Trastuzumab and Interleukin-2 in HER2-positive Metastatic Breast Cancer: A Pilot Study

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ABSTRACT

Purpose: Trastuzumab as a single agent has activity in metastatic breast cancer; however, the mechanism of action for this clinical activity is uncertain. Whereas interruption of erbB family member signaling occurs, trastuzumab also mediates antibody-dependent cellular cytotoxicity in vitro and in vivo. Based on these data, a clinical trial was performed to test whether interleukin (IL)-2, by increasing FcRγIIIα natural killer (NK) cell numbers and cytolytic function in vitro, when added to trastuzumab, can increase efficacy, be safely given, and avoid the use of chemotherapy.

Experimental Design: In this Phase I trial, 10 patients with HER2-overexpressing metastatic breast cancer were treated with IL-2 (1.75 × 10^6 IU/m^2/day, s.c.) for 7 weeks and trastuzumab (4 mg/kg load and then 2 mg/kg weekly) for 6 weeks. Safety, in vitro immune responses, and clinical responses were assessed.

Results: Ten women received a total of 12 cycles of therapy (each cycle lasted 7 weeks). No significant toxicities were seen, and one patient required an IL-2 dose reduction. Among the evaluable patients (10 cycles), the responses were one partial response, five cases of stable disease, and four cases of progressive disease. In vitro immune assays showed NK cell expansion and trastuzumab-mediated increased NK cell killing of breast cancer targets (antibody-dependent cellular cytotoxicity) in a HER2-specific manner but did not correlate with clinical responses.

Conclusions: Trastuzumab + IL-2 is a well-tolerated outpatient regimen that results in NK cell expansion with enhanced in vitro targeted killing of HER2-expressing cells. These preliminary data suggest that this strategy may benefit heavily pretreated metastatic breast cancer patients.

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INTRODUCTION

Growth factors and their receptors are known to play critical roles in development, cell growth, and differentiation. Many receptors possess intrinsic tyrosine kinase activity that is activated upon interaction of the receptor with its cognate ligand. Gene amplification of the human epidermal growth factor receptor 2 (HER2) gene (1, 2) is frequently observed in a number of primary tumors, suggesting that the overexpression of this growth factor receptor may contribute to transformation and tumorigenesis (3). Approximately 25–30% of patients with breast cancers overexpress HER2 (4, 5). In most cases, HER2 protein overexpression is correlated with gene amplification and is associated with poor clinical outcome in patients with lymph node-positive breast cancers.

Several lines of evidence support a direct role for p185HER2 (the HER2 gene product) overexpression in the pathogenesis and poor clinical course of human tumors. Whereas the rat HER2 homologue, the neu proto-oncogene, is associated with the induction of neuroblastomas (6), the overexpression of erbB2 (neu) in transgenic mice induces mammary tumors (7). In addition, specific antibodies to the extracellular domain of the membrane-based protein encoded by the HER2/neu gene will inhibit the growth of tumors that overexpress HER2 (8, 9). These data are consistent with a direct role for the HER2 proto-oncogene in both malignant transformation and enhanced tumorigenicity and indicate a potential target for cancer therapy. Trastuzumab (Herceptin) is a humanized mouse monoclonal antibody that has been approved for treatment in women with metastatic breast cancer.

Whereas trastuzumab has superior efficacy when combined with chemotherapy (10–12), it is clear that single-agent trastuzumab has significant activity in patients with HER2-overexpressing tumors (13–15). In a study of 114 patients who had not received prior chemotherapy for metastatic disease, Vogel et al. have shown that response rates were 26% (CR + PR) and 38% (complete response + PR + SBD > 6 months) for patients who were 2 or 3+ by IHC (16). When only patients who were IHC 3+ or fluorescence in situ hybridization positive were considered, the objective response rates were substantially higher (35% and 34%, respectively), whereas those who were IHC 2+ and fluorescence in situ hybridization negative had no response. Survival benefits in this study were similar to those reported in studies using trastuzumab plus chemotherapy (16).

The potential mechanisms of action of trastuzumab are diverse and have yet to be fully elucidated. We have previously...
shown that trastuzumab, through its IgG1 Fc domain, can enhance ADCC against breast cancer targets overexpressing HER2/neu (17). In addition, Clynes et al. (18) have reported that ADCC may be an important mechanism for trastuzumab-induced responses in vivo because HER2-dependent xenografts in mice treated with anti-Her2/neu antibody were not inhibited in the absence of the FcyRIII.

