EXHIBIT 1

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EXHIBIT 2

VOLUME 17

199

PROGRAM/PROCEEDINGS

AMERICAN SOCIETY OF CLINICAL ONCOLOGY

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THIRTY-FOURTH

Annual Meeting of the

American Society of Clinical Oncology

May 16 - 19, 1998

PROGRAM/PROCEEDINGS

Los Angeles, California

Contents

1998 ASCO CALENDAR OF EVENTS

SATURDAY, MAY 16, 1998

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E8 Hepatocellular Carcinoma—Etiology, Pathogenesis and Management
E13 Inherited Breast Cancer Susceptibility and Testing Prevention
E15 Management of Stage III Non-Small Cell Lung Cancer
E20 Prostate Cancer: What to do After Hormones Fail?
E22 Rectal Cancer
E24 Tumor Vaccines

8:00 am-9:15 am Meet the Professor Sessions

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M15A Management Trends in Low and Intermediate Risk Neuroblastoma: *Robert Castleberry, MD*
M28A Testicular Cancer: *Craig Nichols, MD*
M29A Therapy of Early Stage Colorectal Cancer: *Daniel Haller, MD*

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E14 Interpreting Clinical Trials
E16 New Agents for Colon Cancer
E21 Psychosocial Interventions

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M6A Ductal Carcinoma in Situ: *Monica Morrow, MD*
M7A Endometrial Cancer: *Jonathan Berek, MD*
M12A Management of Brain Metastases: *Jay Steven Loeffler, MD*
M14A Management of Recurrent Low-Grade Lymphoma: *Ama Rohatiner, MD*
M17A Osteogeneic Sarcoma: *Robert Benjamin, MD*
M19A Ovarian Cancer: Molecular Approaches to Management: *Robert Bast, MD*
M23A Prostate Cancer: *Ian Tannock, MD*
M25A Role of Immunotherapy in Renal Cancer: *Ronald Bukowski, MD*
M27A Solid Tumor Cytogenetics: *Jonathan Fletcher, MD*

9:35 am-10:50 am Tumor Panel

TP10 Thymoma

9:35 am-10:50 am Scientific Symposium

SS1 Molecular Targets of Chemoprevention

9:35 am-10:50 am Internet Session

IS1 Introduction to the Internet and the World Wide Web

11:10 am-1:10 pm Presidential Symposium

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2:10 pm-3:10 pm American Cancer Society Lecture

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SATURDAY, MAY 16, 1998 (CONTINUED)

3:35 pm-4:50 pm Education Sessions

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E10 How to Break Bad News to Patients with Cancer
E11 Implications of Genetic Testing for Practicing Physicians
E17 New Approaches to the Treatment of Advanced Bladder Cancer
E18 Non-Hodgkin's Lymphoma
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3:35 pm-4:50 pm Meet the Professor Sessions

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M5A Chronic Myelogenous Leukemia: *Hagop Kantarjian, MD*
M10A Late Complications of Allogeneic Transplant: *Keith Sullivan, MD*
M11A Management of Advanced Breast Cancer: *Andrew Seidman, MD*
M20A Pain Management: *Stuart Grossman, MD*
M22A Primary Extranodal Lymphomas—1998: *Mary Gospodarowicz, MD*
M30A Treatment of Older Breast Cancer Patients: *Hyman Muss, MD*

3:35 pm-4:50 pm Tumor Panel Sessions

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TP4 Glioma—Adult and Pediatric
TP5 Head and Neck Cancers
TP7 Melanoma: Controversies in Advanced and Intermediate Risk Disease

3:35 pm-4:50 pm Internet Session

IS5 Oncology on the Internet—Resources, Tools, and Trends for the Experienced Internet User

3:35 pm-4:50 pm Special Sessions

NCI Listens
The Role of Consumers in Cancer Research and Clinical Trials

SUNDAY, MAY 17, 1998

8:00 am-9:15 am Education Sessions

E1 Adult Acute Myeloid Leukemia
E4 Bone Metastases—Management
E5 Cancer Survivors: Clinical and Research Issues
E8 Hepatocellular Carcinoma—Etiology, Pathogenesis and Management
E15 Management of Stage III Non-Small Cell Lung Cancer
E18 Non-Hodgkin's Lymphoma

8:00 am-9:15 am Meet the Professor Sessions

M3A Childhood Acute Lymphoblastic Leukemia—Treatment of Relapse: *David Poplack, MD*
M13A Management of Esophageal Cancer: *Arlene Forastiere, MD*
M16A Non-Small Cell Lung Cancer: *Everett Vokes, MD*
M18A Outcomes Research and Management: *Jane Weeks, MD*
M24A Resolving Ethical Dilemmas in Cancer Care: *Ezekiel Emanuel, MD, PhD*
M26A Small Cell Lung Cancer—Management Issues: *David Johnson, MD*

8:00 am-9:15 am Tumor Panel Sessions

TP1 Cutaneous Lymphoma
TP3 Esophageal Cancer Combined Modality Therapy
TP4 Glioma—Adult and Pediatric

8:00 am-9:15 am Scientific Symposia

SS1 Molecular Targets of Chemoprevention
SS2 Progress in Gene Therapy

9:35 am-10:50 am Education Sessions

E6 Controversies in the Management of Early Stage Breast Cancer
E9 Hodgkin's Disease
E12 Incorporating Geriatric Principles into Oncology Practice
E14 Interpreting Clinical Trials
E16 New Agents for Colon Cancer
E21 Psychosocial Interventions

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M9B Identifying Patients at Risk for Cancer: *Olufunmilayo Olopade, MBBS*
M15B Management Trends in Low and Intermediate Risk Neuroblastoma: *Robert Castleberry, MD*
M17B Osteogeneic Sarcoma: *Robert Benjamin, MD*
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M21A Pancreatic Cancer: Current and Future Multimodality Treatment Strategies: *Douglas Evans, MD*
M25B Role of Immunotherapy in Renal Cancer: *Ron Bukowski, MD*
M27B Solid Tumor Cytogenetics: *Jonathan Fletcher, MD*
M28B Testicular Cancer: *Craig Nichols, MD*
M29B Therapy of Early Stage Colorectal Cancer: *Daniel Haller, MD*

9:35 am-10:50 am Tumor Panel Session

TP2 Early Stage Prostate Cancer

9:35 am-10:50 am Internet Session

IS2 Introduction to the Internet and the World Wide Web

9:35 am-10:50 am Scientific Symposium

SS3 Viral Pathogenesis of Human Malignancies

11:10 am-12:25 pm Education Sessions

E2 AIDS Associated Malignancies
E4 Bone Metastases—Management
E23 Strategies for Promoting Evidence Based Medicine: Critical Appraisal, Practice Guidelines and the Cochrane Collaboration
E24 Tumor Vaccines

11:10 am-12:25 pm Meet the Professor Sessions

M6B Ductal Carcinoma in Situ: *Monica Morrow, MD*
M7B Endometrial Cancer: *Jonathan Berek, MD*
M8A Gastric Cancer: Update on Clinical Trials and Recent Developments in Clinical Molecular Correlations: *David Kelsen, MD*
M12B Management of Brain Metastases: *Jay Steven Loeffler, MD*
M14B Management of Recurrent Low-Grade Lymphoma: *Ama Rohatiner, MD*
M22B Primary Extranodal Lymphomas—1998: *Mary Gospodarowicz, MD*
M23B Prostate Cancer: *Ian Tannock, MD*

11:10 am-12:25 pm Tumor Panel Sessions

TP5 Head and Neck Cancer
TP7 Melanoma: Controversies in Advanced and Intermediate Risk Disease
TP8 Sarcoma
TP9 The Clinical Challenge of Unknown Primary Tumors
TP10 Thymoma

11:10 am-12:25 pm Scientific Symposium

SS2 Progress in Gene Therapy

11:10 am-1:10 pm Special Session

FECS/ASCO Symposium: Controversies and Challenges in the Management of Rectal Cancer

1:25 pm-2:40 pm Education Sessions

E2 AIDS Associated Malignancies
E3 Alternative Donor Sources in Allogeneic Transplant
E10 How to Break Bad News to Patients with Cancer
E12 Incorporating Geriatric Principles into Oncology Practice
E20 Prostate Cancer: What to Do After Hormones Fail?

