British Journal of Cancer (2010) 103, 1331 – 1334

www.bjcancer.com

Addition of GM-CSF to trastuzumab stabilises disease in trastuzumab-resistant HER2 + metastatic breast cancer patients

YC Cheng[1,2], V Valero[3], ML Davis[3], MC Green[3], AM Gonzalez-Angulo[3], RL Theriault[3], JL Murray[3], GN Hortobagyi[3] and NT Ueno[*,2,3]

[1]Division of Neoplastic Diseases and Related Disorders, Department of Medicine, Medical College of Wisconsin, Milwaukee, WI, USA; [2]Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 448, Houston, TX, USA; [3]Breast Cancer Translational Research Laboratory, Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 1354, Houston, TX 77030, USA

BACKGROUND: One of the proposed mechanisms of trastuzumab-induced regression of human epidermal growth factor receptor 2-positive (HER2 +) tumours includes facilitation of antibody-dependent cell-mediated cytotoxicity (ADCC). Granulocyte-macrophage colony-stimulating factor (GM-CSF) mediates ADCC. We presented our pilot study of adding GM-CSF to trastuzumab in patients with trastuzumab-resistant HER2 + metastatic breast cancer.

METHODS: Patients with HER2 + metastatic breast cancer that progressed after trastuzumab +/− chemotherapy were continued on trastuzumab 2 mg kg^{-1} intravenous weekly and GM-CSF 250 μg m^{-2} subcutaneous daily. Patients were assessed for response every 8 weeks. Treatment was continued until disease progression or intolerable toxicity.

RESULTS: Seventeen patients were evaluable (median age 48 years, range 27–75 years). The median number of metastatic sites was 2 (range 1–3); the most common site was the liver ($n = 10$). The median number of prior regimens for metastatic disease was 2 (range 1–5). No objective disease response was observed, but five patients (29%) had stable disease for a median duration of 15.8 (range 10–53.9) weeks. The most common adverse event was rash at the injection site. No grade 4 or irreversible adverse event was seen.

CONCLUSION: The addition of GM-CSF to trastuzumab alone had a modest clinical benefit and acceptable safety profile in heavily pretreated patients with trastuzumab-resistant HER2 + metastatic breast cancer.

British Journal of Cancer (2010) **103,** 1331 – 1334. doi:10.1038/sj.bjc.6605918 www.bjcancer.com
Published online 28 September 2010

Keywords: granulocyte-macrophage colony-stimulating factor; HER2; metastatic breast cancer; trastuzumab

Metastatic breast cancer is generally considered incurable by standard chemotherapy. Nevertheless, it is a chemosensitive disease. Among patients treated with chemotherapy, median survival is 24 months, and 2–5% patients have disease-free survival longer than 5 years. Multiple prognostic and predictive factors determine the course of the disease and the response to systemic treatment. One of these factors is expression of the human epidermal growth factor receptor 2 (HER2), which is overexpressed in ~20% of breast cancers (Slamon *et al*, 2001).

The *HER2* oncogene is a member of the HER family of tyrosine kinase receptors. Amplification of *HER2* results in overexpression of the HER2 receptor, which correlates with several negative prognostic variables, including oestrogen receptor-negative status, high S-phase fraction, positive nodal status, mutated *p53*, and high nuclear grade (Sjögren *et al*, 1998). Overexpression of the HER2 receptor in turn results in relative resistance to endocrine therapy (Atalay *et al*, 2003) and is correlated with an aggressive form of breast cancer and significantly shorter disease-free and overall survival (Press *et al*, 1993; Seshadri *et al*, 1993; Ravdin and Chamness, 1995; Cobleigh *et al*, 1999; Slamon *et al*, 1987). As overexpression of HER2 receptor is such an important prognostic and predictive factor (Pietras *et al*, 1995), targeting this receptor with tumour-specific passive and active immunotherapeutic treatments is a rational strategy.

