A Phase I Study of Interleukin 12 with Trastuzumab in Patients with Human Epidermal Growth Factor Receptor-2-Overexpressing Malignancies: Analysis of Sustained Interferon γ Production in a Subset of Patients

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ABSTRACT

Purpose: **On the basis of preclinical studies, we hypothesized that interleukin (IL)12 would potentiate the antitumor actions of an antihuman epidermal growth factor receptor-2 (HER2) monoclonal antibody (trastuzumab). We conducted a Phase I trial to determine the safety and optimal biological dose of IL-12 when given in combination with trastuzumab.**

Patients and Methods: **Patients with metastatic HER2-positive malignancies received trastuzumab on day 1 of each weekly cycle. Beginning in week 3, patients also received intravenous injections of IL-12 on days 2 and 5. The IL-12 component was dose-escalated within cohorts of 3 patients (30, 100, 300, or 500 ng/kg). Correlative assays were conducted using serum samples and peripheral blood cells obtained during the course of therapy.**

Results: **Fifteen patients were treated, including 12 with HER2 2+ or 3+ breast cancer. The regimen was well tolerated with IL-12-induced grade 1 nausea and grade 2 fatigue predominating. Evaluation of dose-limiting toxicity and biological end points suggested that the 300 ng/kg dose was both the maximally tolerated dose and the optimal biological dose of IL-12 for use in combination with trastuzumab. Two patients with HER2 3+ breast cancer within the 500 ng/kg dose level experienced grade 1 asymptomatic decreases in left ventricular ejection fraction of 12% and 19% after 3 and 10 months of therapy, respectively. There was one complete response in a patient with HER2 3+ breast cancer metastatic to the axillary, mediastinal, and supraclavicular nodes, and 2 patients with stabilization of bone disease lasting 10 months and >12 months, respectively. Correlative assays showed sustained production of interferon (IFN)γ by natural killer cells only in those patients experiencing a clinical response or stabilization of disease. Elevated serum levels of macrophage inflammatory protein-1α, tumor necrosis factor-α, and the antiangiogenic factors IFN-γ inducible protein-10 and monokine induced by γ were also observed in these patients. Patient genotyping suggested that a specific IFN-γ gene polymorphism might have been associated with increased IFN-γ production. The ability of patient peripheral blood cells to conduct antibody-dependent cellular cytotoxicity against tumor targets *in vitro* did not correlate with clinical response or dose of IL-12.**

Conclusions: **The addition of IL-12 to trastuzumab therapy did not appear to enhance the efficacy of this antibody treatment. Sustained production of IFN-γ and other cytokines were observed in three patients: One who exhibited a complete response and two others who had stabilization of disease lasting over 6 months. Given the small sample size and heterogeneity of the patient population, the effects of IL-12 on the innate immune response to trastuzumab therapy should be further explored in the context of a larger clinical trial.**

INTRODUCTION

Tumor overexpression of human epidermal growth factor receptor-2 (HER2) in breast cancer is associated with a worse histological grade, decreased overall survival, and altered sensitivity to chemotherapeutic regimens (1–3). A humanized monoclonal antibody (mAb) against the extracellular domain of HER2 (rhuMAb HER2, trastuzumab, or Herceptin) potently inhibited the growth of HER2-overexpressing cell lines and xenografts in preclinical models (4). In metastatic breast cancer patients whose tumors overexpress HER2, administration of trastuzumab alone or in combination with paclitaxel produced response rates between 22% and 51% (5–7). In a randomized trial, the combination of trastuzumab and paclitaxel was superior to paclitaxel alone with respect to response rates, time-to-progression, and overall survival (8).

There are several possible mechanisms for the antitumor

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activity of trastuzumab (9). Clynes *et al.* (10) reported that the antitumor effects of trastuzumab in a murine model of breast cancer required the expression of functional Fc receptors by host immune effectors. This report suggested that Fc receptor-dependent mechanisms contribute substantially to the actions of mAbs directed against tumor antigens and implied that coadministration of immune stimulatory cytokines might enhance their effects. Effector cells that bear Fc receptor include natural killer (NK) cells, granulocytes, and monocytes/macrophages (11, 12). Although most immune cells coexpress both activating and inhibitory Fc receptor, NK cells are unique in that they constitutively express only an activating, low-affinity Fc receptor (Fcγ RIIIa or CD16; Ref. 13). Activated NK cells are an important source of interferon γ, and early secretion of this cytokine is critical for the optimization and coordination of the innate and specific immune responses (14). It is also well known that activation of patient NK cells with immune stimulatory cytokines enhances their antibody-dependent cellular cytotoxicity activity against trastuzumab-coated breast cancer targets *in vitro* (15).

We have examined recently NK cell cytokine production in response to Fc receptor stimulation, both alone and in combination with NK cell-activating cytokines such as interleukin (IL)2 and IL-12. Using a novel *in vitro* coculture system, we showed that the combination of IL-12 with trastuzumab-coated tumor stimulated NK cells to secrete greater amounts of immune modulatory cytokines [*e.g.,* interferon-γ, tumor necrosis factor (TNF)α, granulocyte macrophage colony-stimulating factor (GM-CSF), and macrophage inflammatory protein (MIP)1α] as compared with stimulation with either agent alone, a result that could not be duplicated by costimulation with other interleukins such as IL-2 (16). In addition, immune-competent mice challenged with trastuzumab-coated HER2-positive tumor cells and IL-12 exhibited enhanced systemic levels of NK cell-derived cytokines. These preclinical data suggested that coadministration of IL-12 with trastuzumab might lead to enhanced immune activation in patients with HER2-overexpressing cancers.

The current study was designed to determine the safety and optimal biological dose of IL-12 when given in combination with trastuzumab and to examine the immunological response to this treatment. The results show that IL-12 can be given safely with trastuzumab. In addition, cytokine production by NK cells was observed in a subset of patients.

PATIENTS AND METHODS

Eligibility Criteria. A National Cancer Institute (NCI)-sponsored Phase I trial of IL-12 with trastuzumab (T99–0032) was conducted at The Ohio State University Comprehensive Cancer Center from August 1999 to January 2003. The treatment protocol was approved by the Human Institutional Review Board at The Ohio State University Medical Center (OSU-9968). Patients with nonhematological malignancies that expressed HER2 were eligible for enrollment. HER2 expression was defined as 1+, 2+, or 3+ as determined by the standard histological assay currently in clinical use at the time of study initiation, the DAKO HercepTest. Patient tumors were later evaluated in a central lab for HER2/*neu* gene expression by fluorescence *in situ* hybridization (FISH; Ventana Inform; Ref. 17). Study patients were required to be ≥18 years of age and meet the following eligibility criteria: a life expectancy of >6 months; a Karnofsky Performance Status index of ≥70%; normal left ventricular ejection fraction of >50% as measured by echocardiogram or multigated acquisition scan (18); normal organ and bone marrow function; either measurable or evaluable disease; have received at least one prior regimen of chemotherapy for metastatic disease; and be capable of giving their written informed consent in accordance with federal and institutional guidelines. Patients were excluded from participation if they had received prior therapy with trastuzumab or IL-12; were pregnant or nursing; or had a significant concurrent illness such as an underlying immunological disease, uncontrolled or severe cardiac disease, or known seropositivity for viral hepatitis, HIV, or other infectious agents.