1:25 pm-2:40 pm Meet the Professor Sessions

M4B Chronic Lymphocytic Leukemia: *Michael Grever, MD*
M8B Gastric Cancer: Update on Clinical Trials and Recent Developments in Clinical Molecular Correlations: *David Kelsen, MD*
M13B Management of Esophageal Cancer: *Arlene Forastiere, MD*
M16B Non-Small Cell Lung Cancer: *Everett Vokes, MD*
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M21B Pancreatic Cancer: Current and Future Multimodality Treatment Strategies: *Douglas Evans, MD*
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M26B Small Cell Lung Cancer—Management Issues: *David Johnson, MD*

1:25 pm-2:40 pm Tumor Panel Session

TP6 Locally Advanced Cervical Cancer: Innovation and Treatment Optimization

1:25 pm-2:40 pm Special Session

International Symposium: Cancer Around the World

1:25 pm-2:40 pm Scientific Symposium

SS3 Viral Pathogenesis of Human Malignancies

3:20 pm-4:35 pm Education Sessions

E1 Adult Acute Myeloid Leukemia
E7 Germ Cell Tumors
E11 Implications of Genetic Testing for Practicing Physicians
E17 New Approaches to the Treatment of Advanced Bladder Cancer
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E22 Rectal Cancer
E23 Strategies for Promoting Evidence Based Medicine: Critical Appraisal, Practice Guidelines and the Cochrane Collaboration

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M20B Pain Management: *Stuart Grossman, MD*
M39B Treatment of Older Breast Cancer Patients: *Hyman Muss, MD*

3:20 pm-4:35 pm Tumor Panel Sessions

TP6 Locally Advanced Cervical Cancer: Innovation and Treatment Optimization
TP8 Sarcoma
TP9 The Clinical Challenge of Unknown Primary Tumors

3:20 pm-4:35 pm Internet Session

IS6 Oncology on the Internet—Resources, Tools, and Trends for the Experienced Internet User

4:00 pm-6:00 pm Integrated Session

HER-2-Neu in Breast Cancer

4:55 pm-6:10 pm Special Symposia

Forum on Reimbursement: Health Care Legislation, and Other Government Issues
Strategies for Successful Clinical Grant Writing

MONDAY, MAY 18, 1998

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Cancer Genetics
Cancer Vaccine Therapy
Clinical Pharmacology
Head and Neck
Non-Hodgkin's Lymphoma
Non-Small Cell Lung Cancer
Pediatric Oncology-Solid Tumors
Progress in Early Breast Cancer

8:00 am-10:00 am Poster Discussion Sessions

HSR: Quality of Life and Clinical Trials
Melanoma

8:00 am-10:00 am Integrated Symposium

Prostate Cancer: Optimizing Therapy for Different Disease Status

8:00 am-12:00 pm Poster Discussion Sessions

Clinical Pharmacology

8:00 am-12:00 pm General Poster Sessions

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AIDS/Supportive Care
Central Nervous System
Clinical Pharmacology I
Gastrointestinal Cancer I—Upper GI, Pancreatic and Liver Cancer
Gastrointestinal Cancer II—Colorectal Cancer
Gene Therapy
Gynecologic Cancer
Sarcoma

9:35 am-10:50 am Internet Session

IS3 Introduction to the Internet and World Wide Web

11:10 am-12:25 pm Internet Session

IS7 Oncology on the Internet—Resources, Tools, and Trends for the Experienced Internet User

11:15 am-12:00 pm Special Session

Karnofsky Award and Memorial Lecture: Cancer Pain—The Science, Politics, and Ethics, *Kathleen Foley, MD*

1:00 pm-3:30 pm Plenary Session

Plenary Session

1:00 pm-5:00 pm General Poster Sessions

Cancer Vaccines and Dendritic Cells
Clinical Pharmacology II
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Health Services Research

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Advances in Breast Cancer Biology and Treatment
Central Nervous System
Melanoma
Novel Compounds
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Small Cell and Other Lung Issues
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TUESDAY, MAY 19, 1998

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Pediatric Oncology II—Lymphoma, Brain Tumors, and Other Pediatric Issues
Tumor Biology and Cancer Genetics I
Tumor Biology and Cancer Genetics II

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Anti-Emetics

9:00 am-12:00 pm Oral Session (Slide Session)

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IS4 Introduction to the Internet and World Wide Web

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IS8 Oncology on the Internet—Resources, Tools, and Trends for the Experienced Internet User

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1:00 pm-3:00 pm Poster Discussion Sessions

Genitourinary Cancer—Developing Approaches

1:00 pm-5:00 pm Oral Session (Slide Session)

Topics in Breast Cancer

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Gynecologic Cancer
Head and Neck Cancer

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Bone Marrow Transplantation/Cytokines
Clinical Pharmacology IV
Immunotherapy

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ADDITION OF HERCEPTIN® (HUMANIZED ANTI-HER2 ANTIBODY) TO FIRST LINE CHEMOTHERAPY FOR HER2 OVEREXPRESSING METASTATIC BREAST CANCER (HER2+/MBC) MARKEDLY INCREASES ANTICANCER ACTIVITY: A RANDOMIZED, MULTINATIONAL CONTROLLED PHASE III TRIAL. *D. Slamon, B. Leyland-Jones, S. Shak, V. Paton, A. Bajamonde, T. Fleming, W. Eiermann, J. Wolter, J. Baselga, L. Norton. Los Angeles CA, Montreal Canada, Genentech, S. San Francisco, CA, Seattle WA, Munich Germany, Chicago IL, Barcelona Spain, New York NY.*

Herceptin (H), a humanized monoclonal antibody directed against HER2, has single-agent activity in previously-treated HER2+/MBC (JCO 14:737, 1996), and is additive to chemotherapy (CRx) in HER2+ preclinical models. To test H's ability to augment the activity of CRx safely in the clinic, 469 female patients (pts) with HER2+/MBC received doxorubicin-cyclophosphamide (AC) or paclitaxel (T) as first CRx if they had not received prior adjuvant A, or T if previously exposed to A. (A = 60 mg/m², C = 600 mg/m², T = 175 mg/m² × 3 hrs, all CRx q 3 weeks × 6 cycles.) Half the pts (stratified by CRx) were randomized to additionally receive H (4 mg/kg loading, then 2 mg/kg intravenously q week). At a median follow-up of 10.5 months, investigator assessments of time to disease progression (TTP) and response rates (RR) show a significant augmentation of CRx effect by H, without increase in overall severe adverse events (AE):

	Enrolled	TTP (months)	RR(%)	AE(%)
CRx	234	5.5	36.2	66
CRx + H	235	8.6*	62.0**	69
AC	145	6.5	42.1	71
AC + H	146	9.0	64.9	68
T	89	4.2	25.0	59
T + H	89	7.1	57.3	70

*$p < 0.001$ by log-rank test **$p < 0.01$ by X^2 test

A syndrome of myocardial dysfunction similar to that observed with anthracyclines was reported more commonly with AC + H (18% Grade 3/4) than with AC alone (3%), T (0%), or T + H (2%). Review by an independent Response Evaluation Committee and analysis of response duration, time to treatment failure, survival, and quality of life are in progress. In summary, these data indicate that addition of Herceptin to CRx markedly increases clinical benefit, as assessed by RR and TTP. Preliminary analysis of both risk and benefit favors the regimen of Herceptin plus T.

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FACTORS RESPONSIBLE FOR THE UNDERUTILIZATION OF BREAST CONSERVING THERAPY (BCT). *M. Morrow, D.P. Winchester, J.S. Chmiel, J. Moughan, J. Owens, T. Pajak, J. Sylvester and J.F. Wilson. Northwestern University Medical School, Chicago, IL.*

Guidelines for BCT were developed in 1992 and widely disseminated. This study conducted by the American College of Surgeons and the American College of Radiology was undertaken to determine current patterns of care and to evaluate guideline adherence. 17,931 patients with Stage I and II breast cancer treated at 827 institutions in 1994 were studied. Only 7,914 (44.1%) had BCT. 46.7% of BCT patients were under age 60 compared to 40.7% of mastectomy patients ($p < 0.0001$). Significant differences in clinical and pathologic stage were noted between patients undergoing BCT and mastectomy, with 53.6% of 8,312 clinical stage I patients having BCT compared to 32.2% of 4,138 clinical stage II patients and 38.6% of 5,252 patients with no clinical stage data ($p < 0.0001$). Significant differences in prccedure were noted on the basis of both clinical tumor size and nodal status with 52.7% of 9,140 T1 tumors having BCT versus 32.6% of 3,954 T2 tumors ($p < 0.0001$), and 47.2% of 11,435 NO patients versus 31.9% of 920 N+ patients having BCT ($p < 0.0001$). These differences persisted when pathologic stage was considered, with 51.4% of 9,662 pathologic stage I patients having BCT compared to 30.5% of 7,417 pathologic stage II patients ($p < 0.0001$). Patients with favorable histologies (tubular, mucinous, intracystic, n = 840) were more likely to undergo BCT than those with other histologies (n = 17,062; $p < 0.0001$). Radiotherapy (RT) was given to 78.6% of BCT patients. Of 1,155 patients not receiving RT, surgical failure to refer for RT accounted for 51.1%, and patient refusal for 15%. These results indicate that surgeons continue to utilize BCT primarily for patients with favorable breast cancer, in spite of guidelines and data from randomized trials indicating that age, prognosis, and tumor type should not be used as selection criteria for local therapy. This misunderstanding is a major factor responsible for low national rates of BCT.

BREAST CANCER
Progress in Early Breast Cancer
Oral Session, Monday, May 18, 1998

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CIRCULATING INSULIN-LIKE GROWTH FACTOR I LEVEL AND RISK OF BREAST CANCER. *M.N. Pollak, W.C. Willett, G.A. Colditz, D.J. Hunter, D.S. Michaud, B. Deroo, B. Rosner, F.E. Speizer, S.E. Hankinson. Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School (SEH, WCW, DSM, GAC, DJH, BR, FES); Departments of Epidemiology (SEH, WCW, DSM, GAC, DJH) and Nutrition (WCW, DSM), Harvard School of Public Health, Boston MA; Depts. of Medicine and Oncology, Lady Davis Res. Inst. of the Jewish General Hospital and McGill University, Montreal, Quebec, Canada.*

Insulin-like growth factor I (IGF-1) is a mitogenic and anti-apoptotic peptide that influences the proliferative behavior of many cell types, including normal breast epithelial cells. To determine if higher circulating IGF-1 levels are associated with an increased risk of breast cancer, we conducted a nested case-control study within the prospective Nurses' Health Study cohort. We examined plasma levels of IGF-1 and IGF binding protein 3 (IGFBP-3), the major circulating IGF binding protein, in 397 women with invasive breast cancer and 620 age-matched controls. We observed no appreciable association between circulating IGF-1 and breast cancer risk among women who were postmenopausal at blood collection (top versus bottom quintile of IGF-1 level: relative risk (RR) = 0.85; 95% confidence interval (CI) = 0.53–1.39; p-trend = 0.63). However, among premenopausal women, particularly premenopausal women who were 50 years of age or younger at blood collection, we observed a strong positive relationship (top versus bottom tertile comparison: among all premenopausal women RR = 2.33, 95% CI = 1.06–5.16, p-trend = 0.08; among premenopausal women 50 years of age or less, RR = 4.58, 95% CI = 1.75–12.0, p-trend = 0.02). These relative risks were somewhat stronger after accounting for plasma IGFBP-3 levels (RR = 2.88 and 7.28, respectively). These findings have potentially important implications both for identifying women at high risk of breast cancer and for the development of risk reduction strategies. Additional, larger studies of this association are needed to provide more precise estimates of the effect and to investigate the possibility of a relationship between premenopausal IGF-1 levels and postmenopausal breast cancer risk.