We have been interested in different modalities of immunotherapy as an adjuvant to standard cytotoxic therapies (19), and we have gained considerable experience using outpatient, s.c., low-dose IL-2 to increase NK cell numbers, which should enhance ADCC (20, 21). Our conclusion from trials using outpatient single-agent IL-2 s.c. for up to 12 weeks is that the therapy is safe with manageable toxicity. Subcutaneous IL-2 increased the absolute number of circulating NK cells by approximately 10-fold and induced an increase in the function of these lymphocytes against a series of breast cancer targets (17, 22). However, the nonspecific nature of this immune activation has not been shown to have clinical benefit. In this study we were interested in determining whether IL-2 and trastuzumab could be safely coadministered and, by stimulating NK cells numbers and function, serve to enhance ADCC against HER2-bearing breast cancer target cells.

**MATERIALS AND METHODS**

**Clinical Trial.** Patients with a diagnosis of HER2/neu-positive [2+ or 3+] by Herceptest (Dako) advanced breast cancer of any histological type were enrolled in a pilot trial approved by the University of Minnesota Human Subjects Committee and the United States Food and Drug Administration. All of the patients had been treated previously with standard chemotherapy, radiation therapy, or surgery. Patients were required to have a Karnofsky score of at least 70% and a life expectancy of at least 4 months. Evaluable or measurable disease was not required for entry into this pilot study. Adequate organ function and the following hematological parameters were required: Hb ≥ 9 g/dl untransfused; platelet count ≥ 100 × 10^9/liter untransfused; and an absolute neutrophil count of >1.5 × 10^9/liter without growth factors.

Patients with active cardiac disease or symptomatic heart failure requiring medical intervention, ejection fraction of ≤40%, or other valvular abnormalities or arrythmias that may predispose patients to congestive heart failure were excluded. Patients could not be receiving concomitant immunosuppressive medications. All patients had a complete history and physical examination, routine hematological and biochemical testing, chest X-ray, electrocardiogram, and ejection fraction determination by MUGA scan before study entry. Baseline computed tomography or magnetic resonance imaging scans of disease sites and CA27.29 tumor marker were also obtained.

All patients signed a protocol-specific consent. Subcutaneous IL-2 (provided by Chiron Corp., Emeryville, CA) was given at 1.75 × 10^6 IU/m^2/day × 49 days, based on dose-escalation studies performed previously (20). All patients received prophylactic premedications consisting of acetaminophen (650 mg, p.o.), ibuprofen (400 mg, p.o.), and diphenhydramine (50 mg, p.o.) 30 min before and 4 h after each s.c. IL-2 dose. It was recommended that patients administer IL-2 4 h before bedtime.

Patients who developed difficult-to-tolerate constitutional symptoms or fluid retention, which did not constitute dose-limiting toxicity, decreased their IL-2 dose to 1.25 × 10^6 IU/m^2/day. The lower dose was then continued for the rest of the study. If IL-2 was interrupted because of toxicity, it was restarted at 1.25 × 10^6 IU/m^2/day. If IL-2 was interrupted because of other reasons, it was restarted at the same dose. Trastuzumab was begun 1 week after the initial IL-2 dose (day 8) and was infused weekly for six weeks (6 doses). The initial trastuzumab dose was 4 mg/kg (over 90 min), with subsequent weekly doses (on days 15, 22, 29, 36, and 43) of 2 mg/kg (over 30 min). No premedications were given for the trastuzumab therapy, unless reactions occurred. Meperidine (25–50 mg, i.v.) was used for infusion reactions with chills or rigors.

Cardiac function was monitored by MUGA scan midstudy and within 7 days after the treatment was completed. Complete blood and differential counts and chemistry panels were performed weekly while the patient was on the study. Tumor assessment was done on day 1 and at study termination. PR was defined as a reduction of ≥50% in the sum of the products of the perpendicular diameters of all bidimensionally measurable disease when compared with pretreatment measurements. SBD was defined as no change in measurable disease for the duration of the study.