Treatment Schema and Dose Escalation. Consenting patients received an intravenous loading dose of trastuzumab (4 mg/kg) followed 1 week later by a maintenance dose of 2 mg/kg. On the third week and every week thereafter, patients received 2 mg/kg trastuzumab on day 1 of the weekly cycle followed by intravenous IL-12 on days 2 and 5 (Fig. 1). The starting dose of IL-12 was 30 ng/kg (administered intravenous over 5–10 s). Patients received this therapy for 14 weeks at which time they were restaged with appropriate imaging modalities. Successive cohorts of 3 patients received increasing doses of IL-12. However, neither the dose of trastuzumab nor IL-12 was escalated within a given patient. Patients with a clinical response or stable disease at week 14 were allowed to continue therapy for a total of 54 weeks or until disease progression. Cardiac function was monitored via multigated acquisition scan at 3-month intervals.

Dose escalation of IL-12 was initiated after 3 patients had received trastuzumab at the current dose of IL-12 for 3 weeks with no dose-limiting toxicity (defined below). In the absence of dose-limiting toxicity, successive cohorts of patients were entered into the protocol at increasing doses of IL-12. For any dose level, if 1 of the 3 patients demonstrated dose-limiting toxicity, then an additional 3 patients were treated at that dose level. If only 1 of the 6 exhibited dose-limiting toxicity, then the next cohort of 3 patients were entered at the next dose level. If ≥2 of

Week 1:

Day	1	2	3	4	5	6	7
Treatment	▲						

Week 2:

Day	1	2	3	4	5	6	7
Treatment	△						

Weeks 3 – 54:

Day	1	2	3	4	5	6	7
Treatment	△	★			★		

▲ Trastuzumab (4 mg/kg IV)

△ Trastuzumab (2 mg/kg IV)

★ IL-12 (30, 100, 300, or 500 ng/kg IV)

Fig. 1 Treatment schema.

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the 6 demonstrated dose-limiting toxicity, no additional patients were treated at that dose level. The maximum tolerated dose was defined as the dose level of IL-12 one level beneath the dose at which 2 or more of 6 patients showed dose-limiting toxicity. If dose level 4 was completed without dose-limiting toxicity, then 3 additional patients would be accrued at a dose level that was determined by the principal investigator to have been both well tolerated and effective in terms of inducing the desired biological response as measured by correlative assays (optimal biological dose). If dose-limiting toxicity was not reached at this dose level, then the trial would be halted. Because 500 ng/kg intravenous twice weekly was well established as the maximum tolerated dose in previous trials of single-agent IL-12 and IL-12 in combination with IL-2, we did not choose to exceed this dose level in the current trial (19, 20).

Criteria for Dose-Limiting Toxicity and Response Assessment. Toxicity was assessed using the revised NCI Common Toxicity Criteria. Patients who experienced any clearly drug-related grade 3 or greater toxicity (nonhematological) that did not resolve after a 2-week rest period were considered to have experienced a dose-limiting toxicity and were removed from the study. Patients resuming therapy after the resolution of a grade 3 toxicity could continue therapy with the following dose-modification: if the toxicity was felt to be secondary to IL-12, the dose of this component was reduced by 50%; alternatively, if the toxicity was felt to be secondary to trastuzumab, the dose of this component was reduced by 50%. In general, any patient experiencing a clearly drug-related grade 4 toxicity (nonhematological) was removed from the study. However, if a grade 4 toxicity was rapidly reversible and the patient appeared to be deriving some benefit from therapy, treatment was allowed to continue with a dose modification (as outlined above). Irreversible cardiac toxicity, grade 3 or 4 cardiac toxicity, and any new symptoms of cardiac dysfunction (congestive heart failure, arrhythmia, or angina) resulted in removal of the patient from the study. Asymptomatic decreases in left ventricular ejection fraction of ≥20% as compared with baseline, symptomatic decreases of ≥10% as compared with baseline, and left ventricular ejection fraction values <40% were all cause for removal of the patient from the study.

A complete response was defined as disappearance of all symptoms and signs of all known disease, as confirmed by physical examination, laboratory, and radiographic studies. Partial response was defined as a >50% reduction in the sum of the products of the perpendicular diameters of all measured lesions. Progressive disease was defined as a >25% increase in the sum of the products of the perpendicular diameters of all of the measured lesions, any new lesions, or any definitive increase in tumor size. Stable disease was defined as <50% reduction and <25% increase in the sum of the products of the two perpendicular diameters of all measured lesions and no increase in evaluable or nonevaluable disease for a period of 8 weeks. Clinical benefit rate was defined as the percentage of patients exhibiting a complete response, partial response, or stable disease lasting >6 months (21).

Procurement of Patient Serum and Peripheral Blood Mononuclear Cells. Blood for use in the correlative studies was drawn just before each injection of trastuzumab or IL-12 (2 heparinized green-top tubes, ~20 ml). Serum and peripheral blood mononuclear cells (PBMC) were procured from the blood sample within 6 h of phlebotomy. Serum was snap frozen and stored at −70°C, whereas PBMC were cryopreserved and stored at −134°C in liquid nitrogen. During every other cycle, the PBMCs were processed for total RNA. Cells from all of the time points from a given patient were simultaneously thawed and used in immune assays (described below), along with fresh cells from a normal donor and frozen cells from a single normal donor as controls to evaluate interassay variability (≤8%). NK cell viability after thawing was routinely >90%, and NK cells subjected to a single freeze/thaw cycle exhibit only an 8–15% reduction in interferon γ production and antibody-dependent cellular cytotoxicity activity.

Intracellular Flow Cytometry. The production of interferon γ by PBMC derived from cycles 7 and 10 was analyzed using a fluorescein isothiocyanate-conjugated mAb to human interferon γ (BD PharMingen, San Diego, CA) and phycoerythrin-conjugated mAbs to surface markers specific for NK cells (CD56) and T cells (CD3), as described previously (16). The percentage of positively staining cells and mean fluorescence intensity were calculated for interferon γ within the specified cell population.

Real-Time Reverse Transcription-PCR. Real-Time reverse transcription-PCR (RT-PCR) was used to quantify the relative increase in interferon γ transcript after administration of IL-12 (22). Total RNA was isolated from PBMCs ($\sim 5 \times 10^6$) that had been processed with RNA STAT-60 solution (Tel-Test, Inc, Friendswood, TX). The RNA was quantitated, reverse transcribed, and the cDNA product was then used as a template in a real-time RT-PCR amplification reaction, as described previously (16). Fold increases in cytokine transcript were determined using the comparative threshold cycle method (23).

Cytokine Enzyme-Linked Immunosorbent Assays. Patient serum samples were thawed at 37°C and analyzed in triplicate for levels of cytokines (interferon-γ, TNF-α, GM-CSF, and MIP-1α) and antiangiogenic chemokines [interferon γ inducible protein-10 (IP-10) and monokine induced by γ (MIG)] by enzyme-linked immunosorbent assays (ELISA) using commercially available monoclonal antibody pairs (Endogen, Inc., Woburn, MA). On the basis of the manufacturer's guidelines, a standard sandwich ELISA for each human cytokine was developed, and cytokine concentrations were determined by the use of standard curve regression analysis. The lower limit of detection for all ELISAs was 10 pg/ml, except for the GM-CSF ELISA (30 pg/ml).

Antibody-Dependent Cellular Cytotoxicity. Frozen patient PBMCs were thawed (average 90% viable cell recovery), enumerated, and plated into 96-well V-bottomed plates in culture medium supplemented with 10% human AB serum with or without 10 ng/ml recombinant human IL-12. PBMCs were incubated for 18 h at 37°C and then used in a 4-h ^{51}Cr release antibody-dependent cellular cytotoxicity assay *versus* the HER2-overexpressing breast cancer cell line, BT-474. Percentage of specific lysis was calculated as described previously (24). Exogenous trastuzumab was chosen as the mAb source for this assay (as opposed to patient serum obtained following a trastuzumab infusion) so that antibody-dependent cellular cytotoxicity results might reflect differences in effector cell activation

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status rather than interpatient differences in trastuzumab pharmacokinetics.