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CURRENT MANAGEMENT OF AXILLARY LYMPH NODES IN BREAST CANCER: A NATIONAL PATTERNS OF CARE STUDY. *D.R. Brenin, M. Morrow, J. Moughan, J. Owen, J.F. Wilson, and D.P. Winchester. Northwestern University Medical School, Chicago IL.*

Routine axillary lymph node dissection (ALND) for breast cancer patients has become controversial. Factors influencing the performance rates of ALND and axillary irradiation (AI) were evaluated in a joint study of the American College of Surgeons and the American College of Radiology. 17,931 patients with Stage I and II breast cancer treated at 827 institutions in 1994 were studied. 15,992 (93.2%) underwent ALND. The mean ages of patients who did and did not undergo ALND were 60.4 yrs and 73.0 yrs ($p < 0.0001$). Patients with T1a tumors underwent fewer ALND's when compared to patients with larger tumors (81% vs 93%, $p < 0.0001$). Patients with favorable histology (tubular, papillary and mucinous carcinomas) underwent ALND in 87.9% of cases, compared to 93.6% of patients with other histologies ($p < 0.0001$). Women age 70 or older underwent fewer ALND's compared to younger women (86% vs. 97%, $p < 0.0001$).

Multivariate Analysis of ALND Rate

	Odds Ratio (95% CI)	p
Mastectomy vs Lumpectomy	8.5 (6.3–11.4)	0.0001
Age <70 vs ≥70	5.4 (4.1–7.3)	0.0001
Stage T1b, T1c vs T1a	2.4 (1.8–3.2)	0.0001
Non-favorable Histology	1.5 (1.0–2.2)	0.04

ALND rate did not vary between palpable vs. non-palpable tumors nor with tumor grade. 899 patients received AI. Patients not undergoing ALND were more likely to receive AI (27% vs 12%, $p < 0.0001$). In patients who underwent ALND, 1.6% of those with no lymph node metastasis received AI, 8.9% of those with 1–3 nodal metastases received AI, 24.0% of those with 4–9, and 29.9% of patients with ≥10 nodal metastases received AI.

We conclude that the majority of patients with small breast cancers continue to undergo axillary dissection while AI is under-utilized in patients at risk for local regional relapse.

EXHIBIT 3

VOLUME 17 1998

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AMERICAN SOCIETY OF CLINICAL ONCOLOGY

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NEOADJUVANT TAXOTERE AND DOXORUBICIN IN LOCALLY ADVANCED INOPERABLE STAGE III BREAST CANCER/LAIBC/COMPARED TO HISTORICAL STANDARD REPRESENTED BY CMFVP REGIMEN. *M. [illegible], J. Andrasina, J. Kimo, T. Packan, R. Dubinsky, A. Berc, P. Matula, Dpt. of Radiotherapy and [illegible], L. Pasteur University Hospital Kosice, Slovak Republic*

LAIBC is [illegible] heterogeneous group of tumours. Introduction of neoadju[illegible] chemotherapy to multimodality treatment has brought some improvement of 5 year DFS but treatment results remain disappointing. There is strong need to try new combinations of active drugs which may facilitate [illegible] and perhaps also delay recurrence. The pilot study of 17 pts with LAIBC is presented. Response rate and toxicity was evaluated after [illegible] administration of four courses of Taxotere 60mg/m² Day 1 and [illegible] Day 1 q 3 wks. Median age was 44.8 years(34–59) [illegible] PS was 90–100%. [illegible] remission was observed in 10 pts [illegible] and stable disease in 2 pts(17%). Patients were subsequently treated by locoregional RT of 50 Gy in 5 wks. All patients were rendered [illegible] afterwards and 50% had mastectomy and 40% conservative surgery. Toxicity was rather high. Grade 3 and 4 neutropenia was observed in 5 pts (42%), grade 1 thrombocytopenia in 3 pts (25%) and grade 1 liver [illegible] in 2 pts [illegible] 5 pts (42%) developed hypersensitivity reaction [illegible] the first course. Short term results this study were compared to [illegible] standard. The historical group of 87 pts treated by 4 courses [illegible] between 1986–89 was evaluated. Response rate was 76% (46% CR [illegible] PR). Subsequent XRT was followed by surgery in all patients. Toxicity was mild with no grade 3 or 4 hematological events.

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HIGH PROPORTION OF DELETERIOUS MUTATIONS OF BRCA1 AND BRCA2 IN PATIENTS WITH BOTH BREAST AND OVARIAN CANCER. *B. E. Ward, A. Thomas, T.S. Frank, Myriad Genetic Laboratories, Salt Lake City, UT*

The subsequent development of ovarian cancer in patients with breast cancer is highly correlated to the presence of a deleterious mutation in BRCA1 or BRCA2 genes. Previous reports have documented that [illegible] 100% of women with the combined diagnosis have deleterious mutations. In association with the Beta Testing Consortium of Myriad Genetics, a total of over 300 breast cancer patients were tested for the presence of deleterious mutations in BRCA1 and/or BRCA2. Testing was performed via comprehensive sequence analysis utilizing dye primer sequencing to examine over 5,400 DNA base pairs in both the forward and reverse direction for BRCA1 and over 11,10[illegible] base pairs for BRCA2. Frameshift mutations, nonsense mutations and other reported causal mutations are defined as deleterious. Sixteen patients were identified whose referral information documented a diagnosis of breast cancer followed by a diagnosis of ovarian cancer. Familial history was available on eleven patients. Six patients had first or second degree relatives with ovarian cancer, ten patients had first or second degree relatives with breast cancer and one patient had a negative family history. Fourteen of sixteen patients (88%) were diagnosed with a deleterious mutation. Of the 14 deleterious mutations, eight were diagnosed in BRCA1 while six were diagnosed in BRCA2. It is of interest to note that the time interval between diagnosis of breast and ovarian cancer in the two patients who were mutation negative was greater than 14 years. This data demonstrates that in patients with breast cancer who develop ovarian cancer there is a [illegible] increased likelihood of being BRCA1/BRCA2 positive versus BRCA1/BRCA2 negative.

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PHARMACOKINETICALLY GUIDED DOSE ESCALATION STUDY OF ANTI-HER2 MONOCLONAL ANTIBODY IN PATIENTS WITH HER2/NEU-OVEREXPRESSING METASTATIC BREAST CANCER. *T. Watanabe, Y. Tokuda, Y. Sasaki, I. Adachi [illegible] T. Tajima, National Cancer Center Hospital, Tokai University Hospital [illegible] National Cancer Center Hospital East, Tokyo, Isehara and Kashiwa, Japan*

We have conducted a phase I dose escalation study of recombinant [illegible] anti-p185HER2 MAb 4D5 (MKC-454) in 18 patients (pts) with [illegible] resistant metastatic breast cancer. Based on the results of [illegible] preclinical studies, 10µg/ml was set as the target trough [illegible] concentration. Tumor expression of HER2 was determined by immunohistochemical staining using the pt's paraffin-embedded blocks [illegible] at the time of their primary surgery. 3 or 6 patients at each dose level of 1, 2, 4 and 8 mg/kg received MKC-454 as 90-min intravenous infusions. The first dose was followed in 3 weeks by 9 weekly doses.

MKC-454 dose	# of Pts	trough level (µg/ml)	Toxicity grade 2	Toxicity grade 3<	Tumor response
1 mg/kg	[illegible]	9		1 fever, 1 n/v	
2 mg/kg	[illegible]	19	1 fever, 1 pain		1 MR
4 mg/kg	[illegible]	102	1 fever		1 PR
8 mg/kg	[illegible]	248		1 pain	1 MR, 2 PR

[illegible] plasma concentration was achieved with 2 mg/kg weekly intravenous infusions. 4 pts had [illegible] fever and 3 pts had [illegible] grade 2 [illegible] vomiting fever. One pt received 8 mg/kg of MKC-454 suffered [illegible] generalized bone pain. Tumor response was observed in 5 pts. [illegible] examining the efficacy of MAb 4D5 (MKC-454) with [illegible] mg/kg weekly [illegible] infusions is warranted.