The humanised monoclonal antibody trastuzumab was developed as a therapy targeted against HER2 receptor. In patients with HER2-positive (HER2 +) metastatic breast cancer, response rates to trastuzumab monotherapy range from 12 to 34%, median duration of response is 9 months (Cobleigh *et al*, 1999; Nahta *et al*, 2004). Concomitant trastuzumab and chemotherapy are synergistic and have resulted in better response rates, time to disease progression, and overall survival than chemotherapy alone or trastuzumab monotherapy. Therefore, concomitant trastuzumab and chemotherapy is considered a standard of care in HER2 + metastatic breast cancer (Seidman *et al*, 2001; Slamon *et al*, 2001; Esteva *et al*, 2002; Stein *et al*, 2004; Marty *et al*, 2005).

Although the mechanisms by which trastuzumab induces regression of HER2 + tumours are not known definitively, proposed mechanisms include potentiation of chemotherapy (Pegram *et al*, 1999), inhibition of tumour cell proliferation (Baselga *et al*, 1998; Sliwkowski *et al*, 1999), and facilitation of immune function through antibody-dependent cell-mediated cytotoxicity (ADCC) (Lewis *et al*, 1993; Sliwkowski *et al*, 1999;

*Correspondence: Dr NT Ueno; E-mail: nueno@mdanderson.org
Received 18 May 2010; revised 2 August 2010; accepted 25 August 2010; published online 28 September 2010

Repka *et al*, 2003). Thus, ADCC appears to be one of the most important immune effector functions. Cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) may augment ADCC by direct activation of immune cells or by enhancement of tumour-associated antigens on tumour cells (Sondel and Hank, 2001).

We hypothesised that adding GM-CSF to trastuzumab would overcome trastuzumab resistance via enhancing ADCC. We hereby present the results of our pilot study assessing the feasibility, safety profile, and efficacy of adding GM-CSF to trastuzumab in women with trastuzumab-resistant metastatic breast cancer.

PATIENTS AND METHODS

Trial design

All patients provided written informed consent prior to participating in the pilot study, and the study was reviewed and approved by the Institutional Review Board at The University of Texas MD Anderson Cancer Center. Eligible patients were women with metastatic breast cancer who had HER2-overexpressing disease (HER2 3+ by immunohistochemical staining or amplification by fluorescence *in situ* hybridisation) that was progressing after treatment with at least one cycle of trastuzumab with or without chemotherapy. Patients were required to have an Eastern Cooperative Oncology Group performance status score of 0 or 1, an adequate haematological profile, and adequate liver, kidney, and heart function. Patients also were required to have measurable metastatic disease. Patients with only bone disease, only leptomeningeal disease, or only malignant pleural effusion were not eligible.

Treatment

Trastuzumab was intravenously given as follows: a $4\,mg\,kg^{-1}$ loading dose followed by $2\,mg\,kg^{-1}$ every week for 4 weeks (one cycle). A loading dose was not necessary if patients received trastuzumab within 2 weeks before the start of study treatment. Subcutaneous GM-CSF was given at $250\,\mu g\,m^{-2}$ daily until the absolute neutrophil count was $>10\,000\,mm^{-3}$, then was given every other day to maintain the absolute neutrophil count below $10\,000\,mm^{-3}$. When GM-CSF and trastuzumab were given on the same day, GM-CSF was given before trastuzumab infusion. Patients underwent restaging every 8 weeks (two cycles). Granulocyte-macrophage colony-stimulating factor and trastuzumab were continued until disease progression or intolerable toxic effects. The primary end points of the trial were tumour response (including stable disease) and time to progression. Treatment-related toxicity was assessed according to the National Cancer Institute Common Toxicity Criteria version 2.0. Tumour response was assessed according to the Response Evaluation Criteria in Solid Tumours system.

Statistical consideration

A maximum of 18 patients will be entered in the study. A 15% (three patients) improved tumour response (including stable disease) rate in this patient population would provide an impetus towards further investigation of this treatment, including measurement of ADCC.