Genotyping Assays. Genomic DNA was isolated from donor PBMCs and adjusted to 25 μg/ml for use in cytokine genotyping assays. Cytokine genotyping for *transforming growth factor* β (codons 10 and 25), *TNF*-α (promoter region, residue-308), *IL-6* (promoter region, residue −174), *IL-10* (promoter region, residues −1082, −819, and −592), and *interferon* γ (intron 1, residue 847) was accomplished using Cytgen cytokine genotyping trays from One Lambda (Canoga Park, CA) according to the manufacturer's instructions.

Statistics. Statistical analyses of binomial data (response/no response) used Fisher's exact test, whereas analysis of continuous data including uncensored progression-free survival was performed using the nonparametric Mann-Whitney U test. Whereas statistical significance may be achieved with our small sample size, our pilot study was not powered to allow evaluation of inferential hypotheses. Should observed differences reach statistical significance ($P < 0.05$), such findings will only provide indications for future investigation and should not be viewed as clinically unequivocal.

RESULTS

Patient Characteristics and Toxicities. Fifteen patients received treatment. The characteristics of these patients are described in Table 1. The lung, liver, and bone were the most common sites of metastases in these patients. The median number of prior chemotherapy regimens was 2.3 (range, 1–4). The distribution of HER2 expression by tumor type is described in Table 2. The majority of the patients were female breast cancer patients with HER2 2+ (47%) or HER2 3+ (33%) tumors (Table 3). All 5 (100%) of the HER2 3+ tumors and 5 of 7 (71%) HER2 2+ tumors were found to be positive by FISH analysis. The rate of FISH positivity in HER2 2+ tumors was higher than expected from previous data and must be interpreted with some caution, because the Ventana Inform system used in the current trial for the detection of HER2/*neu* amplification had no centromeric 17 probe (control for polysomy 17; Ref. 24).

Table 1 Patient characteristics

Characteristic	
Total patients treated, *n*	15
Age (years)	
Median	52
Range	32–71
Gender, *n*	
Male	1
Female	14
Karnofsky Performance Index, *n*	
90%–100%	4
80%–90%	9
70%–80%	2
No. metastatic sites, *n*	
1	6
2	5
≥3	4
Metastatic site, *n*	
Liver or lung	8
Other	11
No. prior chemotherapy regimens, *n*	
0	0
1	2
2	8
>2	5
Prior trastuzumab, *n*	0

Table 2 Tumor HER2/*neu* levels: a comparison between IHC and FISH

Tumor type	No. of patients	HER2/*neu* by IHC			HER2/*neu* by FISH
		1+	2+	3+	Positive/no. assayed
Breast	12	0	7	5	5/7 (2+), 5/5(3+)
Pancreatic	2	1	1	0	0/1 (1+), 1/1(2+)
Cervical	1	1	0	0	0/0*

Abbreviations: IHC, immunohistochemistry.
* No additional tissue or slides available for analysis.

Table 3 Patient profiles

Patient	Tumor type (HER2 level)	IL-12 dose (ng/kg)	IL-12 dose level	Clinical outcome	No. cycles received
A	Breast (3+)	30	1	PD	11
B	Breast (2+)	30	1	PD	14
C	Pancreatic (1+)	30	1	PD	5
D	Pancreatic (2+)	100	2	PD	14
F	Breast (2+)	100	2	PD	14
E	Breast (3+)	100	2	CR	54
H	Breast (2+)	300	3	PD	14
J	Breast (2+)	300	3	PD	12
K	Breast (2+)	300	3	PD	10
M	Breast (3+)	500	4	SD	34
N	Cervical (1+)	500	4	PD	7
L	Breast (3+)	500	4	PD	14
O	Breast (2+)	300	3	PD	4
P	Breast (3+)	300	3	PD	14
Q	Breast (2+)	300	3	SD	54

Abbreviations: PD, progressive disease; CR, complete response; SD, stable disease.

The most commonly observed toxicities related to treatment (IL-12) were NCI-Common Toxicity Criteria grade 1 nausea and grade 2 fatigue. These events were easily managed, and no patients required dose modifications. The only grade 4 toxicities reported during treatment were for patient A in dose level 1 (dyspnea and fatigue) and for patient D in dose level 2 (abdominal pain/cramping and hepatic pain). These events were felt to be unrelated to the treatment regimen, because these patients had progression of pre-existing lung and hepatic metastases, respectively, during treatment. The most commonly observed grade 2 or 3 toxicities are listed in Table 4. The grade 3 arthralgia observed in patient Q (dose level 3) was felt to be related to metastatic disease in the bone. The remaining grade 3 toxicities, consisting of hepatic enzyme abnormalities and leukocyte counts, did not qualify as dose-limiting toxicity (see "Patients and Methods") and stabilized with proper management. Two patients, both HER2 3+ and receiving the highest IL-12 dose level, experienced a grade 1 asymptomatic decrease in left ventricular ejection fraction by multigated acquisition scan and were removed from the study. Patient L was removed for a 12% decrease in left ventricular ejection fraction (from

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Table 4 Common toxicities

Toxicity	% of total patients	% of patients per IL-12 dose level (grade) 30 (n = 3)	100 (n = 3)	300 (n = 6)	500 (n = 3)	Category of adverse event
Arthralgia	27	33 (2)	33 (2)	17 (**3***)	33 (2)	* Disease-related, resolved
Dyspnea	60	66 (2,**4***)	33 (2)	50 (2)	100 (2)	* Disease-related
Fatigue	93	100 (2,**4***)	66 (2)	100 (2,**3***)	100 (2)	* Disease-related
Hemoglobin	60	33 (2)	66 (2)	50 (2)	100 (2)	
Leukocytes	40	33 (2)	33 (2)	50 (2,3)	33 (3)	
Lymphopenia	66	66 (2,3)	66 (2,3)	66 (2,3)	66 (2)	
Polymorphonuclear leukocytes/granulocytes	47	33 (2)	33 (3)	66 (2,3)	33 (3)	

60% to 48%) after 3 months of therapy. Patient M remained on therapy for a full 10 months until experiencing an asymptomatic 19% decrease in left ventricular ejection fraction (from 54% to 35%). Both patients were asymptomatic with no clinical signs or symptoms of cardiomyopathy at the time that therapy was discontinued. As per protocol, patient M was considered to have experienced a dose-limiting toxicity. After consultation with NCI officials regarding the two cardiac adverse events within the 500 ng/kg dose level (decreases in left ventricular ejection fraction), it was decided that an additional 3 patients would be accrued to the 300 ng/kg IL-12 dose level. Because dose-limiting toxicity was not encountered in >2 of 6 patients in this cohort, the 300 ng/kg IL-12 dose level was chosen as the maximum tolerated dose. Evaluation of biological end points (see Figs. 2, 3, and 6) suggested that the 300 ng/kg dose was also highly effective in the induction of systemic NK cell-derived cytokines and was, therefore, chosen as the optimal biological dose.

There was one complete response in a patient (E) with bulky medistinal, axillary lymphadenopathy that was HER2 3+ and positive for HER2 gene amplification by FISH analysis. This patient received a full year of therapy at dose level 2 and went on to receive trastuzumab alone upon completion of the trial. There were 2 patients (M and Q) who experienced significant stabilization of disease. In patient M with right axillary lymphadenopathy and HER2 3+ overexpression, stable disease was maintained for >10 months until removal from the study for an asymptomatic decrease in left ventricular ejection fraction. In patient Q with FISH+ (HER2 2+) breast cancer metastatic to the vertebrae, lower spine, and pelvis, stable disease was maintained for the full 54 weeks of therapy dictated by the protocol. This patient currently receives trastuzumab alone with elimination of bone pain and marked improvement in mobility. The appearance of sclerotic changes within several of the larger metastatic sites suggested that this patient may have had experienced a response to treatment.

