Symposium article

Phase I and II clinical trials of trastuzumab

J. Baselga

Medical Oncology Service, Hospital General Universitari Vall d’Hebron, Barcelona, Spain

Summary

This report summarizes the efficacy of trastuzumab (Herceptin) based on its completed clinical trial program in patients with HER2-positive metastatic breast cancer for phase I and II studies which have been completed to date and were integral in the submission that led to approval of trastuzumab for clinical use in the USA. There were three small-scale, phase I clinical trials conducted, which were primarily designed to determine the safety and pharmacokinetics of trastuzumab (10–500 mg) administered i.v. as single or weekly doses. This was followed by two phase II clinical trials of fixed-dose trastuzumab either as a single-agent or in combination with cisplatin in 46 and 39 patients, which produced overall response rates of 11.6% and 24.3%, respectively. In a pivotal phase II clinical trial, trastuzumab was administered on a bodyweight-adjusted basis as a single agent to 222 patients with HER2-positive metastatic breast cancer who had relapsed after one or two prior chemotherapy regimens. The overall response rate was 21% when assessed in evaluable patients by the investigators and 15% when analyzed on an intent-to-treat basis by an independent Response Evaluation Committee. The pharmacokinetics of trastuzumab were evaluated in these studies and the results are summarized.

Key words: cisplatin, clinical trial, Herceptin, metastatic breast cancer, pharmacokinetics, trastuzumab

Introduction

The human epidermal growth factor receptor-2 (HER2) is a 185kD transmembrane tyrosine kinase receptor encoded by the HER2 proto-oncogene mapped to chromosome 17q21 [1-4]. The HER2 gene is often referred to as HER2/new or c-erbB-2 in literature. HER2 belongs to a family of four closely related transmembrane tyrosine kinase receptors, designated HER1 to HER4 [3, 5-8], which demonstrate growth-stimulating activity and are involved in normal development and differentiation of mammary tissue [9-12]. HER receptors exist as monomers in the cell membrane but are stabilized by binding to a range of different ligands, e.g., epidermal growth factor (EGF) and heregulin [5, 7, 13], to form active homo- or heterodimers [14-16]. HER2, unlike the other members of the HER family of receptors, does not have a cognate ligand. However, HER2 is the preferred heterodimerization partner within the HER family [17], forming heterodimers which have particularly high signaling potency [18, 19].

A range of in vitro and animal studies support a direct role for HER2 in the oncogenic transformation, tumorigenesis, and increased clinical aggressiveness and metastatic potential of HER2-positive tumors in a range of different human cancers, but most notably breast cancer [5, 20-23]. Overexpression of the HER2 receptor is almost invariably the result of amplification of the HER2 gene [24]. The gene amplification results in an increase in the number of receptors on the cell surface by several orders of magnitude, leading to dysregulation of the normal homeostasis of the HER system, and thus serves to trigger the cell to divide and multiply at an accelerated rate, thereby contributing to tumor growth. The HER2 receptor is overexpressed in up to 30% of human breast cancers and predicts for a worse prognosis, as measured by decreased overall and disease-free survival [25-28], and increased probability of resistance to therapy with hormonal and certain cytotoxic chemotherapeutic agents [29].

The HER2 protein offers a logical and accessible therapeutic target for specific anti-cancer agents, particularly monoclonal antibodies. Many studies have shown that the growth of breast cancer cell lines in vitro, and tumor xenografts in vivo, is inhibited by various anti-HER2 monoclonal antibodies [30-38]. From one series of murine monoclonal antibodies [32], a particular antibody, 4D5, was selected for further development because of its potency in suppressing the growth of human HER2 xenografts in murine models [39].

To overcome the immunogenicity of the murine 4D5 monoclonal antibody during administration in humans, the antigen recognition regions of 4D5 were inserted into a human immunoglobulin Gl [40]. This humanized anti-HER2 monoclonal antibody, trastuzumab (Herceptin), proved to have a higher binding affinity to HER2 (Kd ≈ 0.1 nM) than 4D5. Non-clinical studies suggest that the mechanism of action of trastuzumab includes antagonism of the growth-stimulating properties of overexpressed HER2 and stimulation of antibody-
dependent cell-mediated, and possibly complement-mediated, cytotoxicity [41]. The preclinical anti-tumor activity of trastuzumab has been extensively studied [42-45]. The present report overviews initial phase I and II clinical trials of trastuzumab in women with HER2-positive metastatic breast cancer, in which trastuzumab was used either as a single agent or in combination with cisplatin. These studies and a pivotal phase III combination trial [46] provided the clinical data that supported the approval of trastuzumab in the USA by the FDA. The safety of trastuzumab in these preliminary phase I and II clinical trials is described in detail in the article by Gianni in this supplement [47]. Also, ongoing and planned clinical trials of trastuzumab are described separately [48].