RESULTS

Eighteen patients with progressive HER2+ metastatic breast cancer were eligible, and 17 were evaluable (median age 48 years, range 27–75 years) (Table 1). One patient did not complete the first cycle because of a protocol violation. Among the 17 evaluable patients, 9 had hormone receptor-positive disease. The median number of sites of metastasis was 2 (range 1–3). The most common site of metastasis was the liver ($n=10$). The median number of trastuzumab-containing regimens for metastatic disease was 2 (range 1–5). One patient developed rapidly progressive disease 2 weeks after the start of study therapy and died soon after. Sixteen patients received treatment for at least 8 weeks (two cycles) until disease progression. No disease response was observed, but five patients (29%) had stable disease more than 8 weeks. The median duration was 15.8 weeks (range 10–53.9 weeks). Three of the five patients had hormone receptor-negative disease and four of them had visceral organ involvement. Thirteen patients had grade 1 adverse events; six patients had grade 2 adverse events; and two patients had grade 3 adverse events (fatigue, muscle aches, and paraesthesia). The most common adverse events, in decreasing order of frequency, were rash at the GM-CSF injection site, skin rash, fatigue, and muscle aches (Table 2). No grade 4 or irreversible adverse event was seen.

Table 1 Patient characteristics[a]

Number of patients	
Total	18
Evaluable	17
Age in years, median (range)	48 (27–75)
Initial disease stage	
I	1
II	5
III	6
IV	5
Hormone receptor status	
Positive	9
Negative	8
HER2 receptor positive	18
Neoadjuvant chemotherapy	6
Anthracycline-containing regimen only	4
Anthracycline- and taxane-containing regimen	2
Adjuvant chemotherapy	9
Anthracycline-containing regimen only	2
Anthracycline- and taxane-containing regimen	3
Taxane-containing regimen only	4
Adjuvant radiation therapy	5
Adjuvant hormone therapy	3
Number of systemic regimens after metastasis, median (range)	2 (2–8)
Number of trastuzumab-containing regimens after metastasis, median (range)	2 (1–5)
Number of sites of metastasis, median (range)	2 (1–3)
Sites of metastasis	
Liver	10
Bone	8
Lymph nodes	6
Lung	5
Chest wall	2
Brain	1
Peritoneum	1

[a]Values are numbers of patients unless otherwise specified.

DISCUSSION

Several proposed mechanisms of trastuzumab resistance included downregulation of HER2, upregulation of expression of *PTEN* gene or insulin-like growth factor receptor I gene, and also expression of a truncated form of HER2 receptor – p95HER2 (Lu *et al*, 2001;

Table 2 Toxic effects of treatment

Toxic effect	Total	Grade 1	Grade 2	Grade 3
Fever	3	2	1	0
Nausea	2	1	2	0
Vomiting	1	0	1	0
Sore mouth	2	2	0	0
Diarrhoea	3	3	0	0
Constipation	1	1	0	0
Fatigue	5	5	2	1
Muscle pain	4	3	3	1
Numbness	1	1	1	1
Sore fingers/toes	1	1	0	0
Red eye	1	1	0	0
Rash at injection site	7	6	1	0
Skin rash	6	5	2	0
Itchy hands/feet	1	1	0	0
Headache	2	2	1	0

Scaltriti *et al*, 2007). Our pilot study has demonstrated the potential for therapeutic synergy when trastuzumab is combined with GM-CSF in patients with trastuzumab-resistant HER2+ metastatic breast cancer. The addition of GM-CSF to trastuzumab alone provided clinical benefit in 29% of heavily pretreated patients without causing any grade 4 adverse events.

We tested a different approach to overcoming tumour resistance to trastuzumab: enhancing the effect of trastuzumab through addition of cytokines. One of the antitumour effects of trastuzumab is through the action of innate effector mechanisms, such as ADCC (Drebin *et al*, 1988; Kim *et al*, 2002; Spiridon *et al*, 2002). As a mediator of ADCC, trastuzumab is detected as an abnormality on the HER2 receptor of tumour cells by natural killer (NK) cells, which in turn secrete cytokines and subsequently lead to tumour cell death (Lewis *et al*, 1993; Sliwkowski *et al*, 1999). The GM-CSF is commonly used to augment immune response by increases antigen presentation of monocytes and macrocytes, enhances CD20 expression, stimulates the effector function of myeloid cells (i.e., neutrophils, macrophages, NK cells, and dendritic cells), and enhances cell-mediated immunity (Dranoff, 2004; Niitsu *et al*, 2004; Olivieri *et al*, 2005). The GM-CSF also mediates ADCC via stimulation of macrophages (Kushner and Cheung, 1989; Erbe *et al*, 1990; Liesveld *et al*, 1991; Tarr, 1996), and the ability of GM-CSF to increase the production of granulocytes and mononuclear cells, as well as to enhance their cytotoxic activities against tumour cells, is well documented (Kushner and Cheung, 1989; Erbe *et al*, 1990; Liesveld *et al*, 1991; Ragnhammar *et al*, 1992; Tarr, 1996; Yu *et al*, 1997). The GM-CSF also can affect the migration of granulocytes (Gasson *et al*, 1984; Barker *et al*, 1991), resulting in their increased accumulation at tumour sites (Tseng *et al*, 1999).