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INTRAPERICARDIAL CISPLATIN BASED CHEMOTHERAPY IN BREAST AND LUNG CANCER PATIENTS WITH SYMPTOMATIC MALIGANT PERICARDIAL EFFUSION. *D. Weber, S. Anchisi, F. Alberto, Division d'Oncologie, Hôpital Cantonal Geneva 14, Switzerland*

Maligant pericardial effusion (MPE) is a life threatening complication in advanced breast cancer (BC) and non-small cell lung cancer (NSCLC). Between 1991 and 1997, 4 patients (pts) with BC and 12 pts with NSCLC median age 52.6 yrs (range 30–75) presented with symptomatic MPE (main symptoms: dyspnea, cough and thoracic pain). All pts had positive cytology. MPE was inaugural in 5 NSCLC pts. Eleven pts (69%) presented echographic evidence of pericardial tamponade. Intrapericardial chemotherapy (IPC) consisted in cisplatin 50–80 mg (median 60 mg) (6 pts) or a combination of cisplatin 50–60 mg (median 60 mg) and etoposide 80–[illegible] mg (median 100 mg) (10 pts) followed by 2–8 days (d) of pericardial drainage. IPC was repeated in two patients. Acute toxicity was recorded as fever WHO1 (1 pt), and nausea WHO1 (2 pts). IPC was painless in all pts. No toxic death, catheter infection or renal toxicity occurred. Failure was prospectively defined as recurrent symptomatic and/or echographic pericardial effusion of > 250 ml. No failure were observed with BC and NSCLC with inaugural MPE. Mean survival (MS) was 555d (range 174–1342 d) for BC and 171 d (range 59–308 d) for inaugural NSCLC. With non inaugural NSCLC the failure rate was 50% and MS 62 d (range 8–164). One BC and 1 NSCLC are alive 165 and 316 d after IPC. Eleven pts died (78.6%) from tumor progression without recurrence of MPE or cardiac symptoms. Four BC (100%) and 5 NSCLC (41.7%) received subsequent systemic chemotherapy.

We conclude that cisplatin containing IPC is highly active and non toxic in breast and NSCLC pts with symptomatic MPE. In our pts with breast cancer and non pretreated NSCLC, no failures and no recurrences were recorded following IPC.

AMGKAN02733144

EXHIBIT 4

Phase II Study of Weekly Intravenous Recombinant Humanized Anti-p185^{HER2} Monoclonal Antibody in Patients With HER2/*neu*-Overexpressing Metastatic Breast Cancer

By José Baselga, Debasish Tripathy, John Mendelsohn, Sharon Baughman, Christopher C. Benz, Lucy Dantis, Nancy T. Sklarin, Andrew D. Seidman, Clifford A. Hudis, Jackie Moore, Paul P. Rosen, Thomas Twaddell, I Craig Henderson, and Larry Norton

Purpose: **Breast cancer frequently overexpresses the product of the HER2 proto-oncogene, a 185-kd growth factor receptor (p185^{HER2}). The recombinant humanized monoclonal antibody (rhuMAb) HER2 has high affinity for p185^{HER2} and inhibits the growth of breast cancer cells that overexpress HER2. We evaluated the efficacy and toxicity of weekly intravenous administration of rhuMAb HER2 in patients with HER2-overexpressing metastatic breast cancer.**

Patients and Methods: **We treated 46 patients with metastatic breast carcinomas that overexpressed HER2. Patients received a loading dose of 250 mg of intravenous rhuMAb HER2, then 10 weekly doses of 100 mg each. Patients with no disease progression at the completion of this treatment period were offered a maintenance phase of 100 mg/wk.**

Results: **Study patients had extensive metastatic disease, and most had received extensive prior anticancer therapy. Adequate pharmacokinetic levels of rhuMAb HER2 were obtained in 90% of the patients. Toxicity was minimal and no antibodies against rhuMAb HER2 were detected in any patients. Objective responses were seen in five of 43 assessable patients, and included one complete remission and four partial remissions (overall response rate, 11.6%; 95% confidence interval, 4.36 to 25.9). Responses were observed in liver, mediastinum, lymph nodes, and chest wall lesions. Minor responses, seen in two patients, and stable disease, which occurred in 14 patients, lasted for a median of 5.1 months.**

Conclusion: **rhuMAb HER2 is well tolerated and clinically active in patients with HER2-overexpressing metastatic breast cancers that had received extensive prior therapy. This is evidence that targeting growth factor receptors can cause regression of human cancer and justifies further evaluation of this agent.**

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DURING THE LAST DECADE, proto-oncogenes that encode growth factors and growth factor receptors have been found to play important roles in the pathogenesis of several human malignancies, including breast cancer.[1] The HER2 gene (also known as *neu* and as *c-erbB*-2) encodes a 185-kd transmembrane glycoprotein receptor (p185^{HER2}) that has partial homology with the epidermal growth factor receptor, and that shares with that receptor intrinsic tyrosine kinase activity.[2-4] HER2 is overexpressed in 25% to 30% of human breast cancers[5,6] and predicts for a worse prognosis in patients with primary disease that involves axillary lymph nodes.[5,7,8] Several lines of evidence support a direct role for HER2 in the pathogenesis and clinical aggressiveness of HER2-overexpressing tumors: The introduction of HER2 into nonneoplastic cells causes their malignant transformation.[9,10] Transgenic mice that express HER2 develop mammary tumors.[11] HER2 overexpression is common in ductal carcinomas in situ and in their associated invasive cancers.[12,13] Antibodies directed at p185^{HER2} can inhibit the growth of tumors and of transformed cells that express high levels of this receptor.[14-18]

The latter observation suggests that p185^{HER2} may be a potential target for the treatment of breast cancer or preinvasive breast lesions because these cells commonly overexpress HER2. The murine monoclonal antibody (MAb) 4D5, directed against the extracellular domain of p185^{HER2} (ECD^{HER2}), is a potent inhibitor of growth, in vitro and in xenograft models, of human breast cancer cells that overexpress HER2.[19-21] However, murine antibodies are limited clinically because they are immunogenic. To facilitate further clinical investigations, therefore, MAb 4D5 was humanized. The resulting recombinant humanized anti-p185^{HER2} monoclonal antibody (rhuMAb HER2) was found to be safe and to have dose-dependent pharmacokinetics in two prior phase I clinical trials.

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We now report the results of a phase II study of multiple-dose intravenous administration of rhuMAb HER2 in patients with metastatic breast cancer. The objectives of this trial were to determine the antitumor activity of rhuMAb HER2 in this patient population, as well as to define further the toxicity profile and pharmacokinetics of rhuMAb HER2.

PATIENTS AND METHODS

Preparation and Humanization of rhuMAb HER2 Antibody

MAb 4D5 was initially derived by immunizing mice with cells that expressed high levels of the HER2 gene product, $p185^{HER2}$.[19] MAb 4D5, directed at the extracellular domain of $p185^{HER2}$ (ECD^{HER2}), inhibits the in vitro growth of breast cancer cells that contain high levels of $p185^{HER2}$.[19,20] rhuMAb HER2 was engineered by inserting the complementarity determining regions of MAb 4D5 into the framework of a consensus human immunoglobulin G_1 (IgG_1).[22] The resulting rhuMAb HER2 has high affinity for $p185^{HER2}$ (Dillohiation constant [K_d] = 0.1 nmol/L), markedly inhibits, in vitro and in human xenografts, the growth of breast cancer cells that contain high levels of $p185^{HER2}$, and induces antibody-dependent cellular cytotoxicity (ADCC).[22,23] rhuMAb HER2 is produced by a genetically engineered Chinese hamster ovary (CHO) cell line, grown in large scale, that secretes rhuMAb HER2 into the culture medium. Antibody is purified from the CHO culture media using standard chromatographic and filtration methods. Each lot of antibody used in this study was assayed to verify identity, purity, and potency, as well as to meet Food and Drug Administration requirements for sterility and safety.

Selection of Patients

Patients eligible for this study were adult women whose metastatic breast carcinomas overexpressed HER2 (see later). All patients had measurable disease, a Karnofsky's performance status of at least 60%, and preserved hematologic, liver, renal, and pulmonary function. Patients with lymphangitic pulmonary metastasis, history of brain metastasis, or bone metastases as the only site of measurable disease were excluded. Chemotherapy or additive hormonal therapy within 3 weeks before study entry (6 weeks for mitomycin or nitrosureas) was not permitted. Informed consent was obtained and documented in writing before study entry.

Tumor expression of HER2 was determined by immunohistochemical analysis, as previously described,[5,6] of a set of thin sections prepared from the patient's paraffin-archived tumor blocks. The primary detecting antibody used was murine MAb 4D5, which has the same complementarity determining regions as rhuMAb HER2. Tumors were considered to overexpress HER2 if at least 25% of tumor cells exhibited characteristic membrane staining for $p185^{HER2}$.

Antibody Administration

The pharmacokinetic goal was to achieve rhuMAb HER2 trough serum concentrations greater than 10 μg/mL, a level associated with optimal inhibition of cell growth in the preclinical model.[22] The optimal dose and schedule of rhuMAb HER2 was based on two prior phase I clinical trials, conducted at University of California, Los Angeles, and Memorial Sloan-Kettering Cancer Center, which had documented dose-dependent pharmacokinetics. In this current trial, rhuMAb HER2 was administered intravenously over a period of 90 minutes in the outpatient setting. Each patient received a loading dose of 250 mg of rhuMAb HER2 on day 0, and beginning on day 7,100 mg weekly for a total of 10 doses. At the completion of this treatment period, patients with stable disease or minor, partial, or complete responses were entered onto a maintenance phase of weekly rhuMAb HER2 administration until disease progression.

Evaluation of Toxicity

Toxicity was scored based on a modified National Cancer Institute common toxicity criteria. Complete blood cell counts, urinalysis, coagulation profile, and hepatic enzyme, renal, and electrolyte studies were performed weekly while on the study.