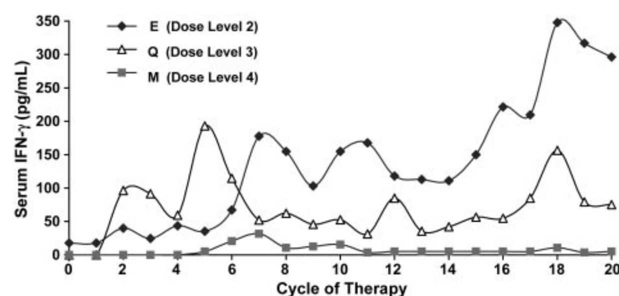

Fig. 2 Serum interferon (*IFN*)γ levels in patients E, M, and Q during first 20 cycles of therapy. Serum samples from each patient were analyzed for IFN-γ content by ELISA. Results represent mean IFN-γ content of triplicate wells. SE was <5% for all data points shown.

Measurement of Interferon γ Production by Patient PBMCs. Correlative studies were conducted to investigate immune cell activation in response to treatment. Of note, the only patients with measurable levels of interferon γ on days 1, 2, and 5 of the treatment cycle were those who experienced a clinical response or significant stabilization of disease (patients E, M, and Q). The remaining 12 patients exhibited no detectable levels of interferon γ (<10 pg/ml) within their sera at these time points. As seen in Fig. 2, levels of interferon γ rose above baseline in these 3 patients within the first 6 cycles of therapy. Patient M exhibited modest elevations in interferon γ levels every 4–5 cycles that dropped down to low but detectable levels (*e.g.,* 10–20 pg/ml) during the interval periods. Although serum interferon γ levels in these patients fell slightly by cycle 30, they remained elevated above baseline throughout the trial (data not shown). Analysis of patient time-to-progression data using the nonparametric Mann-Whitney U test indicated that induction of interferon γ was associated with a statistically significant increase in progression-free survival (P = 0.004); however, this finding must be interpreted with caution given the small sample size.

Analysis by real-time RT-PCR of patient PBMCs taken from an early cycle of therapy coinciding with the initial increases in interferon-γ serum levels revealed that the three patients who experienced a clinical benefit (patients E, M, and Q) exhibited the most dramatic increases in transcript induction during days 1 and 2 of the cycle (P = 0.002 *versus* mean of all other patients). Patients Q and M exhibited ~200-fold increases in interferon γ transcript on day 1 as compared with the average 9.5-fold induction seen in the other patients in dose levels 3 and 4. Interestingly, a nonresponder (patient B) also exhibited an increase in transcript induction but during day 5 of the cycle. This did not correlate with the observed lack of serum interferon γ in this patient nor clinical outcome. Because blood for correlative studies was drawn 72 h after the administration of IL-12, it is possible that less-sustained production of interferon γ may have occurred in nonresponding patients but was not detected. In other trials, IL-12 administration has been associ-

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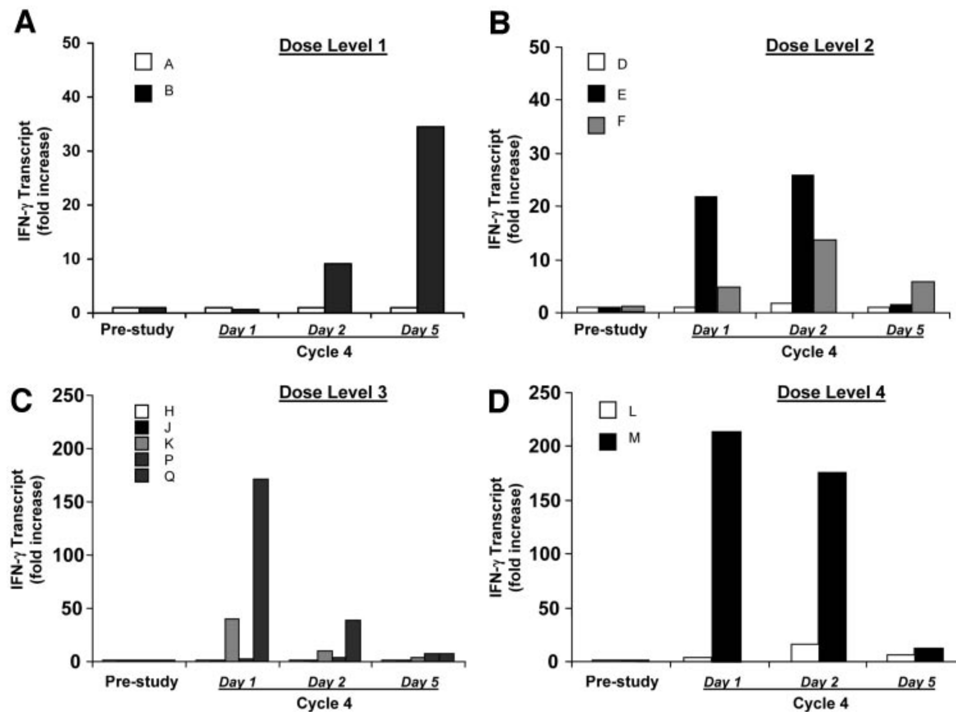

Fig. 3 Interferon (*IFN*)γ gene transcript levels at baseline and during cycle 4. Patient peripheral blood mononuclear cells were examined for levels of IFN-γ mRNA over time by real-time reverse transcription-PCR. Results are shown as fold increase in IFN-γ transcript over prestudy levels during cycle 4 directly after the first full week of interleukin 12 administration, for patients in (*A*) dose level 1, (*B*) dose level 2, (*C*) dose level 3, and (*D*) dose level 4. Patients C, O, and N were not analyzed due to low numbers of cells.

ated with an acute induction of interferon γ followed by a return to baseline within 48–72 h (25, 26). Indeed, our real-time PCR analysis revealed low levels of interferon γ transcript within 4 of the 9 nonresponding patients examined (mean day 1 fold-increase = 6.09 ± 4.2). However, this initial transcript induction was significantly less than in patients E, M, and Q and was not sustained in later cycles.

Intracellular flow cytometry was evaluated to determine which cellular compartment was responsible for the sustained production of interferon γ in later cycles. Of the patients from each IL-12 cohort examined at cycle 10, only those patients who exhibited a complete response or stabilization of disease showed any detectable levels of interferon γ secretion by IL-12-stimulated NK cells. The magnitude of interferon γ secretion, represented by the mean fluorescence intensity, increased significantly above baseline during the cycle for patients E, M, and Q (mean pretherapy mean fluorescence intensity = 10.25 ± 4.4 and post-IL-12 mean fluorescence intensity = 122.65; $P < 0.005$). No interferon γ production was detected within NK cells from the other patients examined. In addition, interferon γ production was not observed within patient T cells from any patient examined (data not shown). PBMC from patients E and Q were harvested directly from a blood sample drawn on day 5 of cycle 44 and stained for intracellular interferon γ with no IL-12 restimulation. Fresh cells for patient M were not available. A striking 100% of NK cells from patient E were actively producing interferon γ, whereas at least 44% of NK cells from patient Q exhibited cytokine production at this time point (Fig. 4). Interferon γ production was undetectable in freshly harvested NK cells from nonresponding patients (data not shown).

