

Review

## Antibody Therapy for Breast Cancer

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**Abstract.** Targeted therapies against tumor biological properties are an essential part of individualized therapy concepts in breast cancer. Next to risk-adapted strategies using conventional chemo- and/or endocrine therapies, antibody therapy has become an additional option. The humanized monoclonal antibody trastuzumab (Herceptin™) is the first novel targeted therapy approved for routine clinical application in advanced breast cancer. Patients with HER2/neu protein overexpression as assessed by immunohistochemistry (IHC) and/or gene amplification as assessed by fluorescence in-situ hybridization (FISH) in their tumors respond well to palliative trastuzumab therapy, either as single agent or in combination with chemotherapy. New combinations with endocrine therapy are currently being evaluated in clinical trials. Trastuzumab therapy is generally well-tolerated. So far, considerable cardiotoxicity was seen only in combination with doxorubicin. Thus, extensive cardiomonitoring is now performed in trials assessing further chemotherapeutic partners. Clinical trials looking at early trastuzumab therapy in the adjuvant (e.g. HERA, BOND 006) or neoadjuvant (e.g. TECHNO) setting are still open for recruitment in Germany. Since only about those 25 % of breast cancers which are HER2/neu-positive are eligible for trastuzumab, novel targeted therapeutics for the remaining HER2/neu-negative tumors are needed. Another therapeutic antibody, 2C4 (Pertuzumab, Omnitarg™), is currently under clinical evaluation. It binds to a different epitope on HER2/neu than trastuzumab and inhibits heterodimerization with other HER receptors. Phase I data showed that 2C4 is well tolerated and clinically active.

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Recruitment to a phase II monotherapy trial in advanced HER2/neu-negative breast cancer has recently been completed at several European centers.

### Background

One in ten women in the western world will develop breast cancer during their life-time, and this incidence is still increasing. Most women newly diagnosed with breast cancer will be treated with systemic therapy, either to reduce the risk of recurrence in case of a primary lesion, or to reduce the tumor burden in case of metastatic disease. For a long time, only estrogen (ER) and progesterone receptors (PR) were available as biological markers for decision making regarding targeted treatment options in breast cancer. Anti-hormonal (endocrine) therapy is generally recommended to patients whose breast cancers overexpress either or both steroid hormone receptors. Such endocrine therapy in combination with conventional chemotherapy, however, is still not sufficient to cure or at least stabilize all patients' disease. Besides, these therapies may have severe side-effects since they do not specifically target tumor tissue.

### History of HER2/neu and development of trastuzumab

Due to the limited availability of specific therapy targets, research on potential therapeutic markers, which are specifically associated with tumor progression and disease aggressiveness, has aroused significant interest and hundreds of markers have been investigated over recent decades.

Among these markers, the HER2/neu oncogene is one of the most promising (Figure 1). In 1985, HER2/neu was isolated and cloned for the first time. It belongs to the four-member family of the closely related growth factor receptors HER1 or EGFR, HER2, HER3 and HER4. This family of receptors is known to be responsible for the growth and differentiation of many normal and transformed epithelial cell types (1). The HER2/neu oncogene encodes a 185 kd

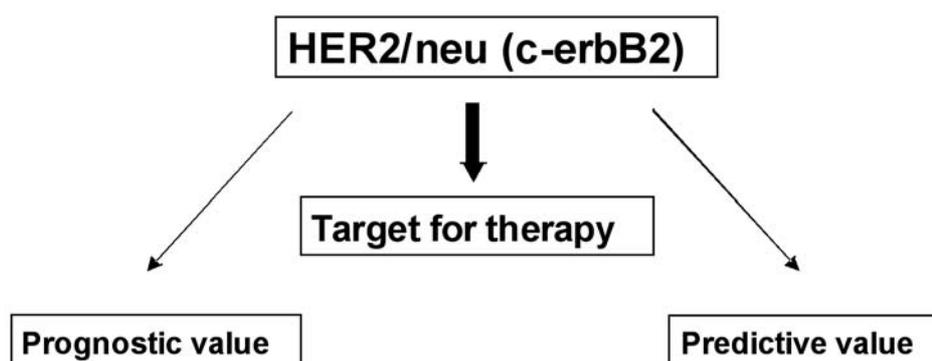


Figure 1. Clinical utility of HER2/neu in breast cancer.