Pharmacokinetics, Determination of Extracellular Domain of $p185^{HER2}$ Levels, and Antibodies Directed Against rhuMAb HER2

Blood samples for pharmacokinetic analysis were collected just before each treatment with rhuMAb HER2 and within the first hour following the end of each rhuMAb HER2 infusion. Serum concentrations of rhuMAb HER2 were determined in a receptor binding assay that detects binding with ECD^{HER2}. The nominal limit of detection for rhuMAb HER2 in serum samples was 156 ng/mL. The presence of antibodies to rhuMAb HER2 was determined with a bridging-type titer enzyme-linked immunosorbent assay (ELISA). Circulating concentrations of ECD^{HER2} shed by patients' tumors were also determined using an ELISA.[24] The pair of antibodies used for the assay were 7C2 as coat and 2C4 as horseradish peroxidase–conjugated antibody; the lower limit of detection for this assay ranged from 2.8 to 8.3 ng/mL (Baly D, Wong WL, unpublished data, November 1994).

Serum levels of rhuMAb HER2 as a function of time were analyzed for each patient using a one-compartment model. Model parameters (volume and the elimination rate constant [K_e]) were estimated for each patient using a maximum-likelihood estimation procedure.[25] rhuMAb HER2 half-life ($t_{1/2}$) was calculated by dividing ln 2 by K_e.

Tumor Response

Tumor response was determined at the completion of the initial 11-week treatment period. All responses were confirmed by an independent extramural evaluation committee composed of an oncologist and a radiologist. Complete response was defined as the disappearance of all radiographically and/or visually apparent tumor, partial response as a ≥ 50% reduction in the sum of the products of the perpendicular diameters of all measurable lesions, minimal response as a ≥ 25% and less than 50% reduction in the diameters, stable disease as no change greater than 25% in the size of measurable lesions, and progressive disease as a ≥ 25% increase in any measurable lesion or the appearance of any new lesion. Although bone metastases were considered not measurable for response, patients had to have at least stability of bone lesions to be considered responders. Patients who had entered the maintenance phase of the study had tumor responses evaluated every 11 weeks, or earlier if clinically indicated. Time to tumor progression was calculated from the beginning of therapy to progression. Confidence limits for response rates were calculated using the exact method for a single proportion.[26]

Table 1. Patient Characteristics

Characteristic	Patients (N = 46) No.	%
Age, years		
Median	50	
Range	30-65	
Karnofsky performance status		
Median	90	
Range	60-100	
Level of HER2 expression*		
25%-50% cells	7	15.2
> 50% cells	39	84.8
Receptor status		
Estrogen receptor-positive (n = 40)	17	42.5
Progesterone receptor-positive (n = 39)	15	38.5
No. of metastatic sites		
1	16	34.5
2	14	30.4
≥ 3	16	34.5
Dominant site of metastasis		
Viscera	37	80.4
Skeleton	1	2.2
Soft tissues	8	17.4
Prior therapy		
Chemotherapy	45	97.8
Adjuvant chemotherapy	26	56.5
Neoadjuvant chemotherapy	4	8.7
Metastatic disease (no. of regimens)		
None	8	17.4
1	9	19.6
2	9	19.6
> 2	20	43.5
Median	2	
Range	0-7	
Hormonal therapy		
Adjuvant tamoxifen	7	15.2
Metastatic disease	21	45.6

*In percent of tumor cells with cytoplasmic membrane staining.

RESULTS

Patients characteristics are listed in Table 1. A total of 46 patients were enrolled onto the study. Their level of tumor overexpression of HER2 was relatively high, with more than 80% of the tumors having more than half of their cells exhibit positive membrane staining. Our patient population had extensive metastatic disease: 34.5% of patients had three or more metastatic sites. Dominant sites of metastases were visceral in 80% of cases (lung in 18, liver in 13, both liver and lung in five, and ovary in one). Only 17.4% of cases had dominant metastases in soft tissues (skin and lymph nodes) and only one patient had bone as the dominant site of disease. The total number of patients with bone disease was 18 (39%). All but one of the patients had received prior chemotherapy, with 82.6% having received at least one regimen for metastatic disease and 63% having received two or more regimens. Of this latter group, four patients had previously received high-dose chemotherapy with hematopoietic stem-cell support.

Data on rhuMAb HER2 pharmacokinetics are available from 45 patients (Table 2). More than 90% of the examined population (41 patients) had rhuMAb HER2 trough levels above the targeted 10-μg/mL level. The mean serum $t_{1/2}$ of rhuMAb HER2 was 8.3 ± 5.0 days. The rhuMAb HER2 serum $t_{1/2}$ was found to be dependent on the presence of circulating ECD^{HER2} released from the tumor into the serum (Table 2). Representative examples of pharmacokinetics profiles are shown in Fig 1. Figure 1A shows the serum levels of rhuMAb HER2 in a patient with undetectable level of circulating ECD^{HER2}; stable, therapeutic serum levels of the drug were maintained in this patient for more than 1 year. Figure 1B shows the serum levels of rhuMAb HER2 in a patient with high levels of circulating ECD^{HER2}; trough levels of rhuMAb HER2 were consistently below detectable levels throughout the treatment course and until disease progression. Antibodies against rhuMAb HER2 (human antihuman antibodies [HAHA]) were not detected in any patients.

Treatment with rhuMAb HER2 was remarkably well tolerated. Of a total of 768 administrations of rhuMAb HER2, only 11 events occurred that were considered to be related to the use of the antibody (Table 3). Fever and chills occurred on five occasions after the first administration of rhuMAb HER2. The fever lasted less than 8 hours in all cases and did not recur on subsequent administrations of the antibody. Three patients experienced chest pain in areas of tumor involvement shortly after the infusion of the first dose of rhuMAb HER2; in one case this required an overnight hospital admission for pain control. The pain did not recur on successive administrations of the antibody. None of the patients whose cancer regression met the formal criteria for complete or partial response had pain at a tumor site after administration of rhuMAb HER2.

The number of patients assessable for treatment response on evaluation day 77 was 43. Three patients were not assessable for response. One had a bacteremic infection of an intravenous catheter that required prolonged administration of antibiotics, which precluded treatment

Table 2. ECD^{HER2}-Dependent Pharmacokinetics of rhuMAb HER2

N	Patient Group	rhuMAb HER2 $t_{1/2}$ (days)
45	All patients	8.3 ± 5.0
40	Circulating ECD^{HER2} < 500 ng/mL	9.1 ± 4.7
5	Circulating ECD^{HER2} > 500 ng/mL	1.8 ± 1.0

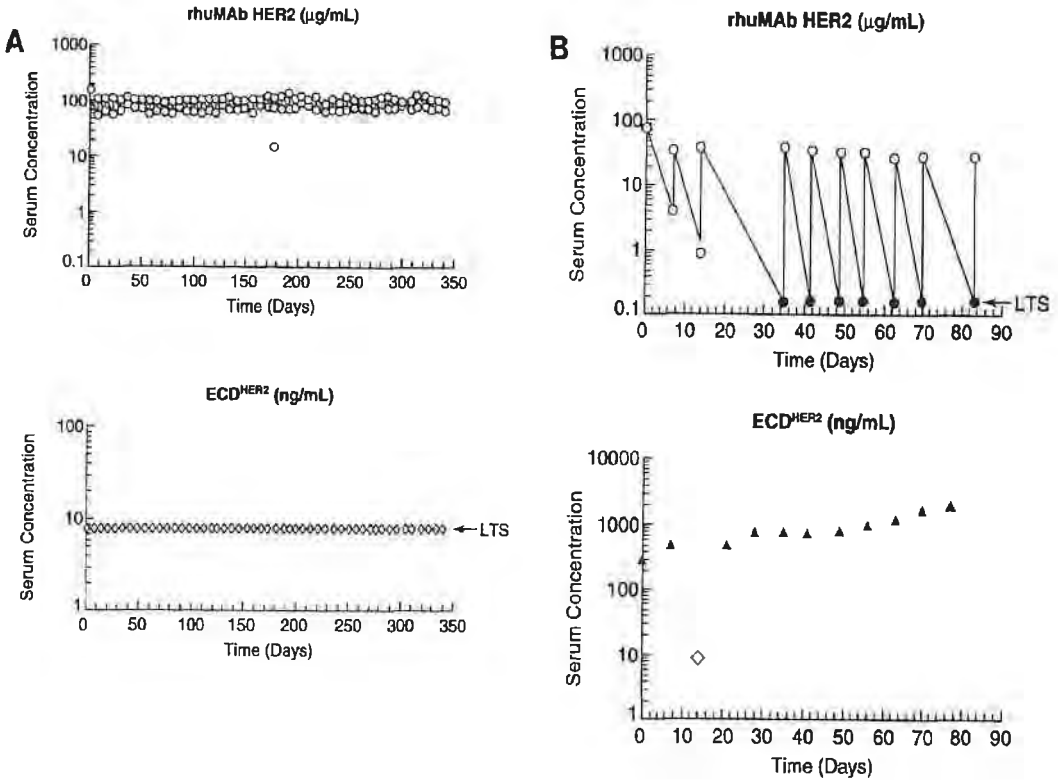

Fig 1. Effect of serum ECD^{HER2} on rhuMAb HER2 pharmacokinetics. Stable serum levels of rhuMAb HER2 in a patient with absence of ECD^{HER2} (A) v suboptimal rhuMAb HER2 serum levels in a patient with high ECD^{HER2} (B). Note that log scales on the Y-axis describing the serum ECD^{HER2} differ among charts. LTS, less than lowest assay standard. (○) observed rhuMAb HER2 serum concentration; (●) LTS for rhuMAb HER2 serum concentration; (▲) ECD^{HER2} serum concentration; (◇) LTS for ECD^{HER2} serum concentration.

with rhuMAb HER2. A second declined to continue on the study for personal reasons. The third died of congestive heart failure associated with prior doxorubicin treatment. Among 43 assessable patients, 5 had tumor responses: one patient had a complete remission and four had partial remissions. Therefore, the overall response rate (complete plus partial responses) for assessable patients is 11.6% (95% confidence interval, 4.36 to 25.9). Details of responses are listed in Tables 4 and 5, and examples of the responses are shown in Fig 2.