Measurement of Antiangiogenic Factors in Patient Serum. IP-10 and MIG are chemokines that can be induced within NK cells and T lymphocytes by interferon γ. They have been shown to act as potent inhibitors of neovascularization in a variety of tumor models (27). Recent trials in humans have shown increased levels of both IP-10 and MIG in the PBMCs of IL-12-treated cancer patients (28). Therefore, these antiangiogenic factors were measured in all of the patients on study. We found that 79% and 53% of patients exhibited consistent increases from baseline throughout cycle 4 in serum MIG and serum IP-10, respectively (data not shown). Similar results were observed during cycle 14. Interestingly, only in patients E, M, and Q (those with high circulating levels of interferon-γ) did these elevations in MIG and IP-10 rise above normal levels over the course of multiple treatment cycles (Fig. 5; data not shown). These data imply that some level of interferon γ induction may have occurred in nonresponding patients in response to the IL-12/trastuzumab regimen. However, it appears that only patients with sustained levels of interferon γ exhibited dramatic increases in the circulating levels of downstream antiangiogenic factors.

Measurement of Other NK Cell-Derived Cytokines in Patient Serum. NK cells are potent producers of other immune stimulatory cytokines such as TNF-α, MIP-1α, and GM-CSF. On the basis of our preclinical data that showed potent secretion of these factors by NK cells costimulated with trastuzumab-coated tumor and IL-12 (16), patient sera were examined for these cytokines. Low levels of TNF-α and MIP-1α were detected at baseline in all 15 of the patients (data not shown). Importantly, only patients E, M, and Q showed increases in the levels of circulating TNF-α and MIP-1α over baseline during the course of therapy (Fig. 6). No

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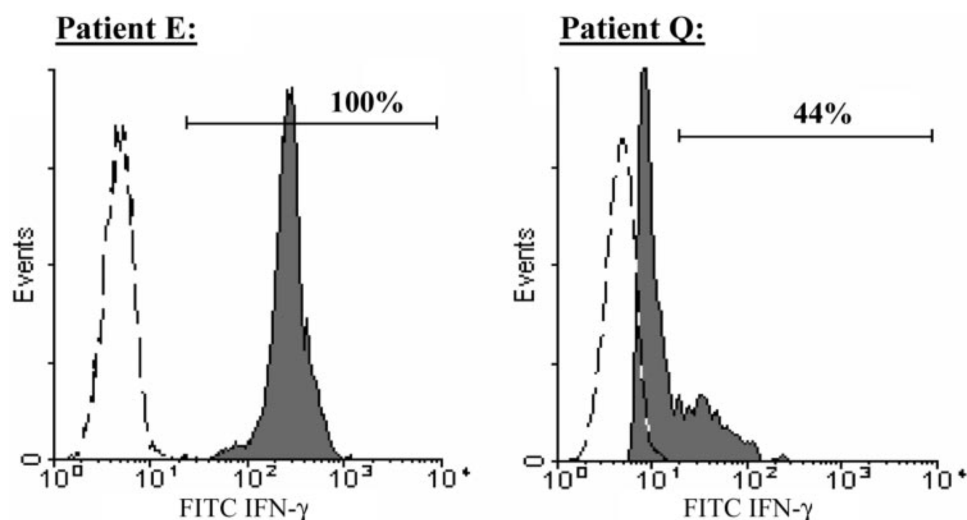

Fig. 4 Interferon (*IFN*)γ protein production by natural killer (NK) cells from patients E and Q during cycle 44. Freshly isolated peripheral blood mononuclear cells from patient E and patient Q were stained for IFN-γ production by NK cells. Percentage within each histogram represents amount of NK cells actively producing IFN-γ within each patient. *FITC,* fluorescein isothiocyanate.

detectable levels of GM-CSF were found at baseline or during therapy for any patient (data not shown).

Analysis of Cytokine Gene Polymorphisms within Patient Immune Cells. The ability of immune effector cells to secrete cytokines in response to trastuzumab plus IL-12 may be determined, in part, by functional polymorphisms within cytokine genes (29). Therefore, the distribution of *interferon* γ and *TNF-*α gene polymorphisms within study patients was determined (Table 5). The distribution of each cytokine genotype within our patient group approximately reflected that found within the population at large (30). Interestingly, patient E (complete response) was the only person found to have the T/T *interferon* γ gene polymorphism, one normally associated with high interferon γ production. Of note, this patient had the highest levels of interferon γ of any patient on the trial. Because only 1 patient on our trial carried this polymorphism, it remains difficult to assess whether this result could predict response to therapy. Patient genotyping of the polymorphisms in the *transforming growth factor* β*, IL-6,* and *IL-10* genes did not show any apparent correlation with response (data not shown). Of note, the 2 patients who experienced stabilization of disease (patients M and Q) carried the A/A *interferon* γ polymorphism associated with low interferon γ production.

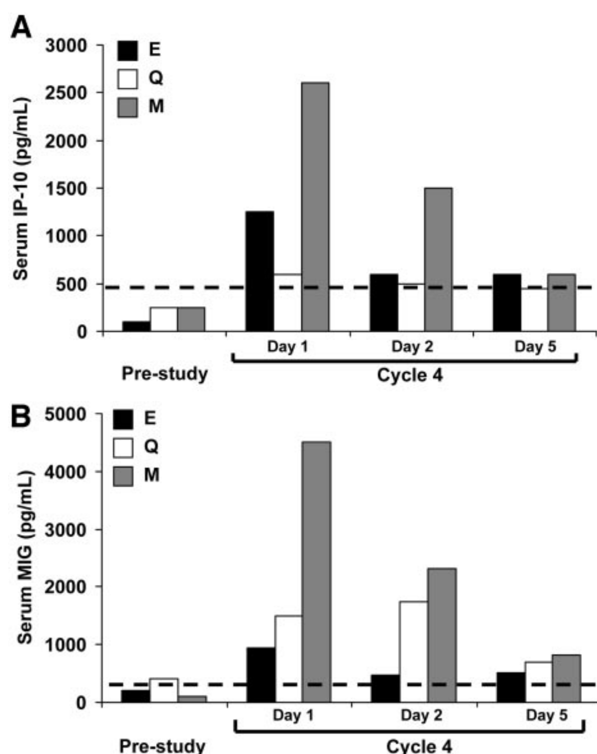

Fig. 5 Levels of serum monokine induced by γ (*MIG*) and interferon γ-inducible protein-10 (*IP-10*) at baseline and during cycle 4. Serum samples from all of the patients on trial were analyzed for the presence of the antiangiogenic factors, IP-10 and MIG. Shown are results from patients E, M, and Q. Results represent mean (*A*) IP-10 and (*B*) MIG of triplicate wells with SE <5%. *Dashed lines* within each graph indicate average levels of the cytokine within normal sera (mean of 10 different normal donors).

Measurement of Trastuzumab-Directed Antibody-Dependent Cellular Cytotoxicity Mediated by Patient PBMCs. The effect of IL-12/trastuzumab on the ability of patient PBMCs to mediate cellular cytotoxicity against trastuzumab-coated, HER2-overexpressing tumors was determined. To examine the effect of addition of IL-12 to the therapeutic regimen, patient PBMCs derived from day 1 (before the trastuzumab dose), day 2 (after trastuzumab, but before the first IL-12 dose), and day 5 (after trastuzumab and the first dose of IL-12) of the weekly cycle were tested. Of the 11 patients evaluable for antibody-dependent cellular cytotoxicity, 9 (82%) exhibited an increase in trastuzumab-directed antibody-dependent cellular cytotoxicity over the course of the cycle, from a mean of 6.0 ± 1.8% at day 1 to 17.3 ± 4.3% at day 5 (Fig. 7). However, the increases in antibody-dependent cellular cytotoxicity did not occur in an IL-12 dose-dependent manner ($P = 1.00$; Fig. 8*A*). In addition, there was no apparent correlation between increases in IL-12-induced antibody-dependent cellular cytotoxicity and clinical benefit ($P = 1.00$). In fact, the mean fold increase in antibody-dependent cellular cytotoxicity from day 1 to day 5 of this representative cycle for the 3 patients who exhibited a