transmembrane glycoprotein with tyrosine kinase activity. Since the original cloning, studies have found that HER2/neu is overexpressed in 20-30% of ductal breast tumors and that overexpression is associated with a shortened median survival compared to women with a normal tumor expression of HER2/neu (2). In 1990, a blocking HER2/neu antibody, muMab 4D5, was produced. Trastuzumab, the humanized version of muMab 4D5, has a high affinity ( $K_d=0.1$  nM) and specificity to the extracellular domain (ECD) of HER2/neu. It was first tested in a phase I-study in 1992 and, in 1998, trastuzumab (Herceptin™) was approved by the FDA as a therapeutic for advanced breast cancer.

### Results of initial phase II and III trastuzumab trials

Phase II and III studies were rapidly initiated after the first successful clinical trials in breast cancer. Trastuzumab single-agent studies (1st-, 2nd-, 3rd-line) and studies of trastuzumab in combination with chemotherapy *vs.* chemotherapy alone were conducted, in the beginning all in metastatic breast cancer.

As first-line monotherapy, weekly trastuzumab with a loading dose of 4 mg/kg body weight and a maintenance dose of 2 mg/kg was associated with an overall response rate (CR, PR) of about 24% and a clinical benefit of 34% (3).

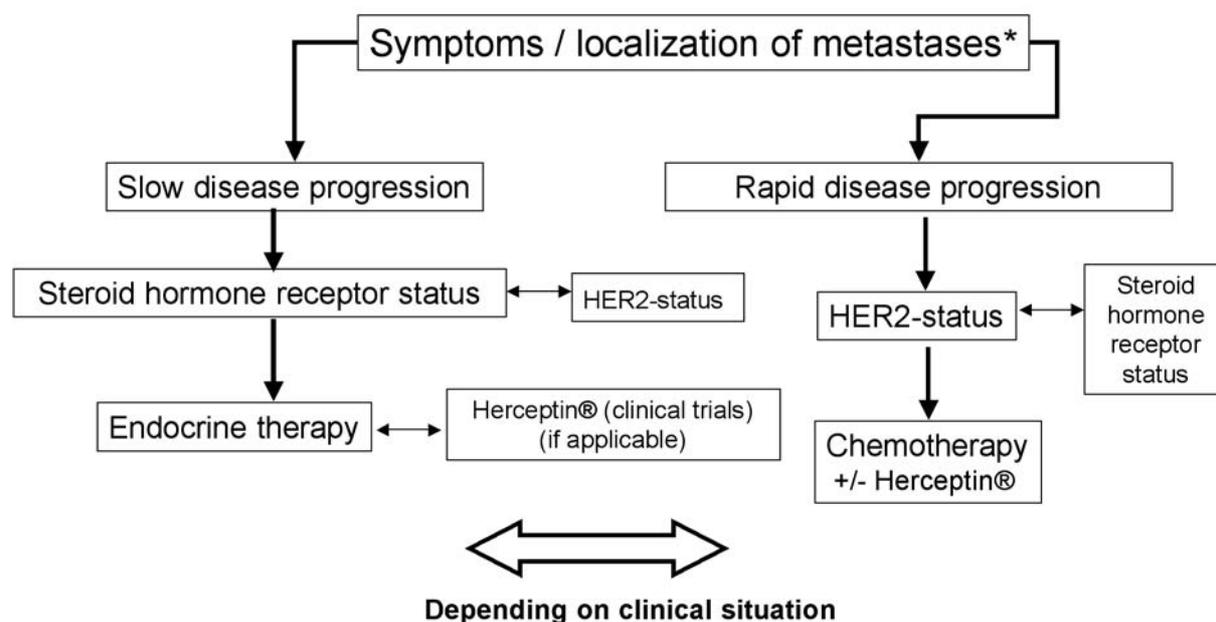
No evidence of a dose-response relationship regarding response, survival, or adverse events was noted (3). However, response to trastuzumab seems to be thoroughly dependent on the degree of HER2/neu-positivity in the primary tumor. Vogel *et al.* (3) noted a response rate of 35% in immunohistochemically (IHC) strongly HER2/neu overexpressing tumors (3+) and 0% in 2+ tumors. In concordance, the response rates also depended on HER/neu gene amplification with a response rate of 35% in FISH-positive and 7% in FISH-negative tumors. Similar response rates for trastuzumab therapy, depending on the

degree of HER2/neu positivity, have since been confirmed by other studies (1). As a single agent therapy in more advanced metastatic breast cancer (2nd- or 3rd-line), weekly trastuzumab was associated with an objective response rate of 15% and a median duration of response of about 9 months (4). Trastuzumab therapy was associated with maintenance of health-related quality of life (QoL), and responders showed a clinically meaningful improvement in QoL parameters (4).