Table 3. rhuMAb HER2-Related Toxicity

Toxicity	Moderate (grade 2)	Severe (grade 3)
Fever and chills	5	
Pain at tumor site	2	1
Diarrhea	2	
Nausea and emesis	1	

NOTE. In number of events of a total of 768 administrations.

Two patients had minor responses and 14 patients had stable disease at day 77. These patients entered a maintenance phase of weekly antibody administration until progression of disease. The median time to progression for the patients with either minor or stable disease was 5.1 months. An additional patient had a greater than 50% reduction in the size of the metastatic disease on her mediastinum and chest wall after 2 weeks of treatment. While the duration of response was greater than 4 weeks, by evaluation day 77 the lesion had begun to regrow from the size of maximal response to therapy. Per protocol guidelines, this patient was therefore considered not to have had a response to therapy, but rather progression of disease.

DISCUSSION

During the last decade, overexpression of the HER2 gene has been shown to play an important role in the pathogenesis and poor prognosis of breast cancer. As a

Table 4. Response Rate Obtained With rhuMAb HER2 in 43 Assessable Patients

Response	No. of Patients	%
Complete response	1	2.3
Partial response	4	9.3
Overall response	5	11.6
Minor response	2	4.6
Stable disease	14	32.6
Progression of disease	22	51.2

consequence, strategies directed at interference with HER2 expression or the function of its protein, $p185^{HER2}$, have been anticipated to have therapeutic value. Extensive preclinical studies have shown that certain MAbs directed against $p185^{HER2}$ can inhibit growth of HER2-overexpressing tumor cells.[15-19] This study provides the first clinical evidence of the antitumor activity of one of these agents, rhuMAb HER2.

Of 43 patients with $p185^{HER2}$-positive tumors assessable for response after treatment with rhuMAb HER2, five experienced a complete or partial remission, for an overall response rate of 11.6%. One additional patient had a greater than 50% shrinking of her cancer that lasted more than 1 month, but was not considered a responder by our protocol definition. The objective antitumor responses observed were of clinical importance, since two patients had regression of cancers in the liver and one patient achieved a pathologically-proven complete response of chest wall disease, which has persisted for 24 months. Our patients were selected to have many sites of metastatic involvement, one of the most dire prognostic characteristics regarding response to therapy. This selection was the consequence of the rule that patients with disease involving only bone were ineligible for accrual, because bone is the solitary site of initial metastatic involvement in up to 60% of cases.[27] It is reasonable to hypothesize that the percentage of patients who show objective tumor regression to rhuMAb HER2 will be higher when patients with less extensive breast cancer are treated, since laboratory studies have shown that the response to antireceptor antibodies is greater with lower tumor burden.[28] It would be also of interest to analyze the response rate to rhuMAb HER2 in a patient population with no prior chemotherapy for stage IV disease, since prior experience has shown that untreated patients usually respond better to new anticancer drugs.[29]

Another important point about the probability of response to rhuMAb HER2 concerns the observation that 37% of patients achieved minimal responses or stable disease. In the laboratory, rhuMAb HER2 or the parent antibody 4D5 has been noted to be cytostatic, which causes growth arrest, rather than cytocidal, which causes cell death. In clinical trials of many anticancer drugs, particularly chemotherapy, the achievement of stable disease is not considered a reliable measure of anticancer activity. However, with rhuMAb HER2, stable disease may be an authentic reflection of the biologic action of the drug, which differs markedly from conventional anticancer agents. The unusually long durations of minimal responses and stable disease seen in our trial may relate to this distinction. These data are specially interesting in light of the absence of significant toxicity observed here, for in a setting in which palliation is a main objective, quality of life while on treatment should be a main end point.

The dose and schedule of rhuMAb HER2 administration used in this protocol provided adequate serum concentrations in all patients, except in those with circulating levels of tumor-shed ECD^{HER2} at serum concentrations ≥

Table 5. Characteristics of Patients Who Achieved a Response to Treatment

Patient No.	HER2*	Site of Metastatic Disease	Prior Systemic Therapy	Best Response	Duration of Response (months)
1	3+	Chest wall	Doxorubicin	Complete response†	> 24
2	3+	Liver	Doxorubicin, mitoxantrone, paclitaxel	Partial response	6.7
3	2+	Mediastinum	CMFVP, doxorubicin, tamoxifen, paclitaxel	Partial response	7.7
4	3+	Liver + retroperitoneal lymph nodes + bone	CMF, docetaxel	Partial response	1
5	2+	Chest wall	Paclitaxel	Partial response	3.4

Abbreviations: CMFVP, cyclophosphamide, methotrexate, fluorouracil, vincristine, and prednisone; CMF, cyclophosphamide, methotrexate, and fluorouracil.

*By immunohistochemistry: 2+, 25% to 50% of tumor cells with cytoplasmic membrane staining; 3+, > 50% of tumor cells with cytoplasmic membrane staining.

†Patient's complete response was pathologically proven with several biopsies at tumor site. Patient bone scan, head, thoracic, abdominal, and pelvic computed tomographic scans are negative.

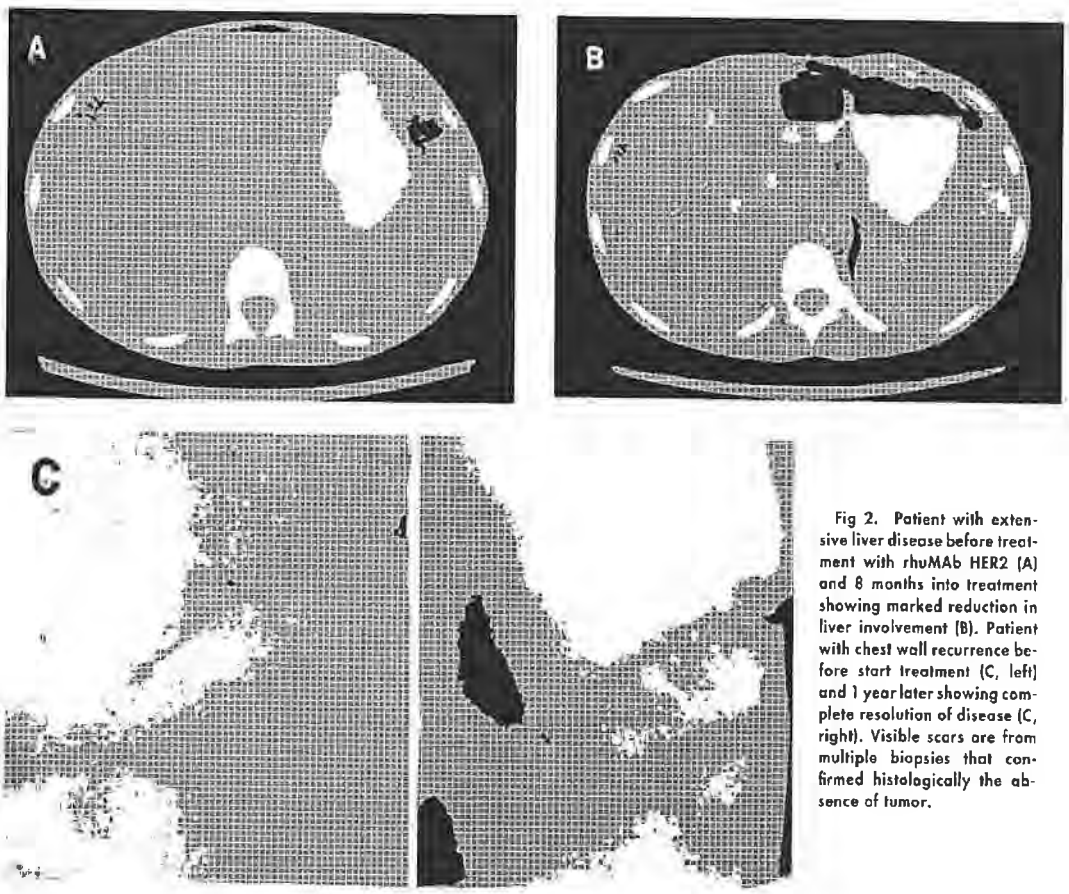

Fig 2. Patient with extensive liver disease before treatment with rhuMAb HER2 (A) and 8 months into treatment showing marked reduction in liver involvement (B). Patient with chest wall recurrence before start treatment (C, left) and 1 year later showing complete resolution of disease (C, right). Visible scars are from multiple biopsies that confirmed histologically the absence of tumor.

500 ng/mL. ECD^{HER2} is known to be released by some breast cancer cells that overexpress HER2,[30-32] and elevated ECD^{HER2} serum levels have been previously reported in patients with breast cancer.[31,33,34] The most likely explanation for the short serum $t_{1/2}$ values and subtherapeutic trough levels of rhuMAb HER2 in this group of patients is that in the presence of ECD^{HER2} in the serum, antigen-antibody complexes form and are rapidly cleared from the circulation. Of interest, no anticancer responses were observed in the group of patients with serum concentrations of $ECD^{HER2} \geq 500$ ng/mL. Hence, the interpretation of results of future trials of agents that bind to or exert their function through $p185^{HER2}$ should take ECD^{HER2} release from the tumors into account; at present, patients with high levels of ECD^{HER2} should continue to participate in these studies.