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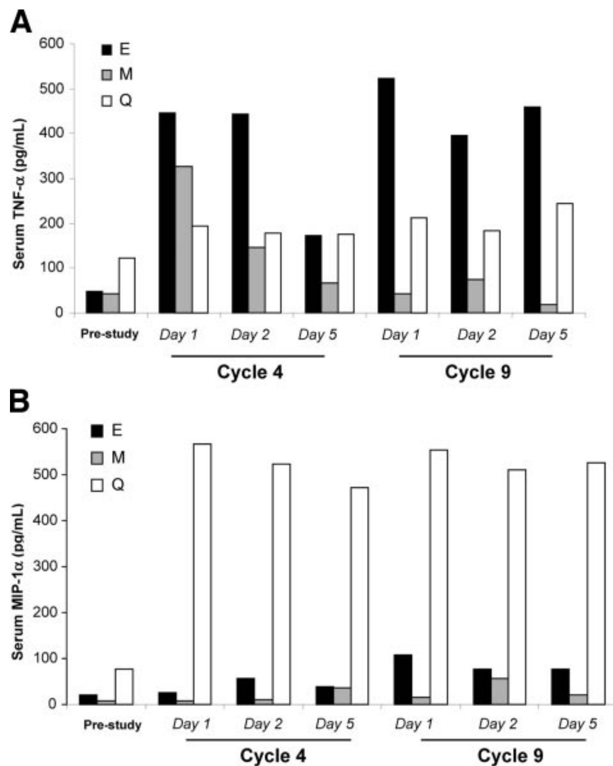

Fig. 6 Levels of serum tumor necrosis factor (*TNF*) α and serum macrophage inflammatory protein (*MIP*) 1α at baseline and during cycles 4 and 9. Serum samples of all 15 patients on trial were analyzed for (*A*) TNF-α and (*B*) MIP-1α content by ELISA. Shown are results from patients E, M, and Q. Results represent mean cytokine value of triplicate wells.

clinical benefit (21) from the regimen (E, M, and Q) was 2.6 as compared with a 8.5-fold increase in antibody-dependent cellular cytotoxicity exhibited by nonresponders (Fig. 8*B*).

DISCUSSION

In this Phase I trial, the toxicity associated with administration of IL-12 with trastuzumab to patients with HER2-overexpressing malignancies was evaluated. The most commonly observed toxicities related to the addition of IL-12 to the trastuzumab regimen were NCI-Common Toxicity Criteria grade 2 or 3 dyspnea, fatigue, hepatic enzyme abnormalities, and leukocyte counts. These events were easily managed, and no patients required dose modification. However, the development of a grade 1 asymptomatic decrease in left ventricular ejection fraction in patient M receiving the highest IL-12 dose was considered a dose-limiting toxicity according to the protocol document (see "Patients and Methods"). After an additional 3 patients were treated at the 300 ng/kg IL-12 dose level without dose-limiting toxicity, the 300 ng/kg IL-12 dose level was chosen as the maximum tolerated dose. Because this dose level was also chosen as the optimal biological dose based on independent biological end points, the recommended dose of IL-12 in our regimen is 300 ng/kg intravenous twice weekly. Interestingly, the addition of low-dose IL-12 to standard trastuzumab therapy did not seem to exacerbate the toxicities most commonly observed with a weekly schedule of single-agent trastuzumab (infusion-related fever and chills, nausea, and vomiting). However, 2 of 3 patients within the high-dose IL-12 cohort (500 ng/kg) experienced decreases in left ventricular ejection fraction, an event that is observed with trastuzumab monotherapy in ~1% of patients with no previous anthracycline exposure and 7% of patients with previous anthracycline exposure (31). Patients L and M on the current trial were removed from therapy for grade 1 decreases in left ventricular ejection fraction of 12% and 19% at weeks 12 and 36, respectively. The observed changes in left ventricular ejection fraction were asymptomatic and only appreciated upon a scheduled multigated acquisition scan. Patient L had previously received a 4-cycle regimen of cyclophosphamide with the anthracycline doxorubicin. Patient M had received only one prior chemotherapeutic regimen that contained docetaxel. Due to these prior treatments, it is not clear whether the addition of IL-12 to the trastuzumab regimen led to the increased cardiac toxicity. However, only 1 of the affected patients exhibited elevated levels of interferon γ, which suggested that the development of cardiac dysfunction might not have been related directly to the secretion of NK cell-derived cytokines. Ongoing studies examining the relationship between NK cell cytokine production and trastuzumab-induced cardiac toxicity may lend additional insight to this question.

Although the current trial was not designed to assess efficacy, our results reveal an overall objective response rate of 7% (1 of 15) and a clinical benefit rate (defined previously as complete response, partial response, or stable disease lasting >6 months) of 20% (3 of 15; Ref. 21). The response rate in our HER2 3+ breast cancer patients, who were also HER2-positive by FISH, was 20% (1 of 5), compared with a response rate of 34% (95% confidence interval, 24–46%) reported for HER2 FISH-positive breast cancer patients in a large Phase II study of single-agent trastuzumab as first-line therapy (21). In a pretreated patient group, the objective response rate to single-agent trastuzumab therapy in the context of a multinational Phase II

Table 5 Patient cytokine gene polymorphisms

Patient	Clinical outcome	*IFN-γ* genotype	*TNF-α* genotype
A	PD	A/A	G/G
B	PD	T/A	G/A
C	PD	T/A	G/G
D	PD	A/A	G/G
F	PD	T/A	G/G
E	CR	T/T	G/G
H	PD	A/A	G/G
J	PD	A/A	G/G
K	PD	T/A	G/G
M	SD	A/A	G/A
N	PD	T/A	G/G
L	PD	T/A	G/A
O	PD	T/A	G/G
P	PD	T/A	G/G
Q	SD	A/A	G/A

Abbreviations: PD, progressive disease; CR, complete response; SD, stable disease; A/A, low IFN-γ; T/A, intermediate IFN-γ; T/T, high IFN-γ; G/G, low TNF-α; G/A and A/A, high TNF-α.

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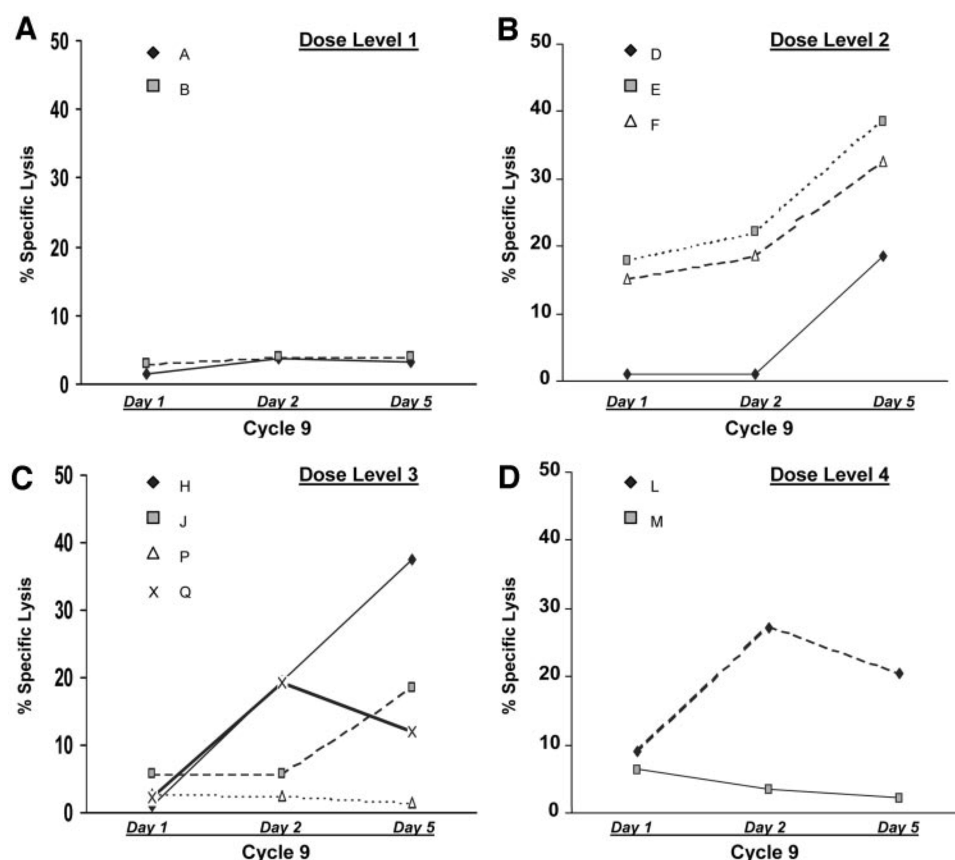