**Phase I clinical trials**

There were three open-label, phase I clinical trials in patients with refractory (grade 4) HER2-positive metastatic breast cancer (Table 1), which were primarily designed to determine the safety, maximum tolerated dose and pharmacokinetics of trastuzumab [49]. The weekly schedule of i.v. infusion was based on the expected clearance of trastuzumab calculated from preclinical studies and was continued until disease progressed. The dose of cisplatin used was 50 or 100 mg/m². As is usual in such studies, relatively low numbers of patients were recruited in these phase I clinical trials (n = 15–17) and the enrollment was completed in short period of time. Trastuzumab was well tolerated at all doses from 10–500 mg i.v. and a maximum tolerated dose was not reached. In the single-agent trials, serious adverse events were recorded in four patients, but none were considered related to the administration of trastuzumab. Trastuzumab was also well tolerated in combination with cisplatin and the frequency and nature of adverse events was similar to that previously described during clinical experience with cisplatin alone. With respect to pharmacokinetics, the serum half-life of trastuzumab increased with increasing dose, ranging from about one day with the lowest dose (10 mg) to two weeks with the highest dose (500 mg). The pharmacokinetics of trastuzumab were unaffected by coadministration with cisplatin.

**Phase II clinical trials**

After the completion of phase I studies, the clinical trial program for trastuzumab rapidly advanced to two small-scale, phase II clinical trials, which were conducted simultaneously – a single-agent trial and a combination trial with cisplatin. Later, a large-scale pivotal clinical trial was performed (Table 2). These studies were all performed in patients with HER2-positive metastatic breast cancer. The results of trastuzumab safety from these trials are described in detail elsewhere in this supplement [47]. The pharmacokinetics of trastuzumab observed in these three trials are described and discussed in the next section.

**Single-agent study**

A total of 46 patients were initially recruited. Trastuzumab was administered in an outpatient setting by slow i.v. infusion over 90 minutes: the initial dose was 250 mg followed by 100 mg weekly for 10 doses [50, 53]. This dose could be continued as a maintenance weekly dose if patients did not show disease progression. Patients had to have adequate liver, kidney, lung and bone marrow function, Karnofsky performance status had to be ≥60%, and patients with bone only disease were excluded. HER2 positivity was determined by an immunohistochemistry (IHC) assay using a murine

### Table 1. Phase I, open-label clinical trials of trastuzumab [49].

<table>
<thead>
<tr>
<th>First patient recruited</th>
<th>Last patient recruited</th>
<th>Number of patients recruited</th>
<th>Age range (years)</th>
<th>Treatment</th>
<th>Herceptin® dose</th>
<th>Number of patients recruited</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 November 1992</td>
<td>4 March 1993</td>
<td>17</td>
<td>30–71</td>
<td>Trastuzumab alone</td>
<td>Weekly 10–500 mg i.v.</td>
<td>17</td>
</tr>
<tr>
<td>6 October 1992</td>
<td>26 October 1992</td>
<td>15</td>
<td>40–71</td>
<td>Trastuzumab + cisplatin</td>
<td>Weekly 10–500 mg i.v.</td>
<td>15</td>
</tr>
</tbody>
</table>

### Table 2. Phase II, open-label clinical trials of trastuzumab.

<table>
<thead>
<tr>
<th>First patient recruited</th>
<th>Last patient recruited</th>
<th>Number of patients recruited</th>
<th>Age range (years)</th>
<th>Treatment</th>
<th>Trastuzumab + cisplatin</th>
<th>Trastuzumab dose followed by weekly i.v.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baselga et al. [50]</td>
<td>15 March 1993</td>
<td>46</td>
<td>30–65</td>
<td>Trastuzumab alone</td>
<td>250 mg initial dose followed by 100 mg weekly i.v.</td>
<td></td>
</tr>
<tr>
<td>Pegram et al. [51]</td>
<td>31 March 1993</td>
<td>39</td>
<td>29–75</td>
<td>Trastuzumab + cisplatin</td>
<td>75 mg/m² weekly i.v.</td>
<td></td>
</tr>
<tr>
<td>Cobleigh et al. [52]</td>
<td>12 June 1995</td>
<td>222</td>
<td>28–81</td>
<td>MBC with 1 or 2 prior CT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CT - chemotherapy treatment; MBC - metastatic breast cancer.
anti-HER2 monoclonal antibody and $\geq 25\%$ reactive cells was considered as a cutoff value for positivity, while in practice $83\%$ patients had $\geq 50\%$ reactive cells. Median age was 50 years (range 30–65 years), median Karnofsky performance status was 90%, and 43% of patients were estrogen-receptor (ER) positive. The population had extensive metastatic disease ($35\%$ had $\geq 3$ metastatic sites) with the dominant sites being visceral (80%). Fifteen percent of patients had received tamoxifen for adjuvant disease and 46% had received hormonal therapy for metastatic disease. Prior chemotherapy had been intensive: 65% had received (neo-)adjuvant chemotherapy and 83% had received chemotherapy for metastatic disease, with 63% receiving $\geq 2$ chemotherapy regimens for metastatic disease.