Currently, GM-CSF is most often used in cancer treatment as a stimulant of leukocyte production to protect against infection (Jones *et al*, 1996; Beveridge and Miller, 1998). However, as GM-CSF can augment immune effector cell functions (Kushner and Cheung, 1989; Erbe *et al*, 1990; Liesveld *et al*, 1991; Tarr, 1996), it also may enhance the therapeutic effect of monoclonal antibodies, such as trastuzumab.

Several experimental and clinical studies have demonstrated the antineoplastic effects of GM-CSF alone or in combination with cytokines and/or monoclonal antibodies (Ragnhammar, 1996). The GM-CSF has been shown to enhance anti-G_{D2}-mediated ADCC by granulocytes in disease-free subjects and in patients with neuroblastoma (Yu *et al*, 1997; Batova *et al*, 1999). A recent pilot trial found that continuous, low-dose GM-CSF had substantial activity (objective response rate 37%) in heavily pretreated patients with either metastatic breast cancer or female genital tract cancer (Kurbacher *et al*, 2005). Enhancement of ADCC of human peripheral blood mononuclear cells by GM-CSF has been described (Grabstein *et al*, 1986; Thomassen *et al*, 1989), and GM-CSF in conjunction with monoclonal antibodies has been used in clinical trials for the treatment of colorectal carcinoma (Mellstedt *et al*, 1991; Ragnhammar *et al*, 1992) and neuroblastoma (Yu *et al*, 1997). Trial results found that GM-CSF augmented ADCC activity of mononuclear cells and granulocytes against both colorectal cancer cells and neuroblastoma; therapeutic efficacy was demonstrated in these trials (Mellstedt *et al*, 1991; Ragnhammar *et al*, 1992; Yu *et al*, 1997). In this study, we have initially designed to continue the trial in a phase II setting including measurement of the ADCC activities under the influence of GM-CSF and trastuzumab. However, we were not able to accrue patients further to the study due to other competing trials in our institution.

Although targeted therapies, such as combinations of trastuzumab and chemotherapy, have been widely investigated for the treatment of metastatic breast cancer, the role of cytokines, such as GM-CSF, as an immunological stimulant in combination with monoclonal antibodies, has been less well examined. Our pilot study has demonstrated the potential for therapeutic synergy with the combination of GM-CSF and trastuzumab. Administration of GM-CSF is simple, safe, and feasible. Although no disease response was seen in our pilot study, our finding that this simple approach stabilised breast cancer is clinically significant in this setting of metastatic disease. This trastuzumab plus GM-CSF regimen needs further evaluation in combination with chemotherapy or other biological agents in the management of metastatic breast cancer. Further, there is a need to determine whether ADCC activities are measured.

ACKNOWLEDGEMENTS

We thank Stephanie Deming for her excellent help in developing this manuscript. This research work was supported in part by Bayer (to NTU).