Fig. 7 Levels of trastuzumab-directed antibody-dependent cellular cytotoxicity mediated by patient immune cells during the course of cycle 9. Patient peripheral blood mononuclear cells from each treatment day of cycle 9 were tested for trastuzumab-mediated antibody-dependent cellular cytotoxicity in the presence of interleukin 12 against the HER2-overexpressing human breast cancer cell line, BT-474. Data represent mean percentage of specific lysis (SE <5%) of trastuzumab-treated BT-474 *versus* polyclonal IgG-treated BT-474. Percentage of specific lysis against the HER2-negative cell line, MDA-468, was <5%, regardless of the presence of interleukin 12 (data not shown).

trial was reported previously as 18% for patients with HER2 3+ breast cancer (32). Thus, the addition of IL-12 to trastuzumab therapy did not appear to significantly enhance the efficacy of this antibody treatment. Although our analysis indicated that induction of interferon γ was associated with a statistically significant increase in progression-free survival ($P = 0.004$), this result must be interpreted with caution given the small sample sizes involved.

Elevated and sustained levels of circulating interferon γ as well as other NK cell-derived cytokines such as TNF-α and MIP-1α were observed in the patient who had a complete response and in 2 others with stable disease that lasted 10 and 12 months, respectively. Levels of effector molecules downstream of interferon γ, such as the antiangiogenic factors IP-10 and MIG, were also elevated in these patients. This simultaneous release of a broad array of cytokines suggested a unique pattern of NK cell activation in response to IL-12 plus trastuzumab that could be induced in a subset of cancer patients. As single agents, neither interferon γ nor TNF-α have demonstrated significant antitumor activity in the Phase I/II setting (33, 34). In the case of TNF-α, significant toxicity has been encountered even in good risk patients. Production of these cytokines by endogenous, antibody-activated NK cells may represent an alternate approach to the treatment of malignancies that traditionally have not been amenable to immune-based therapies. This is of great interest, as increases in IL-12-induced antibody-dependent cellular cytotoxicity measured *ex vivo* were observed even in patients that progressed while on therapy. Other recent clinical trials in breast cancer combining cytokines with trastuzumab therapy have also shown that clinical response may not correlate with antibody-dependent cellular cytotoxicity (24, 35). Taken together, these findings suggest that the immunological antitumor actions of trastuzumab therapy may not depend entirely on the induction of antibody-dependent cellular cytotoxicity by Fc receptor-bearing cells and additionally imply that the cytokine response after antibody administration might be manipulated to elicit an antitumor response. However, given the lack of increased activity associated with the IL-12/trastuzumab combination and the failure to observe sustained cytokine production in 12 of 15 patients, it is possible that this regimen may not be of any clinical utility. Furthermore, given the small sample size, it is not possible to conclude that cytokines or antiangiogenic factors released by NK cells were instrumental in the outcomes of the 3 patients who exhibited a clinical benefit. Given the synergy that can be obtained by coadministration of trastuzumab with cytotoxic agents and the results obtained in our Phase I trial, we are currently performing a Phase I trial of IL-12, trastuzumab, and paclitaxel in patients bearing HER2-overexpressing breast adenocarcinomas (NCI No. 84). We hypothesize that the addition of IL-12 to the trastuzumab/paclitaxel regimen will enhance its effectiveness via the induction of cytokine production.

Of note, it has been shown that the sustained production of innate immune cytokines can be associated with clinical re-

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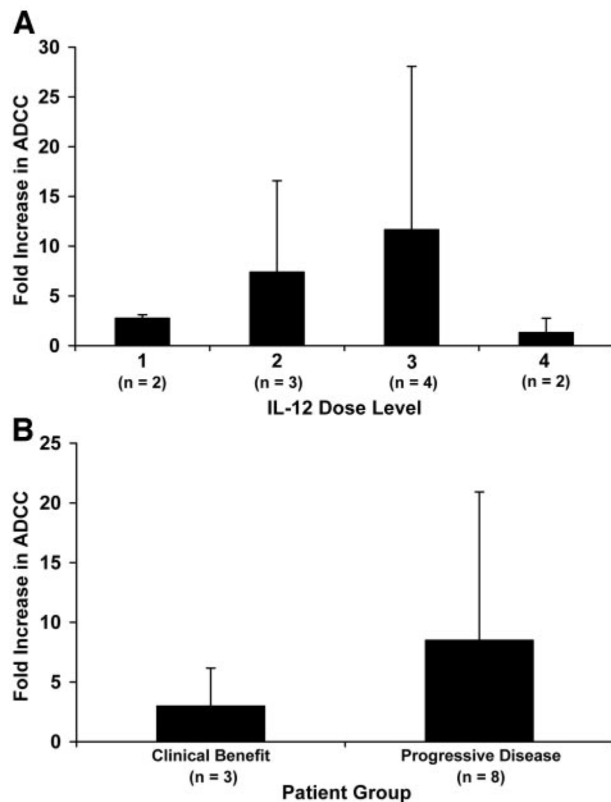

Fig. 8 Analysis of changes in antibody-dependent cellular cytotoxicity (*ADCC*) levels with respect to interleukin (*IL*) 12 dose level and clinical benefit. *A,* fold increase in ADCC from day 1 to day 5 of cycle 9 grouped by dose level of IL-12. Each *bar* represents the mean fold increase of *n* patients, as indicated. *B,* fold increase in ADCC from day 1 to day 5 of cycle 9 grouped by patients exhibiting clinical benefit *versus* those with progression of disease.

sponse. Gollob *et al.* (19) showed that the ability of renal cell or malignant melanoma patients to maintain production of interferon γ, IL-15, and IL-18 during the course of twice-weekly IL-12 therapy correlated with clinical outcome. The critical role of interferon γ in the eradication of established carcinomas in the context of anti-HER antibodies has been established recently in a preclinical model of HER2-overexpressing cancer (36). Mice that had been inoculated with a HER2 vaccine for the induction of circulating anti-HER antibodies required both Fc receptor- and interferon γ-dependent immune mechanisms to clear a subsequent challenge of HER2-overexpressing tumor cells. The failure of IL-12 to improve the objective response rate to trastuzumab in the current Phase I trial suggests that it may not be a simple matter to induce an immune response to antibody-coated tumor targets in humans. However, the finding that a sustained NK cell-derived cytokine response (*e.g.,* interferon-γ, MIG, IP-10, TNF-α, MIP-1α) could be induced in a subset of patients represents a novel finding that can serve as the basis for additional investigation.