Three patients were not assessed for clinical response: one patient required prolonged antibiotic administration for bacteremia which precluded trastuzumab administration, another patient withdrew for personal reasons, and one patient died from congestive heart failure associated with prior doxorubicin treatment. A total of 43 patients were therefore assessable for response. One patient had a complete response (CR), which has lasted more than six years, and four patients had a partial response (PR). The overall response (OR = CR + PR) was therefore $11.6\%$ (95% confidence interval (CI): 4.4%–25.9%). Two patients had a minor response and fourteen had stable disease (total 37% with minimal response), while the remaining twenty-two patients had disease progression. The median time to disease progression was 5.1 months.

**Combination study with cisplatin**

A total of 39 patients were initially recruited. Trastuzumab was administered in an outpatient setting by slow i.v. infusion over 90 minutes: the initial dose was 250 mg followed by 100 mg weekly for a further 8 doses [51, 54]. Cisplatin 75 mg/m² was administered by slow i.v. infusion over 60 minutes on days 1, 29 and 57. Cisplatin doses were always given 24 hours after administration of trastuzumab. Trastuzumab plus cisplatin was continued as maintenance therapy if patients did not show disease progression, using trastuzumab 100 mg i.v. weekly plus cisplatin 75 mg/m² every four weeks until disease progression or prohibitive toxicity occurred. Eligibility criteria for entry included age 18–75 years, presence of measurable metastatic disease, documented progression of disease while on chemotherapy (true chemotherapy-refractory patients), Karnofsky performance status $> 60\%$, and a HER2-positive status ($2+$ or $3+$ on IHC assay). The mean age of the patients was 50 years (range 29–75 years) and 86% had a Karnofsky performance status $> 90\%$. A high grade of HER2 tissue overexpression ($3+$) was seen in the majority of patients ($82\%$), and 65% had measurable serum levels of shed HER2 extracellular domain (ECDHER2) before treatment with trastuzumab. Only 35% of patients were ER positive and 69% were post-menopausal. The disease burden was generally very high, with 46% of patients having $\geq 3$ metastatic sites. The main metastatic sites were lung (49%), lymph node (49%), bone (46%), chest wall/skin (44%), liver (36%), and breast (10%). The population had been heavily pretreated prior to entry: 90% had received $\geq 2$ prior chemotherapeutic regimens for metastatic disease which had failed. Furthermore, 69% had received prior radiotherapy and 54% hormonal therapy.

For the 37 patients assessable for response, eight patients had a PR during the main study plus one additional patient during the maintenance phase, giving an overall response rate of 24.3% (95% CI: 12.4%–41.6%). No patient had a CR. Three patients had a minor response, six had stable disease, and nineteen had disease progression. The median response duration was 5.3 months (range 1.6–18.0 months).

**Pivotal single-agent phase II trial**

This multicenter, open-label, single-arm trial of single-agent trastuzumab was conducted at 54 centers [52]. All the patients had HER2-positive ($2+$ or $3+$ on IHC assay) metastatic breast cancer which had relapsed following one or two prior cytotoxic chemotherapy regimens for stage IV disease. Unlike the preceding phase II clinical trials, the dose of trastuzumab was calculated on a bodyweight basis. It was administered i.v. in an outpatient setting as an initial 4 mg/kg dose over 90 minutes followed by 2 mg/kg weekly. If the infusion was well tolerated, subsequent infusion periods were shortened to 30 minutes. Treatment was continued until disease progression, and thereafter could be continued, the dose doubled, or discontinued at the discretion of the investigator. The primary efficacy endpoint was overall response rate (CR + PR) determined using intent-to-treat analysis by an independent Response Evaluation Committee (REC). Secondary efficacy endpoints were duration of response, time to disease progression, time to treatment failure and survival.