There are several possible mechanisms, not mutually exclusive, that could explain the clinical results observed. An important fact is that rhuMAb HER2 induces a marked downregulation of $p185^{HER2}$.[19] Antibody-induced downregulation of $p185^{HER2}$ has been shown to induce reversion of the transformed phenotype in HER2-transformed cells.[34] By a similar mechanism, the continuous exposure to rhuMAb HER2 at adequate concentrations achieved in our trial could be reversing the malignant phenotype of the clinical cancers by downregulating their level of $p185^{HER2}$. Another possibility is that the known partial agonistic effects of rhuMAb HER2[35] could result in the activation of a signal transduction pathway that leads to inhibition of tumor-cell proliferation. Both of these potential antitumor mechanisms would require, in addition to receptor expression, intact receptor function. Little is known about the functional status of $p185^{HER2}$ in breast tumor specimens, but it is conceivable that not all overexpressing tumors have functional receptors. In support of this view is the observation that HER2-overexpressing tumor-cell lines that are not growth-inhibited by anti-$p185^{HER2}$ antibodies have been described and well charac-

terized[36]; it is noteworthy that some of these antibody-resistant tumor cells also overexpressed truncated forms of ECD^{HER2}. Furthermore, in vitro studies suggest that those breast cancer cell lines that have the highest basal level of $p185^{HER2}$ phosphorylation are the most growth-inhibited by anti-$p185^{HER2}$ antibodies.[20] If this were the case in the clinic, the recently produced antiserum that specifically recognizes only overexpressed tyrosine-phosphorylated $p185^{HER2}$ might prove useful in predicting the subset of $p185^{HER2}$-positive tumors most likely to respond to rhuMAb HER2.[8]

Another possible mechanism of action concerns the observation that rhuMAb HER2 is a potent inducer of ADCC.[22] However, while this immune-mediated mechanism might play a role in the observed clinical responses, ADCC is obviously not involved in the pronounced growth-inhibitory effects of the antibody in vitro.

The observed activity of rhuMAb HER2 against advanced breast cancers that overexpress HER2 provides the first clinical evidence that anti–growth factor receptor-directed strategies may be useful in the treatment of human breast cancer. Therefore, continued research with this agent and other HER2-targeted treatment strategies appears warranted. The response to rhuMAb HER2 in a less heavily pretreated population and in those with less extensive metastatic disease would be of interest since both parameters have historically correlated with a higher response to drugs,[29] and this same principle may apply to antibody-based therapy.[37] In preclinical studies, both in vitro and in xenografts, rhuMAb HER2 markedly potentiated the antitumor effects of several chemotherapeutic agents, including cisplatin, doxorubicin, and paclitaxel,[23,38] without increasing their toxicity. Laboratory studies of the mechanism of this effect and clinical trials of such combination therapy are currently in progress.

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EXHIBIT 5

Phase II Study of Receptor-Enhanced Chemosensitivity Using Recombinant Humanized Anti-p185$^{HER2/neu}$ Monoclonal Antibody Plus Cisplatin in Patients With HER2/*neu*-Overexpressing Metastatic Breast Cancer Refractory to Chemotherapy Treatment

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Purpose: To determine the toxicity, pharmacokinetics, response rate, and response duration of intravenous (IV) administration of recombinant, humanized anti-p185^{HER2} monoclonal antibody (rhuMAb HER2) plus cisplatin (CDDP) in a phase II, open-label, multicenter clinical trial for patients with HER2/*neu*-overexpressing metastatic breast cancer.

Patients and Methods: The study population consisted of extensively pretreated advanced breast cancer patients with HER2/*neu* overexpression and disease progression during standard chemotherapy. Patients received a loading dose of rhuMAb HER2 (250 mg IV) on day 0, followed by weekly doses of 100 mg IV for 9 weeks. Patients received CDDP (75 mg/m^2) on days 1, 29, and 57.

Results: Of 37 patients assessable for response, nine (24.3%) achieved a PR, nine (24.3%) had a minor response or stable disease, and disease progression occurred in 19 (51.3%). The median response duration was 5.3 months (range, 1.6-18). Grade III or IV toxicity was observed in 22 of 39 patients (56%). The toxicity profile reflected that expected from CDDP alone with the most common toxicities being cytopenias (n = 10), nausea/vomiting (n = 9), and asthenia (n = 5). Mean pharmacokinetic parameters of rhuMAb HER2 were unaltered by coadministration of CDDP.

Conclusion: The use of rhuMAb HER2 in combination with CDDP in patients with HER2/*neu*-overexpressing metastatic breast cancer results in objective clinical response rates higher than those reported previously for CDDP alone, or rhuMAb HER2 alone. In addition, the combination results in no apparent increase in toxicity. Finally, the pharmacology of rhuMAb HER2 was unaffected by coadministration with CDDP.

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THE HER2/*neu* GENE encodes a 185-kd transmembrane protein that is a member of the type I family of growth factor receptors. Amplification of this gene is found in approximately 25% of human breast cancers and results in overexpression of the 185-kd encoded receptor tyrosine kinase, which is homologous to the epidermal growth factor receptor (EGFR). Overexpression of p185$^{HER2/neu}$ is an independent predictor of both relapse-free and overall survival in patients with breast cancer.[1-4] In addition, overexpression of this gene has prognostic significance in patients with ovarian,[2] gastric,[5] endometrial,[6] and salivary gland malignancies.[7] In breast cancer, overexpression of HER2/*neu* is also associated with a number of other adverse prognostic factors that include advanced pathologic stage,[4] number of metastatic axillary lymph nodes,[2] absence of estrogen and progesterone receptors,[8] increased S-phase fraction,[9] DNA ploidy,[10] and high nuclear grade.[11] A role for the HER2/*neu* alteration in metastasis has also been suggested given the increased occurrence of visceral metastasis[12] and micrometastatic bone marrow disease in patients with HER2/*neu* overexpression.[13] Like many other cell-surface receptors, a soluble form of the extracellular domain (ECD) of p185$^{HER2/neu}$ can be shed from the surface of tumor cells and is detectable in the sera of experimental animals that bear HER2/*neu*-overexpressing xenografts, as well as in the sera of approximately 20% to 25% of patients with locally advanced or metastatic breast cancer.[14-16] Patients with elevated serum levels of shed HER2/*neu* ECD have a decreased response to hormonal therapy and shortened overall survival compared with patients without shed HER2/*neu* ECD.[14,16]

A murine monoclonal anti-HER2 antibody, 4D5, known to have antiproliferative activity against HER2/*neu*-overex-

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pressing human breast carcinoma cells in vitro and against breast cancer xenografts with HER2/*neu* overexpression in vivo, was humanized, which resulted in a human immunoglobulin (IgG1) molecule with retained murine sequences only in the complementarity determining regions. The resultant molecule has improved binding affinity to the extracellular domain of HER2/*neu* (kd = 0.1 nM *v* 0.3 nM for murine 4D5) and similar growth inhibitory activity against HER2/*neu*-overexpressing cell lines and xenografts.[17]

Previous work has shown that treatment with monoclonal antibodies directed against EGFR in combination with the cytotoxic drug cisplatin (CDDP) resulted in a marked reduction in both size and number of human epidermoid carcinoma xenografts that overexpressed EGFR.[18] Using a similar experimental approach, we have shown a synergistic, cytocidal effect against cell lines and xenografts with HER2/*neu* overexpression by using monoclonal anti-HER2/*neu* antibodies plus CDDP.[19] The mechanism of this effect appears to involve a decreased capacity of HER2/*neu*-overexpressing cells to repair CDDP-induced DNA adducts after pretreatment with anti-HER2/*neu* antibodies.[19-21] This activity, which we have termed receptor-enhanced chemosensitivity (REC), has potential clinical application based on the fact that (1) the dose-effect relationship of the anti-HER2/*neu* antibody plus CDDP is synergistic, (2) this synergistic effect is specific for cells that overexpress the HER2/*neu* receptor, (3) the combination of CDDP plus anti-HER2/*neu* antibody results in a two-log increase in cell killing, and (4) the combination yields pathologic complete remissions against HER2/*neu*-overexpressing human breast carcinoma xenografts in athymic mice.[19]

Pursuant to these preclinical observations, a series of phase I clinical trials were initiated and conducted at the University of California at Los Angeles to determine the safety and pharmacology of the murine monoclonal antibody 4D5, as well as the recombinant, humanized anti-$p185^{HER2}$ antibody monoclonal (rhuMAb HER2), both alone and in combination with CDDP. These studies showed that the pharmacokinetics of rhuMAb HER2 were predictable, and that the doses delivered achieved a target trough serum concentration of 10 to 20 μg/mL, which is associated with antitumor activity in preclinical models. In addition, administration of this anti-HER2/*neu* antibody was safe; the only toxicity was low-grade fever that occurred with the first infusion and/or pain at the site of known tumor deposits in a minority of patients. Moreover, these studies showed that rhuMAb HER2 was not immunogenic in contrast to murine monoclonal antibody 4D5. Finally, the phase I studies showed that the combination of rhuMAb HER2 and CDDP showed significant antitumor efficacy, with four of 15 patients who achieved objective responses, which included three partial responses and one sustained complete remission that lasted in excess of 5.5 years without subsequent treatment. Based on these findings, we designed the current phase II trial with the following objectives: (1) to determine the overall response rate and response duration of intravenous (IV) rhuMAb HER2 plus CDDP in an open-label, multicenter clinical trial for patients with HER2/*neu*-overexpressing metastatic breast cancer who have shown disease progression while undergoing standard chemotherapy treatment; (2) to document the tolerance and toxicity of rhuMAb HER2 plus CDDP; and (3) to determine the pharmacokinetics of rhuMAb HER2 when administered in combination with CDDP.