REFERENCES

Atalay G, Cardoso F, Awada A, Piccart MJ (2003) Novel therapeutic strategies targeting the epidermal growth factor receptor (EGFR) family and its downstream effectors in breast cancer. *Ann Oncol* **14:** 1346 – 1363

Barker E, Mueller BM, Handgretinger R, Herter M, Yu AL, Reisfeld RA (1991) Effect of a chimeric anti-ganglioside G_{D2} antibody on cell-mediated lysis of human neuroblastoma cells. *Cancer Res* **51:** 144 – 149

Baselga J, Norton L, Albanell J, Kim YM, Mendelsohn J (1998) Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/*neu* overexpressing human breast cancer xenografts. *Cancer Res* **58:** 2825 – 2831

Batova A, Kamps A, Gillies SD, Reisfeld RA, Yu AL (1999) The Ch14.18-GM-CSF fusion protein is effective at mediating antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity *in vitro*. *Clin Cancer Res* **5:** 4259 – 4263

Beveridge RA, Miller JA, Kales AN, Binder RA, Robert NJ, Harvey JH, Windsor K, Gore I, Cantrell J, Thompson KA, Taylor WR, Barnes HM, Schiff SA, Shields JA, Cambareri RJ, Butler TP, Meister RJ, Feigert JM, Norgard MJ, Moraes MA, Helvie WW, Patton GA, Mundy LJ, Henry D, Mason B, Staddon A, Ford P, Katcher D, Houck W, Major WB, Gemma NW, Kay G, Priest E, Sowroy P, Bank B, Leibach S, Reisel H, Grad G, Warren RD, Ueno WM, Smith LF, Dobrzynski RF, Sheridan MJ (1998) A comparison of efficacy of sargramostim (yeast-derived RhuGM-CSF) and filgrastim (bacteria-derived RhuG-CSF) in the therapeutic setting of chemotherapy-induced myelosuppression. *Cancer Invest* **16:** 366 – 373

Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, Wolter JM, Paton V, Shak S, Lieberman G, Slamon DJ (1999) Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER-2 overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J Clin Oncol* **17:** 2639 – 2648

Dranoff G (2004) Cytokines in cancer pathogenesis and cancer therapy. *Nat Rev Cancer* **4:** 11 – 22

Drebin JA, Link VC, Greene MI (1988) Monoclonal antibodies specific for the *neu* oncogene product directly mediate anti-tumor effects *in vivo*. *Oncogene* **2:** 387 – 394

Erbe DV, Collins J, Shen L, Graxiano RF, Fanger MW (1990) The effect of cytokines on the expression and function of Fc receptors for IgG on human myeloid cells. *Mol Immunol* **27:** 57 – 67

Esteva FJ, Valero V, Booser D, Guerra LT, Murray JL, Pusztai L, Cristofanilli M, Arun B, Esmaeli B, Fritsche HA, Sneige N, Smith TL, Hortobagyi GN (2002) Phase II study of weekly docetaxel and trastuzumab for patients with HER-2-overexpressing metastatic breast cancer. *J Clin Oncol* **20:** 1800 – 1808

Gasson JC, Weisbart RH, Kaufman SE, Clark SC, Hewick RM, Wong GG, Golde DW (1984) Purified human granulocyte-macrophage colony stimulating factor: direct action on neutrophils. *Science* **226:** 1339 – 1342

Grabstein KH, Urdal DL, Tushinski RJ, Mochizuki DY, Price VL, Cantrell MA, Gillis S, Conlon PJ (1986) Induction of macrophage tumoricidal activity by granulocyte-macrophage colony-stimulating factor. *Science* **232:** 506 – 508

Jones SE, Schottstaedt MW, Duncan LA, Kirby RL, Good RH, Mennel RG, George TK, Snyder DA, Watkins DL, Denham CA, Hoyes FA, Rubin AS (1996) Randomized double-blind prospective trial to evaluate the effects of sargramostim versus placebo in a moderate-dose fluorouracil, doxorubicin, and cyclophosphamide adjuvant chemotherapy program for stage II and III breast cancer. *J Clin Oncol* **14:** 2976 – 2983

Kim KM, Shin EY, Moon JH, Heo TH, Lee JY, Chung Y, Lee YJ, Cho HM, Shin SU, Kang CY (2002) Both the epitope specificity and isotype are important in the antitumor effect of monoclonal antibodies against Her-2/*neu* antigen. *Int J Cancer* **102:** 428 – 434

Kurbacher CM, Kurbacher JA, Cramer EM, Rhiem K, Mallman PK, Reichelt R, Reinhold U, Stier U, Cree IA (2005) Continuous low-dose GM-CSF as salvage therapy in refractory recurrent breast or female genital tract carcinoma. *Oncology* **19**(4 suppl 2)**:** 23 – 26