**In Vitro Studies.** PBMCs were obtained after Ficoll-Hypaque density centrifugation. Patient samples were tested fresh, without further manipulation. Normal donor PBMCs were further enriched for NK cells using a MACS column, as specified by the manufacturer (Miltenyi, Auburn, CA), resulting in a purity of >85% CD56^+CD3^- NK cells. The human breast cancer cell line SKBR-3 and the chronic myelogenous leukemia-derived cell line K562 (American Type Culture Collection, Manassas, VA) were cultured using established conditions. Cytotoxicity assays were performed at the indicated E:T ratios using patients’ PBMCs or normal donors’ NK cells against cell lines in a 4-h 51Cr release assay (17). Trastuzumab (Herceptin; Genentech, Inc., San Francisco, CA) at a concentration of 1 µg/ml, patient serum, or normal serum was added to targets 30 min before each assay and remained for the 4-h incubation. Specific lysis was calculated as reported previously (17).

Results of experimental points obtained from multiple experiments were reported as mean ± 1 SE. Significance levels were determined by two-sided Student’s t test analysis.

**RESULTS**

Ten patients with metastatic breast cancer were enrolled into this study and received a total of 12 cycles of trastuzumab and IL-2. The median age was 51 years (range, 32–69 years). The majority of the patients had visceral disease (60%), and the remaining patients had bone and skin lesions. All patients received prior therapy and had progressive metastatic disease. Sixty percent of patients received prior chemotherapy, and 30% received two or more regimens. Fifty percent of patients had previously received regimens containing trastuzumab with or without chemotherapy. Forty percent of patients progressed after prior hormonal therapy. Among the 10 patients, 3 had primary metastatic disease, and 7 patients progressed to metastatic disease from primary stage II or III breast cancer after...
either (neo-) adjuvant chemotherapy (n = 5) or hormonal therapy (n = 2).

All patients were evaluable for toxicity. One patient developed mild fluid retention after 3 weeks of treatment and required an IL-2 dose reduction to 1.25 × 10^6 IU/m^2/day for the remainder of the study. The majority of patients received more than 80% of the planned therapy (Table 1). No unexpected toxicities were observed. Both hematological and nonhematological toxicities were minimal. One patient developed grade III anemia (Hb 7.1 from initial Hb 9.3 at study onset). No cardiac toxicity was observed from the combination therapy (Fig. 1). Two patients experienced nonhematological grade III toxicity: one patient had dyspnea, which was thought to be tumor related, that resolved on therapy, and one patient had constipation thought to be due to opioid analgesic treatment.

Two patients (two cycles) were not evaluable for response. One patient withdrew after 3 weeks due to personal reasons (cycle 10), and 2 months later she re-enrolled and completed the study, receiving 100% of the planned therapy (cycle 11). One patient had no measurable disease at study entry (cycle 4). Among the 10 evaluable cycles of trastuzumab and IL-2, the responses were 1 PR, 5 cases of SBD (4 with a decline in CA27.29), and 4 cases of PD.

The patient who obtained a PR (cycle 1) had previously received a peripheral stem cell transplant for treatment of metastatic disease and nine subsequent distinct chemotherapy regimens, including trastuzumab alone and in combination with chemotherapy, without any response. She had an objective response to the trastuzumab and IL-2 regimen with near complete resolution of the soft tissue metastatic lesions on her back. This response lasted for 2 months after therapy was discontinued and subsequently progressed. She did not respond to further chemotherapy. After obtaining Food and Drug Administration and institutional review board approval for retreatment with trastuzumab and IL-2, she received a second cycle of this therapy and had SBD and a decline in her CA27.29 (cycle 2). The interval of time between her cycle 1 and cycle 2 was 12 months.

PBMCs and serum were collected from patients on study. The absolute number of circulating NK cells increased in vivo by approximately 10-fold by day 28 of therapy, irrespective of clinical response (Fig. 2). The absolute number of T cells did not change throughout the monitoring period. PBMCs were tested against HER2/neu-negative K562 targets and HER2/neu-positive SKBR-3 targets before study entry and during therapy. To test whether patient-derived NK cells could be “pre-armed” with trastuzumab in vivo to kill targets in vitro, patient-derived cells were tested alone (control) or in the presence of patient and normal human serum. The nonspecific lytic activity against K562 targets was significantly increased on therapy, as expected with the increased NK cell numbers. Incubation with trastuzumab, patient serum, or normal human serum did not alter this nonspecific K562 cytotoxicity (Fig. 3). In the absence of trastuzumab or patient serum, 24 ± 5.1% of HER2/neu-overexpressing SKBR-3 targets were lysed by patient cells on therapy (>28 days) compared with the 6 ± 4% prestudy (baseline), representative of increased killing due to s.c. IL-2 alone. In contrast to K562 cells, specific lysis of SKBR-3 was further enhanced by the addition of trastuzumab (52 ± 6.3%) or patient serum (58 ± 7.5%), compared with normal human serum (16 ± 4.8%), which contributed no added activity (P < 0.01; Fig. 3). Similar but less augmented killing was seen against MCF-7 targets with low expression of HER2/neu (data not shown).