A total of 222 patients were enrolled and 213 received at least one dose of trastuzumab. Nine patients were not treated for various reasons. Baseline patient characteristics and demographics were: mean age of the patients was 50 years (range 28–81 years); 55% had ER-negative tumors, 78% were grade 3+ HER2 positive; 37% had a disease-free interval $< 12$ months; 36% had $\geq 3$ metastatic sites; and 72% had liver or lung metastases. About two-thirds (68%) had received prior adjuvant chemotherapy, and all had received prior therapy for metastatic disease (94% anthracyclines, 67% taxanes, 71% radiotherapy, 57% hormonal therapy, and 26% high-dose chemotherapy with bone marrow or stem-cell support).

The overall objective response rate assessed by the REC on intent-to-treat analysis was 15% (95% CI: 11%–21%); this included 8 CRs and 26 PRs. The overall response rate according to investigators when analyzing patients who had received at least 1 dose of trastuzumab was 22% (95% CI: 16%–28%); this included 9 CRs and
37 PRs. In addition, there were 12 minor responses (6%), 62 patients with stable disease (29%), and 93 patients with progressive disease (44%). The median duration of response as determined by the REC was 9.1 months (range 1.6–26 months) and the median duration of survival was 13 months (range 0.5–30 months) (Figure 1). Among all treated patients, the median time to disease progression was 3.1 months (range 0–28 months) and the median time to treatment failure was 2.4 months (range 0–28 months). The median time to treatment failure among the 34 responders was 11 months (range 2–28 months) compared with the median time to treatment failure for prior cytotoxic chemotherapy in these patients of 5.4 months (range 0–27.4 months).

The efficacy of trastuzumab was generally seen consistently across all subgroups. Patients with grade 3+ HER2 overexpression tended to have a higher response rate than those with 2+ overexpression (18% vs. 6%, \( P = 0.06 \)). Patients whose time to first relapse was > 6 months had a higher response rate than those who relapsed earlier (20% vs. 9%, \( P = 0.03 \)). Tumor responses were seen in 11% of patients with liver metastases and in 26% of patients who had received stem-cell or bone marrow support following high-dose chemotherapy. Univariate analysis showed that there were correlations between the time to disease progression and certain patient characteristics: the median time to disease progression was longer among patients who exhibited 3+ HER2 overexpression (3.3 vs. 1.9 months, \( P = 0.0034 \)), who had relapsed > 6 months after treatment (3.4 vs. 2.1 months, \( P = 0.0045 \)), who had a Karnofsky performance status \( \geq 90\% \) compared with < 90% (3.2 or 3.5 vs. 2.0 months, \( P = 0.0068 \)), and who had one or two compared with three or more metastatic sites (3.5 or 3.2 vs. 2.3 months, \( P = 0.001 \)). Multivariate analysis showed that three factors significantly (\( P < 0.05 \)) affected time to disease progression: number of metastatic sites at entry, level of HER2 overexpression, and time to first relapse.

Health-related quality of life (HRQL) was assessed at baseline and at week 12 in 154 patients using the EORTC Quality of Life Questionnaire-C30. The results have been reported in detail [55]. In the subset of patients with a clinical response to trastuzumab, there was a clinically meaningful improvement in HRQL. For all patients, trastuzumab was associated with maintained HRQL until disease progression.

Pharmacokinetics

Blood samples were taken for pharmacokinetic analysis just before the administration of each dose and within the first hour following the end of infusion of each dose of trastuzumab in most patients in all phase II clinical trials. Serum concentrations of trastuzumab and shed ECD\(^{HER2}\) concentrations were determined using enzyme-linked immunosorbent assays (ELISA).

The pharmacokinetic results for the two small-scale, phase II clinical trials are summarized in Table 3 [51]. The minimum desired trough concentration of trastuzumab of > 10 \( \mu g/ml \), which was selected from in vitro studies [40], was achieved in the majority of patients (58 of 77; 75%). The pharmacokinetics of trastuzumab were unaffected by the coadministration with cisplatin. However, the serum half-life of trastuzumab was reduced because of increased clearance in patients with high circulating ECD\(^{HER2}\) levels (cutoff values < and \( \geq 5 \) \( \mu g/ml \), respectively). Figure 2 shows an example of the differences in serum trastuzumab concentration fluctuation in representative patients with high and low shed ECD\(^{HER2}\) concentration, respectively [50]. There was a strong inverse correlation (\( r = -0.79, P = 0.0001, n = 184 \)).

![Figure 1](https://example.com/image1.png)  
**Figure 1** Kaplan–Meier plots of (a) the duration of response in responders (CR + PR) and (b) survival in all patients enrolled in the pivotal phase II, single-agent trial of trastuzumab (Reproduced with permission from Cobleigh et al. [52]).

