers of response. They found that the ER-positive/PR-negative and the HER-2 amplified sub-groups showed the greatest inhibition of tumour cell proliferation (72).

Improving response to EGFR inhibitors such as gefitinib will require better definition of the patient groups that are likely to benefit from EGFR inhibition such as the ER-positive, EGFR-positive population and the HER-2-positive, EGFR-positive population.

Erlotinib. Erlotinib (Tarceva™, OSI Pharmaceuticals) is also a small molecule inhibitor of EGFR and also binds reversibly to the ATP binding site of EGFR. Similar to gefitinib, erlotinib has also shown good preclinical activity against breast cancer cells *in vitro* and in tumour xenografts (73, 74). However, also similar to gefitinib, preliminary phase II trials of erlotinib in combination with chemotherapy have shown disappointing response rates in first- or second-line treatment (75). Erlotinib combined with docetaxel produced a partial response rate of 55% compared to response rates of 29-53% which have previously been reported for docetaxel alone in metastatic breast cancer (76). A number of studies of erlotinib in combination with chemotherapy and hormone therapies are ongoing in breast cancer.

Cetuximab. Cetuximab (Erbitux™, ImClone Systems Incorporated) is a monoclonal antibody to EGFR. Cetuximab binds to EGFR with high affinity, competes for ligand binding and blocks activation of the receptor tyrosine kinase by EGF or TGFα. It also induces antibody-mediated receptor dimerisation resulting in receptor down-regulation, which may be important for its growth inhibitory effects. Results of a phase I trial of cetuximab in combination with paclitaxel in advanced breast cancer were recently reported. However, based on prohibitive dermatological toxicities and disappointing preliminary efficacy, the combination of paclitaxel and cetuximab was not considered promising in this population (77). A phase II study of cetuximab in combination with carboplatin, in triple negative breast cancer, is currently ongoing.

Dual Targeting of EGFR and HER-2 in Breast Cancer

Evidence from cell line models and from patient samples suggest that dual targeting of EGFR and HER-2 may be beneficial for HER-2 overexpressing breast cancer. In HER-2 overexpressing cell lines, 2 EGFR coexpression, interactions between EGFR and HER-2, as well as the presence or absence of growth factors influence the response to trastuzumab (63, 78). Immunohistochemical analysis of EGFR expression in HER-2-positive breast tumours suggest that EGFR is frequently expressed in HER-2-positive tumours and co-expression of EGFR and HER-2 is associated with a worse prognosis (62, 79). Combinations of the EGFR inhibitors, gefitinib or cetuximab with trastuzumab have been tested in cell line models and show that the response to trastuzumab was enhanced by dual targeting of EGFR and HER-2 (80, 81). Based on the preclinical data, a phase I/II study of trastuzumab and gefitinib in HER-2 overexpressing breast cancer was completed (82). Disappointingly, few responses were observed and only in previously untreated patients

(2/28), and time to progression was shorter than reported for trastuzumab alone. However, Normanno *et al.* (83) argued that the dose used in the phase II part of the study may have been too low to see any benefit. A phase I/II trial to determine the best dose of docetaxel given in combination with gefitinib and trastuzumab for metastatic breast cancer is currently open. Following completion of a phase I trial (84), a phase II trial of erlotinib in combination with trastuzumab as first-line treatment for HER-2-positive metastatic breast cancer is currently ongoing, as is a phase I study of cetuximab in combination with trastuzumab.

We and others have shown that combined treatment with trastuzumab and the dual kinase inhibitor, lapatinib, exhibits synergy in HER-2 overexpressing breast cancer cells (85, 49) and a number of trials of combinations of trastuzumab and lapatinib alone or with chemotherapy are underway.

Future Directions

The role of HER-2 antagonists in the treatment of HER-2-positive breast cancer is now well established. Trastuzumab has had a significant impact on improving the outcome for patients with HER-2-positive breast cancer and is now approved for the treatment of both metastatic and early-stage breast cancer. Very promising results are also emerging from clinical trials with lapatinib. A key area in the future development of HER-2 antagonism in breast cancer will be identifying and overcoming inherent and acquired resistance to HER-2 inhibitors.

The future of EGFR antagonists is less certain and requires significant translational and clinical research in order to better define the patient populations that will benefit from EGFR inhibition. EGFR inhibition may play an important role in overcoming resistance to endocrine therapies and may provide the first targeted therapy option for the treatment of triple negative breast cancer. Dual targeting of EGFR and HER-2 may provide added benefit for a subset of HER-2-positive patients whose tumours also express EGFR.

Combinations of HER-2 and EGFR inhibitors with other targeted therapies are also under investigation in breast cancer, for example combinations with anti-angiogenic therapies such as bevacizumab. Several newer HER-2 and EGFR antagonists are also in the early stages of preclinical development. The key to the clinical success of any of these novel targeted therapeutic agents is the identification of the appropriate patient population and the development of predictive markers of response and resistance.

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