In summary, the current study shows that twice-weekly IL-12 at doses of up to 300 ng/kg can be given safely in combination with weekly trastuzumab. Production of interferon γ and other cytokines by NK cells of responding and stabilized patients suggested that the efficacy of this regimen might be associated with induction of a sustained innate immune response. These results should be interpreted with some caution, however, due to the small size of the patient group. Nevertheless, our data provide additional support for investigational studies examining the administration of immune stimulatory cytokines in combination with trastuzumab for the treatment of HER2-overexpressing malignancies.

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REFERENCES

1. Revillion F, Bonneterre J, Peyrat JP. ERBB2 oncogene in human breast cancer and its clinical significance. Eur J Cancer 1998;34:791–808.

2. Tzahar E, Yarden Y. The ErbB-2/HER2 oncogenic receptor of adenocarcinomas: from orphanhood to multiple stromal ligands. Biochim Biophys Acta 1998;1377:M25–37.

3. Baselga J, Seidman AD, Rosen PP, Norton L. HER2 overexpression and paclitaxel sensitivity in breast cancer: therapeutic implications. Oncology (Huntingt) 1997;11:43–48.

4. Carter P, Presta L, Gorman CM, et al. Humanization of an anti-p185HER2 antibody for human cancer therapy. Proc Natl Acad Sci USA 1992;89:4285–9.

5. Vogel C, Cobleigh MA, Tripathy D, et al. First-line, single-agent Herceptin(trastuzumab) in metastatic breast cancer: a preliminary report. Eur J Cancer 2001;37 Suppl 1:S25–9.

6. Seidman AD, Fornier MN, Esteva FJ, et al. Weekly trastuzumab and paclitaxel therapy for metastatic breast cancer with analysis of efficacy by HER2 immunophenotype and gene amplification. J Clin Oncol 2001;19:2587–95.

7. Esteva FJ, Valero V, Booser D, et al. Phase II study of weekly docetaxel and trastuzumab for patients with HER-2-overexpressing metastatic breast cancer. J Clin Oncol 2002;20:1800–8.

8. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344:783–92.

9. Baselga J, Albanell J. Mechanism of action of anti-HER2 monoclonal antibodies. Ann Oncol 2001;12 Suppl 1:S35–41.

10. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets. Nat Med 2000;6:443–6.

11. Perussia B. Fc receptors on natural killer cells. Curr Top Microbiol Immunol 1998;230:63–88.

12. McKenzie SE, Schreiber AD. Fc gamma receptors in phagocytes. Curr Opin Hematol 1998;5:16–21.

13. Robertson MJ, Ritz J. Biology and clinical relevance of human natural killer cells. Blood 1990;76:2421–38.

14. Fehniger TA, Shah MH, Turner MJ, et al. Differential cytokine and chemokine gene expression by human NK cells following activation with IL-18 or IL-15 in combination with IL-12: implications for the innate immune response. J Immunol 1999;162:4511–20.

15. Carson WE, Parihar R, Lindemann MJ, et al. Interleukin-2 enhances the natural killer cell response to Herceptin-coated Her2/neu-positive breast cancer cells. Eur J Immunol 2001;31:3016–25.

16. Parihar R, Dierksheide J, Hu Y, Carson WE. IL-12 enhances the natural killer cell cytokine response to Ab-coated tumor cells. J Clin Investig 2002;110:983–92.

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17. Press MF, Slamon DJ, Flom KJ, Park J, Zhou JY, Bernstein L. Evaluation of HER-2/neu gene amplification and overexpression: comparison of frequently used assay methods in a molecularly characterized cohort of breast cancer specimens. J Clin Oncol 2002;20:3095–105.

18. Agarwala S, Kumar R, Bhatnagar V, Bajpai M, Gupta DK, Mitra DK. High incidence of adriamycin cardiotoxicity in children even at low cumulative doses: role of radionuclide cardiac angiography. J Pediatr Surg 2000;35:1786–9.

19. Gollob JA, Mier JW, Veenstra K, et al. Phase I trial of twice-weekly intravenous interleukin 12 in patients with metastatic renal cell cancer or malignant melanoma: ability to maintain IFN-gamma induction is associated with clinical response. Clin Cancer Res 2000;6:1678–92.

20. Gollob JA, Veenstra KG, Parker RA, et al. Phase I trial of concurrent twice-weekly recombinant human interleukin-12 plus low-dose IL-2 in patients with melanoma or renal cell carcinoma. J Clin Oncol 2003;21:2564–73.

21. Vogel CL, Cobleigh MA, Tripathy D, et al. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 2002;20:719–26.

22. Bernard PS, Wittwer CT. Real-time PCR technology for cancer diagnostics. Clin Chem 2002;48:1178–85.

23. Chang JT, Chen IH, Liao CT, et al. A reverse transcription comparative real-time PCR method for quantitative detection of angiogenic growth factors in head and neck cancer patients. Clin Biochem 2002; 35:591–6.

24. Fleming GF, Meropol NJ, Rosner GL, et al. A phase I trial of escalating doses of trastuzumab combined with daily subcutaneous interleukin 2: report of cancer and leukemia group B 9661. Clin Cancer Res 2002;8:3718–27.

25. Robertson MJ, Cameron C, Atkins MB, et al. Immunological effects of interleukin 12 administered by bolus intravenous injection to patients with cancer. Clin Cancer Res 1999;5:9–16.

26. Gollob JA, Mier JW, Atkins MB. Clinical use of systemic IL-12 therapy. Cancer Chemother Biol Response Modif 2001;19:353–69.

27. Strieter RM, Polverini PJ, Arenberg DA, Kunkel SL. The role of CXC chemokines as regulators of angiogenesis. Shock 1995;4: 155–60.

28. Bukowski RM, Rayman P, Molto L, et al. Interferon-gamma and CXC chemokine induction by interleukin 12 in renal cell carcinoma. Clin Cancer Res 1999;5:2780–9.

29. Pravica V, Asderakis A, Perrey C, Hajeer A, Sinnott PJ, Hutchinson IV. In vitro production of IFN-gamma correlates with CA repeat polymorphism in the human IFN-gamma gene. Eur J Immunogenet 1999; 26:1–3.

30. Asderakis A, Sankaran D, Dyer P, et al. Association of polymorphisms in the human interferon-gamma and interleukin-10 gene with acute and chronic kidney transplant outcome: the cytokine effect on transplantation. Transplantation 2001;71:674–7.

31. Sparano JA. Cardiac toxicity of trastuzumab (Herceptin): implications for the design of adjuvant trials. Semin Oncol 2001;28:20–7.

32. Cobleigh MA, Vogel CL, Tripathy D, et al. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 1999;17:2639–48.

33. Rinehart J, Malspeis L, Young D, Neidhart J. Phase I/II trial of human recombinant beta-interferon serine in patients with renal cell carcinoma. Cancer Res 1986;46:5364–7.

34. Feldman ER, Creagan ET, Schaid DJ, Ahmann DL. Phase II trial of recombinant tumor necrosis factor in disseminated malignant melanoma. Am J. Clin Oncol 1992;15:256–9.

35. Repka T, Chiorean EG, Gay J, et al. Trastuzumab and Interleukin-2 in HER2-positive metastatic breast cancer: A pilot study. Clin Cancer Res 2003;9:2440–6.

36. Curcio C, Di Carlo E, Clynes R, et al. Nonredundant roles of antibody, cytokines, and perforin in the eradication of established Her-2/neu carcinomas. J Clin Investig 2003;111:1161–70.

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A Phase I Study of Interleukin 12 with Trastuzumab in Patients with Human Epidermal Growth Factor Receptor-2-Overexpressing Malignancies: Analysis of Sustained Interferon γ Production in a Subset of Patients

Robin Parihar, Padma Nadella, Adrian Lewis, et al.

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