Table 3. Summary of mean (± SD) pharmacokinetics of trastuzumab administered as a single agent and in combination with cisplatin. (Reproduced with permission from Pegram et al. [51]).

<table>
<thead>
<tr>
<th>Trial</th>
<th>( t_{1/2B} ) (days)</th>
<th>( C_{\text{max}} ) (( \mu g/ml ))</th>
<th>( C_{\text{min}} ) (( \mu g/ml ))</th>
<th>( C_{\text{ss}} ) (( \mu g/ml ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trastuzumab alone</td>
<td>9.2 ± 5.3 (n = 39)</td>
<td>&lt; 0.5</td>
<td>113 ± 35 (n = 46)</td>
<td>54 ± 32 (n = 45)</td>
</tr>
<tr>
<td></td>
<td>2.9 ± 3.2 (n = 6)</td>
<td>≥ 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trastuzumab + cisplatin</td>
<td>11.0 ± 4.4 (n = 30)</td>
<td>&lt; 0.5</td>
<td>121 ± 84 (n = 37)</td>
<td>85 ± 18 (n = 37)</td>
</tr>
<tr>
<td></td>
<td>4.0 ± 2.6 (n = 7)</td>
<td>≥ 0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: \( C_{\text{ss}} \) - peak serum concentration; \( C_{\text{min}} \) - trough serum concentration; \( C_{\text{ss}} \) - steady-state serum concentration; \( t_{1/2B} \) - elimination half-life; ECD\(^{HER2}\) - shed HER2 antigen (extracellular domain of the HER2 receptor).
between trough serum concentration of trastuzumab and shed ECD\textsuperscript{HER2} in the study where trastuzumab and cisplatin were coadministered (Figure 3) \cite{51}. There appeared to be no correlation between shed ECD\textsuperscript{HER2} concentration and clinical response, although data may have been insufficient to detect any statistically significant effect.

In the pivotal phase II clinical trial mean volume of distribution for trastuzumab was 38.0 ml/kg, which is similar to serum volume. The mean steady-state serum concentration was 59.7 \textmu g/ml and mean elimination half-life was 6.2 days, which are relatively similar to the values reported in the previous studies. Mean trough and maximum serum concentrations of trastuzumab were 25.0 \textmu g/ml and 100.3 \textmu g/ml, respectively. The trough serum concentration tended to increase with time to a plateau of about 70 \textmu g/ml at week 20 onwards, but analysis was confounded by the decreasing number of patients analyzed over time. For the subset of 37 patients who had full data through to week 36, trough serum concentration increased with time and tended to plateau at about week 12. Mean trough concentrations of trastuzumab at weeks 7 and 8 were significantly ($P < 0.001$) higher in complete (70.3 \textmu g/ml) and partial (58.4 \textmu g/ml) responders compared with non-responders (44.3 \textmu g/ml). Shed ECD\textsuperscript{HER2} was below detectable limits in serum in 38% of patients. Median shed serum ECD\textsuperscript{HER2} level was significantly higher in patients with tumor grade 3+ compared with those with grade 2+ HER2 overexpression (16.2 vs. 3.4 ng/ml, $P < 0.0001$). There was no significant correlation between ECD\textsuperscript{HER2} levels and treatment response status.

**Conclusion**

Phase I and II clinical trial results of trastuzumab in patients with metastatic breast cancer have demonstrated that it is effective as a single agent in all HER2-positive patient subgroups and shows promise in combination with cisplatin. Trastuzumab produced clear responses in terms of classic criteria and also in terms of a meaningful duration of treatment response. The results of these studies, together with the pivotal combination phase III clinical trial, formed the clinical trial program which provided the basis for approval of trastuzumab by the FDA in the USA for the treatment of patients with HER2-positive metastatic breast cancer. The current trials have also allowed progression to ongoing and planned clinical trials of trastuzumab as first-line, single-agent therapy and in combination with a range of established and newer chemotherapeutic agents.

**Note**

Dr Baselga has reported that he serves on a Roche advisory board for Herceptin and has received research support from Genentech, Inc.

**References**


17. Graus-Porta D, Beerli RR, Daly JM, Hynes NE. ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signalling. EMBO J 1997; 16: 1647–55.


antibody to HER-2 receptor and DNA-reactive drugs. Oncogene 1998; 17: 2235–49.

Correspondence to:
J. Baselga, MD
Medical Oncology Service
Hospital General Universitari Vall d’Hebron
Psg. Vall d’Hebron, 119–129
08035 Barcelona
Spain
E-mail: baselga@hg.vhebron.es