Another registration trial compared chemotherapy alone, anthracyclines (AC) or paclitaxel (if pre-treated with anthracyclines), *versus* chemotherapy plus trastuzumab as first-line therapy ( $n=469$ , median follow-up 30 months) (5). Inclusion criteria for the trial were metastatic breast cancer (MBC), HER2/neu over-expression, no prior chemotherapy for MBC and a  $KPS \geq 60\%$ . In this trial, patients treated with trastuzumab up-front had a significantly longer median overall survival (25.1 compared to 20.3 months,  $p=0.046$ ,  $RR=0.80$ ), in spite of the fact that the majority of patients with disease progression under chemotherapy alone had taken advantage of the possibility of a cross-over to trastuzumab therapy. The overall response rate was 50% in patients receiving chemotherapy and trastuzumab (56% with AC and 42% with paclitaxel) and only 32% with chemotherapy alone. Moreover, the overall quality of life was similar at the beginning of treatment and significantly better from week 32 onwards in patients receiving trastuzumab therapy.

### Side-effects and tolerability of trastuzumab therapy

Trastuzumab therapy was generally well tolerated in the registration trials. Rare serious infusion-associated side-effects, as well as other more common moderate side-effects such as flu-like symptoms, fever, pain, and asthenia, usually occur within the first two hours of the first infusion and generally respond well to supportive care (4).



\*bone metastases: + bisphosphonates

Figure 2. Decision tree for choice of therapy in metastatic breast cancer. Next to steroid hormone receptor status, HER2/neu status has a key role in deciding on targeted therapy.

Preclinical studies did not anticipate the increase of cardiac dysfunction associated with trastuzumab therapy, particularly when used in combination with anthracyclines which was first seen in the combination therapy registration trial (5). Twenty-seven percent of the patients treated with AC and trastuzumab (n=143) suffered from symptomatic or asymptomatic cardiac dysfunction, compared to only 8% of patients treated with AC alone (n=135). Among patients treated with paclitaxel and trastuzumab (n=91), 13% suffered from cardiac dysfunction, compared to only 1% with paclitaxel monotherapy (n=95). The incidence of NYHA class III or IV cardiac dysfunction was highest (16%) in the group treated with AC + trastuzumab compared to only 3% among patients treated with AC alone, or even less in both the paclitaxel groups. The only significant risk factor associated with cardiac dysfunction was older age. As a monotherapy, trastuzumab was associated with a cardiac event rate in 4.7% (n=10) of metastasized patients, 9 of the 10 patients having received prior anthracycline therapy (4).

The mechanism of trastuzumab-related cardiotoxicity is still unknown. Consequently, trastuzumab should not yet be used together with anthracyclines outside of a clinical trial and proper assessment of potential cardiac risk

factors should be undertaken before initiation of trastuzumab therapy. Moreover, next to hypersensitivity to the agent itself or mouse proteins, contraindications to trastuzumab include severe and clinically symptomatic pulmonary dysfunction.

#### Identification of patients suitable for trastuzumab treatment

Today, women with metastatic breast cancer receive endocrine therapy, chemotherapy, and/or Herceptin™, depending on their clinical situation, HER2/neu status and steroid hormone receptor status of the tumor tissue, metastasis localization and rapidity of disease progression (Figure 2). In order to identify the appropriate treatment options, the question of how HER2/neu overexpression can be detected is essential for patient management in breast cancer today. In Germany, the proposed algorithm is standardized by quality-assured IHC as a basic standard test (e.g. DAKO Herceptest®), with subsequent confirmation by fluorescence *in situ* hybridization (FISH) for certain results. The clinically used IHC results are the so-called DAKO-score (0, 1+, 2+, 3+) with scores of 0 or 1+ rated as HER2/neu-negative. In case of a 3+ IHC score, the tumor is considered as HER2/neu-positive with definite HER2/neu overexpression. If the IHC score is 2+, FISH