We further studied serum samples from patients on study against normal donor NK cells. Mononuclear cells were enriched for NK cells using immunomagnetic beads. Serum from patients before study entry and from samples collected on day 28, before the next trastuzumab dose (a trough serum), were added to normal NK cells and SKBR-3 targets. Two patterns were seen. In patients who had never received prior trastuzumab, there was a marked increase in serum ADCC activity on therapy compared with prestudy. Surprisingly, in patients 2–8 weeks off from a previous course of trastuzumab, serum ADCC activity was still maintained (Fig. 4). This suggests that the biological half-life of this activity may last many weeks between cycles.

### Table 1. IL-2 and trastuzumab delivered and response to therapy

<table>
<thead>
<tr>
<th>Cycle</th>
<th>IL-2/trastuzumab completed</th>
<th>HER2 status</th>
<th>Response (disease)</th>
<th>Response (CA27.29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100%/100%</td>
<td>2+</td>
<td>PR</td>
<td>40 to 38</td>
</tr>
<tr>
<td>2</td>
<td>100%/100%</td>
<td>2+</td>
<td>SBD</td>
<td>384 to 318</td>
</tr>
<tr>
<td>3</td>
<td>33%/33%—off after 16 days for PD</td>
<td>3+</td>
<td>PD</td>
<td>43 to 35</td>
</tr>
<tr>
<td>4</td>
<td>100%/100%</td>
<td>3+</td>
<td>NE</td>
<td>43 to 35</td>
</tr>
<tr>
<td>5</td>
<td>81%/66%—off after 34 days for PD</td>
<td>3+</td>
<td>PD</td>
<td>n/a</td>
</tr>
<tr>
<td>6</td>
<td>100%/100%</td>
<td>3+</td>
<td>SBD</td>
<td>60 to 49</td>
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<tr>
<td>7</td>
<td>86%/83%</td>
<td>2+</td>
<td>PD</td>
<td>58 to 61</td>
</tr>
<tr>
<td>8</td>
<td>100%/100%</td>
<td>3+</td>
<td>SBD</td>
<td>200 to 117</td>
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<tr>
<td>9</td>
<td>100%/100%</td>
<td>3+</td>
<td>SBD</td>
<td>n/a</td>
</tr>
<tr>
<td>10</td>
<td>49%/42%—off after 24 days for personal reasons</td>
<td>2+</td>
<td>NE</td>
<td>75 to n/a</td>
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<tr>
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<td>100%/100%</td>
<td>2+</td>
<td>SBD</td>
<td>157 to 134</td>
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<tr>
<td>12</td>
<td>82%/83%</td>
<td>2+</td>
<td>PD</td>
<td>n/a</td>
</tr>
</tbody>
</table>

*a* Cycles 1 and 2 were the same patient; cycles 10 and 11 were the same patient.  
*b* Patients were considered evaluable for response if they had measurable disease prestudy and were able to be assessed at study termination.  
*c* Cycle 4 was not evaluable (NE) for response because she had no measurable disease.  
*d* Cycle 6 required an IL-2 dose reduction after 3 weeks of treatment.  
*e* Cycle 10: CA27.29 was not available (n/a) when patient discontinued treatment.
trastuzumab doses and supports consideration of increasing the trastuzumab interval between infusions.

DISCUSSION

One in eight women will be affected by breast cancer at some time in their lifetime. Although progress has been made with newer chemotherapy agents and the combination of chemotherapy with monoclonal antibodies, current therapy for patients with metastatic disease is still inadequate. Chemotherapy agents can have considerable morbidity, and ultimately, the disease becomes chemotherapy resistant. Trastuzumab is a humanized antibody that recognizes HER2/neu on malignant cells; however, its exact mechanism of action is less well understood (23). The inhibitory effects of trastuzumab on breast cancer may be due to actions such as: (a) enhancement of c-erbB-2 receptor degradation (24); (b) inhibition of cell cycle progression and suppression of apoptosis-regulatory molecules such as Akt and phosphatidylinositol 3'-kinase (25); and (c) suppression of angiogenesis (26) and metastasis (27). One of the proposed mechanisms of trastuzumab antitumor action is through ADCC (17, 18).