Kushner BH, Cheung NK (1989) GM-CSF enhances 3F8 monoclonal antibody-dependent cellular cytotoxicity against human melanoma and neuroblastoma. *Blood* **73:** 1936 – 1941

Lewis GD, Figari I, Fendly B, Wong WL, Carter P, Gorman C, Shepard HM (1993) Differential responses of human tumor cell lines to anti-p185^{HER2} monoclonal antibodies. *Cancer Immunol Immunother* **37:** 255 – 263

Liesveld JL, Frediani D, Winslow JM, Duerst RE, Abboud CN (1991) Cytokine effects and role of adhesive proteins and Fc receptors in human macrophage-mediated antibody dependent cellular cytotoxicity. *J Cell Biochem* **45:** 381 – 390

Lu Y, Zi X, Zhao Y, Mascarenhas D, Pollak M (2001) Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). *J Natl Cancer Inst* **93:** 1852 – 1857

Marty M, Cognetti F, Maraninchi D, Snyder R, Mauriac L, Tubiana-Hulin M, Chan S, Grimes D, Antón A, Lluch A, Kennedy J, O'Byrne K, Conte P, Green M, Ward C, Mayne K, Extra JM (2005) Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group. *J Clin Oncol* **23:** 4265 – 4274

Mellstedt H, Frödin JE, Ragnhammar P, Masucci G, Ljungberg A, Hjelm AL, Fagerberg J, Lindemalm C, Osterborg A, Wersäll P (1991) Therapy of colorectal carcinoma with monoclonal antibodies (Mab17-1A) alone and in combination with granulocyte monocyte-colony stimulating factor (GM-CSF). *Acta Oncol* **30:** 923 – 931

Nahta R, Hung MC, Esteva FJ (2004) The HER-2-targeting antibodies trastuzumab and pertuzumab synergistically inhibit the survival of breast cancer cells. *Cancer Res* **64:** 2343 – 2346

Niitsu N, Hayama M, Okamoto M, Khori M, Higashihara M, Tamaru J, Hirano M (2004) Phase I study of rituximab-CHOP in combination with GM-CSF in patients with follicular lymphoma. *Clin Cancer Res* **10:** 4077 – 4082

Olivieri A, Lucesole M, Capelli D, Gini G, Montanari M, Candela M, Troiani E, Scortechini I, Poloni A, Leoni P (2005) A new schedule of CHOP/rituximab plus granulocyte-macrophage colony-stimulating factor is an effective rescue for patients with aggressive lymphoma failing autologous stem cell transplantation. *Biol Blood Marrow Transplant* **11:** 627 – 636

Pegram M, Hsu S, Lewis G, Pietras R, Beryt M, Sliwkowski M, Coombs D, Baly D, Kabbinavar F, Slamon D (1999) Inhibitory effects of combinations of HER-2/*neu* antibody and chemotherapeutic agents used for treatment of human breast cancers. *Oncogene* **18:** 2241 – 2251

Pietras RJ, Arboleda J, Reese DM, Wongvipat N, Pegram MD, Ramos L, Gorman CM, Parker MG, Sliwkowski MX, Slamon DJ (1995) HER-2 tyrosine kinase pathway targets estrogen receptor and promotes hormone-independent growth in human breast cancer cells. *Oncogene* **10:** 2435 – 2446

Press MF, Pike MC, Chazin VR, Hung G, Udove JA, Markowicz M, Danyluk J, Godolphin W, Sliwkowski M, Akita R, Paterson MC, Slamon DJ (1993) HER-2/*neu* expression in node negative breast cancer: direct tissue quantitation by computerized image analysis and association of overexpression with increased risk of recurrent disease. *Cancer Res* **53:** 4960 – 4970

Ragnhammar P (1996) Anti-tumoral effect of GM-CSF with or without cytokines and monoclonal antibodies in solid tumors. *Med Oncol* **13:** 167 – 176