analysis should be performed to ensure amplification of the HER2/neu oncogene. An IHC 2+ tumor with FISH detected gene amplification (about 24% of 2+ tumors) is also considered HER2/neu-positive and the patient will be suitable for trastuzumab therapy. The concordance of HER2/neu gene amplification and protein overexpression is highest in IHC 3+ tumors (89%) and lowest in IHC 1+ (7%) or IHC 0 (3%) tumors (6). Consequently, general FISH testing does not seem cost-effective and a combined IHC-FISH algorithm, as described above, is frequently used.

In spite of the fact that the DAKO Herceptest<sup>®</sup> is still the most successful test to assess HER2/neu overexpression, a central review of the first 104 cases of the adjuvant Herceptin<sup>™</sup> NSABP B-31 protocol demonstrated that even such standardized IHC is not completely reliable in a decentralized testing environment (7): eighteen percent of HER2/neu-positive tumors that had obtained a DAKO-score of 3+ in the participating center (non-reference laboratory) turned out to be IHC 0-2+ and FISH-negative, when re-tested in the central laboratory. If the initial 3+ score had been obtained in a community reference laboratory, only 4% were tested negative centrally by both IHC and FISH. If other IHC assays had initially been used in a non-reference laboratory, 35% of the initially positive cases were tested negative by both methods in the central laboratory. Similar discrepancies between community and central test results were observed by the adjuvant Herceptin<sup>™</sup> Breast Intergroup Trial (8).

Thus, the standardized Herceptest<sup>®</sup> appears to be the preferable IHC test option, but continuous quality control and thorough internal standardization are necessary before laboratory results are applied for clinical treatment decisions. Hospitals with small volume HER2/neu testing need to consider the option of sending their HER2/neu testing to central large volume laboratories, which may be more cost-effective and ensure better quality of test results than de-centralized small volume testing.

### Open clinical questions with regard to Herceptin<sup>™</sup>

Even though Herceptin<sup>™</sup> is now firmly established as a valid treatment option for MBC, several clinical questions are still left unanswered, including:

- how to distinguish potential responders from non-responders
- when to start the therapy (and on which schedule)
- how long to continue administering it
- what kind of chemotherapy it can be appropriately combined with and, last but not least,
- whether Herceptin<sup>™</sup> should be used as adjuvant therapy.

With regard to *response prediction*, a phase II study indicated that first-line trastuzumab monotherapy (n=111) achieves the best response in FISH-positive or IHC 3+

tumors. The combined response rate noted in IHC 2+ and 3+ tumors was 26%, that of IHC 3+ alone 35%, and the response in FISH-positive tumors was 34% (3). Retrospective analysis of the combination registration trial hinted at a slightly better response to chemotherapy plus trastuzumab compared to chemotherapy alone in FISH-positive patients compared to the IHC-positive patients as assessed by the original clinical trial assay (CTA) (6). A recent phase II study of weekly trastuzumab and docetaxel data saw changes in the HER2/neu extracellular domain (ECD) corresponding to treatment response. In addition, patients with elevated serum HER2/neu ECD at baseline had a significantly higher response rate (76%) than patients with low levels at baseline (33%) (9). Burstein *et al.* (10) suggested that a lack of decline in ECD during cycle 1 could be predictive for tumor progression, but not baseline levels or decrease with therapy. Further data are necessary in order to determine whether ECD levels could serve as reliable predictors for response to trastuzumab therapy or whether they mainly indicate tumor burden. Molecular tumor analysis may also be able to identify responders more accurately in the future. So far, until new validated data on response prediction are available, the combined algorithm of IHC and FISH results, described above, is the most feasible for choosing patients suitable for trastuzumab therapy.