The primary objective of this study was to determine the safety of trastuzumab combined with an already established dose of daily, outpatient, s.c. IL-2. There were no unexpected toxicities, and the primary side effect was hematological, with mild anemia and neutropenia. No cardiac toxicity was observed in careful monitoring of pre-, mid-, and post-cycle cardiac ejection fractions. The secondary objective of the study was to evaluate in vivo and in vitro immune responses induced by trastuzumab in combination with s.c. IL-2. We documented increased NK cell numbers and augmented killing of breast cancer targets through ADCC. This HER2/neu-specific ADCC only requires signaling through FcRyIII (CD16) and does not require cooperation from intercellular adhesion molecule 1/integrin or CD2 leukocyte function-associated antigen-3 interactions between NK cells and breast cancer targets, as we have shown previously (17).

In this study we show that (a) NK cells can be safely expanded in vivo with outpatient IL-2 therapy, (b) trastuzumab augments NK cell killing of breast cancer targets in a HER2/neu-specific manner, and (c) trough patient serum contains physiological levels of trastuzumab capable of mediating ADCC.

Because we did not analyze the effect of trastuzumab alone, it is worth noting that patients previously pretreated with trastuzumab had detectable levels of ADCC when tested with normal NK cells. This correlates with recent pharmacokinetic data showing the serum half-life of trastuzumab administered weekly to be 28.5 days (28), suggesting that longer intervals between trastuzumab administration may be as efficacious as the weekly doses. Our data show effective biological activity (i.e., ADCC) with trastuzumab at a concentration of 1 µg/ml, which concurs with activity shown in previous studies using concentrations of 1–10 µg/ml (29). In addition, because we did not compare trastuzumab alone with trastuzumab + IL-2, it will be difficult to determine whether enhancement of ADCC by trastuzumab alone would have elicited objective responses or

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Fig. 1 Trastuzumab and IL-2 therapy did not cause cardiotoxicity. Ejection fractions were monitored by MUGA before treatment (Pre-Tx; n = 11), midway through therapy (Mid-Tx; n = 9), and within 7 days after treatment was completed (Post-Tx; n = 6). Shown are the mean ± SE of ejection fractions at each time point. There were no statistical differences between time points.

Fig. 2 s.c. IL-2 increases NK cell effectors. The absolute NK cell (CD56+/CD3-) and T-cell (CD3+) numbers were determined by multiplying the absolute lymphocyte count from the complete blood count with the percentage of each respective cell type. Percentages were determined by flow cytometry from the lymphocyte gated window of the mononuclear cell population.

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clinical benefit, but patient 1 is instructive. This patient was heavily pretreated with stem cell transplantation and had PD through both trastuzumab alone and trastuzumab in combination with chemotherapy.

In this small cohort of patients, we did not observe any correlation between obtaining a clinical response or toxicity and the biological end points, including NK cell expansion or degree of ADCC activity. These data are similar to the findings of a recently published study of escalating doses of trastuzumab and daily IL-2 in solid tumor patients, which showed no correlation between clinical responses and in vitro cytotoxicity (30). It is also possible that trastuzumab and IL-2 may synergize in stimulating other effector functions of the NK cells, such as cytokine production (22). Recently, Parihar et al. (31) have demonstrated that Herceptin-coated human breast cancer cells cultured with purified human NK cells in the presence of IL-12 induced potent cytokine secretion, such as IFN-γ, tumor necrosis factor α, and granulocyte macrophage colony-stimulating factor. In addition, preliminary results from a Phase I clinical trial by the same group demonstrate a correlation between the increased levels of NK-derived cytokines IFN-γ and antiangiogenic factors and clinical response to therapy with trastuzumab and IL-12 (32). Others have also shown that trastuzumab combinations with cytokines, as well as the recently developed fusion molecules of anti-HER2 antibodies linked to IL-2 or IL-12, may be important in augmenting antitumor immunity (33, 34).

In summary, trastuzumab and IL-2 are well tolerated as outpatient therapy, and this regimen avoids the toxicities of chemotherapy. A PR and 5 cases of SBD in heavily pretreated patients were noted during the short duration of the study. As
more humanized and chimeric monoclonal antibodies come into clinical practice, it will be important to determine whether ADCC together with other effector mechanisms has a significant role in mediating objective tumor responses. To further validate this approach, a larger study using trastuzumab and IL-2 is in progress, with a longer treatment duration, in patients with metastatic breast cancer.

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