Ragnhammar P, Masucci G, Frödin JE, Hjelm AL, Mellstedt H (1992) Cytotoxic functions of blood mononuclear cells in patients with colorectal carcinoma treated with mAb 17-1A and granulocyte/macrophage-colony stimulating factor. *Cancer Immunol Immunother* **35:** 158 – 164

Ravdin PM, Chamness GC (1995) The c-*erbB*-2 proto-oncogene as a prognostic and predictive marker in breast cancer: a paradigm for the development of other macromolecular markers—a review. *Gene* **159:** 19 – 27

Repka T, Chiorean EG, Gay J, Herwig KE, Kohl VK, Yee D, Miller JS (2003) Trastuzumab and interleukin-2 in HER2-positive metastatic breast cancer: a pilot study. *Clin Cancer Res* **9:** 2440 – 2446

Scaltriti M, Rojo F, Ocaña A, Anido J, Guzman M, Cortes J, Di Cosimo S, Matias-Guiu X, Ramon y Cajal S, Arribas J, Baselga J (2007) Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. *J Natl Cancer Inst* **99:** 628 – 638

Seidman AD, Fornier M, Esteva FJ, Tan L, Kaptain S, Bach A, Panageas KS, Arroyo C, Valero V, Currie V, Gilewski T, Theodoulou M, Moynahan ME, Moasser M, Sklarin N, Dickler M, D'Andrea G, Cristofanilli M, Rivera E, Hortobagyi GN, Norton L, Hudis CA (2001) Weekly trastuzumab and paclitaxel therapy for metastatic breast cancer with analysis of efficacy by HER2 immunophenotype and gene amplification. *J Clin Oncol* **19:** 2587 – 2595

Seshadri R, Firgaira FA, Horsfall DJ, McCaul K, Setlur V, Kitchen P (1993) Clinical significance of HER-2/*neu* oncogene amplification in primary breast cancer. *J Clin Oncol* **11:** 1936 – 1942

Sjögren S, Inganäs M, Lindgren A, Homberg L, Bergh J (1998) Prognostic and predictive value of c-*erb*B-2 overexpression in primary breast cancer, alone and in combination with other prognostic markers. *J Clin Oncol* **16:** 462 – 469

Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/*neu* oncogene. *Science* **235:** 177 – 182

Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* **344:** 783 – 792

Sliwkowski MX, Lofgren JA, Lewis GD, Hotaling TE, Fendly BM, Fox JA (1999) Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). *Semin Oncol* **26**(4 suppl 12)**:** 60 – 70

Sondel PM, Hank JA (2001) Antibody-directed, effector cell-mediated tumor destruction. *Hematol Oncol Clin North Am* **15:** 703 – 721

Spiridon CI, Ghetie MA, Uhr J, Marches R, Li JL, Shen GL, Vitetta ES (2002) Targeting multiple Her-2 epitopes with monoclonal antibodies results in improved antigrowth activity of a human breast cancer cell line *in vitro* and *in vivo*. *Clin Cancer Res* **8:** 1720 – 1730

Stein S, DeMichele A, Domchek S, Fox K (2004) Gemcitabine and trastuzumab combinations for patients with metastatic breast cancer overexpressing HER2/*neu*. *Clin Breast Cancer* **4**(suppl 3)**:** S117 – S120

Tarr PE (1996) Granulocyte-macrophage colony-stimulating fctor and the immune system. *Med Oncol* **13:** 133 – 140

Thomassen MJ, Barna BP, Rankin D, Wiedemann HP, Ahmad M (1989) Differential effect of recombinant granulocyte macrophage colony-stimulating factor on human monocytes and alveolar macrophages. *Cancer Res* **49:** 4086 – 4089

Tseng SH, Hsieh CL, Lin SM, Hwang LH (1999) Regression of orthotopic brain tumors by cytokine-assisted tumor vaccines primed in the brain. *Cancer Gene Ther* **6:** 302 – 312

Yu AL, Batova A, Alvarado C, Rao VJ, Castleberry RP (1997) Usefulness of a chimeric anti-GD2 (ch14.18) and GM-CSF for refractory neuroblastoma: a POG phase II study [abstract]. *Proc Am Soc Clin Oncol* **16:** 513a. A1846