With regard to the question *when to start Herceptin<sup>™</sup> therapy*, data from combination trials with chemotherapy (5, 11), as well as single agent trials (3, 4), indicate that Herceptin<sup>™</sup> therapy should be initiated in MBC as early as possible: response rates are higher in first-line than in second- or third-line therapy, and patients who receive up-front chemotherapy together with trastuzumab have a significant survival advantage over patients who receive either only upon progression or not at all. A recent study (M77001) compared first-line docetaxel plus trastuzumab *versus* docetaxel alone. Remarkably, patients who started on chemotherapy plus trastuzumab had an estimated median survival advantage of about 1 year over patients who just received chemotherapy alone, and even about a 6-months, advantage over patients who crossed over to trastuzumab after progression on chemotherapy alone (11). Thus, HER2/neu-positive patients should receive Herceptin<sup>™</sup> as soon as distant metastases occur and chemotherapy is warranted (Figure 2). Clinical trials are under way to examine the feasibility and benefit of combining trastuzumab with endocrine therapy in MBC. With regard to *scheduling*, Herceptin<sup>™</sup> is currently approved for the weekly schedule (4 mg/kg loading and 2 mg/kg maintenance dose), but pharmacological data suggest that 3-weekly intervals (8 mg/kg loading and 6 mg/kg maintenance dose) are also possible (11).

A well-established principle in oncological therapy is to discontinue a certain therapy upon disease progression under this therapy. However, the mode of action of novel

targeted therapies such as antibodies differs quite substantially from conventional chemo- or endocrine therapy. Thus, it is still an unsolved question *how long to administer Herceptin™ therapy, i.e. whether to discontinue Herceptin™ therapy upon progression under Herceptin™ and chemotherapy or whether just to change the chemotherapy and continue the antibody therapy*. Cell culture assays showed that cell growth subdued at first, started re-growing after withdrawal of trastuzumab (12). This effect was independent of the presence of concurrent chemotherapy. Judging from this *in vitro* data, it may seem advisable to administer long-term trastuzumab treatment to patients. To test this clinically extremely interesting hypothesis, a new trial "treatment beyond progression" (GBG 26) was recently opened (PI: Gunter von Minckwitz, Frankfurt, Germany) ([www.germanbreastgroup.de](http://www.germanbreastgroup.de)). This trial compares the continuation of trastuzumab together with a change in chemotherapy (*i.e.* capecitabine plus trastuzumab) *versus* a change of chemotherapy plus discontinuation of the antibody therapy (*i.e.* capecitabine alone) as second-line therapy after disease progression while or after treatment with taxanes and Herceptin™.

So far, clinical use of trastuzumab has only been approved together with paclitaxel as first-line therapy in MBC or as a monotherapy in more advanced disease. However, there is quite promising *in vitro* data suggesting possible synergism of trastuzumab with other chemotherapeutic agents (13). Recently, the *in vitro* data about a beneficial combination of trastuzumab with platin compounds has been confirmed *in vivo* by several clinical trials (14, 15). At ASCO 2003, a phase II trial comparing weekly paclitaxel, carboplatin and trastuzumab *versus* a 3-weekly regimen using the same drugs, showed quite promising response data (15): patients receiving the weekly regimen had a more favorable disease outcome with a 78% overall response rate (CR, PR) and a 2-year survival probability of 81%. The suspected synergistic mode of action using trastuzumab and docetaxel has also been clinically validated by the recent M77001 trial, showing an overall response rate of 61% for the combination *versus* merely 36% for the chemotherapy alone (11). In addition, trastuzumab and vinorelbine are also rather promising combination partners not just *in vitro* (16) but also *in vivo*, with a documented 68% overall response rate in first-line therapy (10). Thus, *in vitro* data validated by clinical evidence suggest that there are quite a number of very potent *chemotherapeutic combination partners* for trastuzumab. Additional therapeutic indications are currently being investigated (17-19).

Primary breast cancer is generally regarded as a potentially systemic disease with single tumor cell dissemination already at the time of primary therapy being the rationale for adjuvant systemic therapy after loco-

regional treatment. Thus, the obvious question is whether early use of antibody therapy might not be more effective than its late application in stages where overt metastases and thus a higher tumor load, are present. In view of the promising data from MBC, several clinical trials are currently looking at the efficacy of *adjuvant Herceptin™*. In Germany, there are three trials available in primary breast cancer, one in the preoperative "neoadjuvant" setting (TECHNO, an AGO trial; PI: Michael Untch, Munich, Germany), and two international adjuvant trials (HERA and BOND 006 / BCIRG 006). Whereas adjuvant chemotherapy, is part of the randomization in the BOND 006 trial, HERA tests no trastuzumab *versus* 1 year *versus* 2 years of the antibody after adjuvant chemotherapy, according to the policy of the participating centers. There is very good acceptance of the adjuvant Herceptin™ trials by patients and physicians world-wide, and first safety analyses show that there seem to be no unjustifiable toxicities, particularly no excess cardiotoxicity associated with adjuvant use of the antibody (20).

#### **Antibody therapy for HER2/neu-negative breast cancer patients**

In general, only 20-30% of breast cancer patients will have HER2/neu-positive tumors and, thus, be eligible for Herceptin™ therapy (1, 21). Consequently, in spite of the promising clinical results of Herceptin™, there is currently no antibody therapy available for the majority of breast cancer patients.

Recently, an antibody treatment has been suggested for HER2/neu-negative tumors: RhuMab 2C4 (pertuzumab, Omnitarg™) is a humanized antibody which inhibits *in vitro* cell growth in HER2/neu-negative human breast cancer cells (22). The antibody's binding affinity is similar to that of trastuzumab and it is also directed against the ECD of HER2/neu, but uses a different epitope binding site. Ligand-less HER2/neu depends on activation by dimerization or association with members of the HER-family and the new antibody seems to sterically inhibit this interaction and, thereby, formation of HER-ligand complexes.

In a phase I trial with pertuzumab, including different cancer types such as breast, ovarian, or prostate cancer, 9 out of 21 patients had stable disease after up to 14 cycles (42 weeks) and one patient had a partial response after the second cycle (23). Side-effects such as diarrhea (33%), anemia (19%), fever (14%), lower abdominal pain (14%) were all NCI-CTC grade I or II. Considering the side-effects seen with trastuzumab, cardiotoxicity was of special concern, but in this case only one out of 21 patients had a significant drop of LVEF, whereas 19 out of 21 patients had no alteration. Subsequently, a European multicenter, randomized phase II study was initiated comparing two

different dosing schedules of pertuzumab in MBC. Recruitment ended in late 2003 and, hopefully, more clinical data on this new antibody will be available soon.

## Summary

Over the last thirty years, breast cancer treatment has steadily gained in effectiveness and improvements in early detection as well as in conventional loco-regional and systemic therapy have led to a significant decrease in breast cancer mortality (24). However, still too many women will eventually die of the disease. Therefore, in order to increase the effectiveness of systemic treatment, targeted therapies have been extensively investigated over the past two decades.

One of the most promising therapeutic targets discovered so far is the HER2/neu gene. Treatment with trastuzumab (Herceptin™), a humanized monoclonal antibody, directed against the HER2/neu extra cellular domain, substantially reduces tumor growth in metastatic breast cancer. Consequently, Herceptin™ was approved as the first therapeutic antibody for breast cancer treatment in 1998. In MBC, trastuzumab therapy should be started as early as possible, preferably in combination with first-line chemotherapy. In addition to the approved combination partner paclitaxel, several potentially successful combination partners have recently been validated by clinical trials. The antibody is generally well tolerated and the majority of side-effects will become apparent at the time of first application. Consequent cardiac monitoring is advisable and combination with anthracyclines outside of clinical trials should be avoided. Considering the promising results in the palliative setting, further investigation of this drug for adjuvant therapy seem more than appropriate. The optimism of patients and physicians regarding targeted therapies in the adjuvant setting is documented by the excellent recruitment rate in the adjuvant Herceptin™ trials world-wide. Further international efforts towards standardization and quality control of the HER2/neu assays for clinical routine are still warranted. In addition, patient selection for treatment needs to be optimized, and new predictors of response are needed.

For the majority of breast cancer patients who are HER2/neu-negative, a novel antibody, 2C4 or pertuzumab, which inhibits HER2/neu hetero-dimerization with other members of the HER family, has recently become available in early stage clinical trials.

In conclusion, by means of Herceptin™, antibody therapy has become an essential part of routine breast cancer therapy over the last 5 years. More indications for its clinical use are currently being investigated and different antibodies are already part of early phase clinical trials. Thus, antibody therapy and other tumor-biological therapeutics are promising future options for substantially improving breast cancer therapy and, thereby, patient survival.

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