PATIENTS AND METHODS

Eligibility Criteria

Women aged from 18 to 75 years with a primary histologic diagnosis of invasive breast cancer, with radiographically or visually measurable and assessable metastatic disease documented by physical examination or radiographic findings, were considered for enrollment. Patients were required to have evidence of overexpression (2 + to 3 +) of the HER2/*neu* proto-oncogene in their malignant cells as determined by immunohistochemical analysis (Roche Biomedical Laboratories, Research Triangle Park, NC), and were required to have documentation of objective tumor progression while receiving active chemotherapy for breast cancer. No therapy of any kind (cytotoxic, cytokine, or hormonal) was allowed within the 3 weeks before study entry. In addition, no therapy with mitomycin or nitrosoureas was allowed within 6 weeks of study entry. A Karnofsky performance status (KPS) greater than 60%; life expectancy of 3 months or greater; normal serum calcium level (≤ 10.5 mg/dL); and preserved cardiac, renal (serum creatinine level ≤ 1.5 mg/dL, creatinine clearance ≥ 60 mL/min, ≤ 2 + proteinuria), hepatic (bilirubin level ≤ 1.5 mg/dL), pulmonary (forced expiratory volume in 1 second ≥ 70% of predicted value), hematologic (WBC count ≥ 3,000/μL, granulocyte count ≥ 1,500/μL, platelet count ≥ 125,000/μL), and coagulation (prothrombin time < 14 seconds, partial thromoplastin time < 35 seconds) function were all required. All patients signed a written, internal review board–approved, informed consent document. Patients were excluded for active infection, pregnancy or lactation, significant cardiac disease (New York Heart Association class III or IV), known hemorrhagic diathesis, hepatic metastases that involved greater than 50% of the liver parenchyma, lymphangitic pulmonary metastasis, CNS metastasis, bone-only metastasis, prior treatment with CDDP or other cisplatin analogues, previous therapy with a monoclonal or polyclonal antibody, or concomitant use of any investigational agent.

Study Design

Eligible patients received a 250-mg loading dose of rhuMAb HER2 IV day 0, followed by 100 mg IV weekly for a total of eight doses. Patients also received CDDP 75 mg/m^2 day 1 of treatment, with repeat doses on days 29 and 57. Clinical response was assessed on day 70. Responsive patients or patients with stable disease were eligible for entry onto a maintenance phase program after day 70. The maintenance

phase protocol consisted of rhuMAb HER2 100 mg IV weekly plus CDDP 75 mg/m² IV every 4 weeks until disease progression or prohibitive toxicity ensued.

Treatment Plan

A baseline pretreatment evaluation that included a complete history and physical examination, 12-lead ECG, chest radiograph, serum pregnancy test, complete blood count, urinalysis, creatinine clearance, serum chemistries (which included hepatic function tests), coagulation studies, hepatitis serologies, audiologic testing, pulmonary function tests, and baseline tumor measurements was performed within 2 weeks before study entry. Study patients were monitored weekly by physical examination, complete blood counts, serum chemistries, and coagulation studies. All rhuMAb HER2 doses were administered in 250 mL of 0.9% sodium chloride solution infused IV over 90 minutes. Vital signs were recorded before each dose, at the end of the infusion, and 1 hour postinfusion. Serum samples were collected just before and 1 hour after each rhuMAb HER2 dose for pharmacokinetic analysis of rhuMAb HER2, presence of shed $p185^{HER2}$ ECD, and detection of anti–rhuMAb HER2 antibodies. All CDDP doses were administered 1 day after scheduled rhuMAb HER2 doses, consisted of CDDP 75 mg/m² diluted in 500 mL of 0.9% sodium chloride solution, and were administered IV over 60 minutes after hydration with a minimum of 500 mL of 5% dextrose/0.9% sodium chloride solution. After CDDP administration, patients received an additional 500 mL of 5% dextrose/0.9% sodium chloride solution. Additional hydration, mannitol, furosemide, and electrolyte solutions were administered as medically indicated. Antiemetic therapy consisted of dexamethasone 20 mg IV before CDDP administration and ondansetron 0.15 mg/kg IV before CDDP administration and 1.5 and 3.5 hours after the CDDP infusion. A graded toxicity scale based on the modified National Cancer Institute criteria was used to assess toxicity. Dose modification of CDDP to 50% of the original dose was performed for grades I to II nephrotoxicity or grades III to IV gastrointestinal toxicity. For any other grades III to IV toxicity, treatment was reinstituted at doses of CDDP of 50 mg/m² and rhuMAb HER2 of 50 mg IV after resolution of toxicity. Response criteria were defined as follows: complete response, disappearance of all radiographically and/or visually apparent tumor; partial response, reduction of at least 50% in the sum of the products of the perpendicular diameters of all measurable lesions with no new lesions detected; minor response, a reduction of 25% to 49% in the sum of the products of the perpendicular diameters of all measurable lesions with no new lesions detected; stable disease, not meeting the criteria for response or progression; and progressive disease, objective evidence of an increase of 25% in any measurable lesion or the appearance of any new lesion. All objective responses were assessed by an independent response evaluation committee comprised of a medical oncologist and radiologist who were otherwise not involved in the conduct of this study.

*Detection of HER2/*neu *Oncogene Overexpression in Clinical Tissue Specimens*

Patterns of HER2/*neu* expression were evaluated by a modification of published immunohistochemical techniques that used a murine monoclonal antibody (4D5) directed against HER2/*neu*.[2,22] Four-micron sections from formalin-fixed, paraffin-embedded tissues were cut and mounted on positively charged slides. Tissue sections were deparaffinized and endogenous peroxidase activity was quenched with 1% hydrogen peroxide in methanol. Sections were digested with 1 mg/mL of protease in phosphate buffered saline (PBS) and allowed to incubate with horse serum to block nonspecific antibody binding. Primary antibody (4D5; Genentech, Inc, South San Francisco, CA) was applied (10 μg/mL) and sections were allowed to incubate at 4°C for 18 hours. Sections were washed with PBS and treated with a biotinylated antimouse secondary antibody (Vector Laboratories, Inc, Burlingame, CA). After rinsing with PBS, sections were incubated with avidin-biotinylated enzyme complex (Vector Laboratories, Inc). Sections were then rinsed in PBS, and antibody binding was detected by staining with a diaminobenzidine/hydrogen peroxide chromogen solution. Sections were rinsed in deionized water, counterstained in Harris hematoxylin, dehydrated through graded alcohols, cleared in xylene, and coverslipped. The scoring system for interpretation of HER2/*neu* immunostaining is as follows: 0, 10% or less of tumor cells show any level of positive staining; 1 +, barely perceptible light membranous rimming that may not totally encircle the cell membrane; 2 +, light to moderate membranous rimming that totally encircles the membrane; and 3 +, moderate to strong membrane rimming that totally encircles the membrane.

Pharmacokinetics of rhuMAb HER2

The concentration of rhuMAb HER2 in serum was measured by means of an enzyme-linked immunosorbent assay (ELISA) with the ECD of $p185^{HER2}$ as the coat antigen. In this ELISA format, 100 μL of $p185^{HER2}$ (Genentech, Inc) was added to MaxiSorp 96-well microtiter plates (Nunc, Roskilde, Denmark) at 1 mg/mL in 0.05 mol/L of sodium carbonate, pH 9.6. After overnight incubation at 2° to 8°C, the plates were washed three times with ELISA wash buffer (PBS that contained 0.05% Tween-20) using a Biotek EL304 platewasher (Bio-tek Instruments, Inc, Winooski, VT). The plates were then blocked with 200 μL per well of ELISA diluent (PBS that contained 0.5% bovine serum albumin [BSA]; 0.05% Tween-20; and 0.05% Proclin300, pH 7.2) for 1 to 2 hours at ambient temperature with agitation. After blocking, plates were washed again three times with ELISA wash buffer. Subsequently, 100 μL of standards, samples, or controls were added to duplicate wells and allowed to incubate for 1 hour at ambient temperature. The standard curve range for the assay is 1.56 to 100 μg/mL. After the sample/standard incubation, the plates were washed six times with ELISA wash buffer and 100 μl of goat antihuman IgG Fc-horseradish peroxidase (HRP), freshly diluted to its optimal concentration in ELISA diluent, was added to the plates. After a 1-hour incubation, the plates were washed six times in ELISA wash buffer and 100 μL of PBS, pH 7.2, that contained 2.2 mmol/L of orthophenylene diamine (Sigma Chemical Co, St Louis, MO) and 0.012% (vol/vol) hydrogen peroxide (Sigma Chemical Co) were added to each well. When color had fully developed, the reaction was stopped with 100 μL per well of 4.5 mol/L of sulfuric acid. The absorbencies of the well contents were read at 492 nm minus 405 nm reference absorbance using an automatic plate reader (Molecular Devices, Palo Alto, CA). A four-parameter curve fit program was used to generate the standard curve, from which sample and control concentrations were interpolated.

Detection of Anti-rhuMAb HER2 Antibodies in Serum

An antibody titer ELISA was developed to measure the presence and titer of antibodies against rhuMAb HER2 in human sera. The positive control in the ELISA was an antiserum prepared against rhuMAb HER2 in cynomulogous monkeys. The negative control was a human serum pool prepared from a panel of healthy donors. Briefly, 100 μL of rhuMAb HER2 was added to 96-well microtiter plates at 1 μg/mL in 0.05 mol/L of sodium carbonate buffer, pH 9.6. After overnight

incubation at 4°C, plates were washed with ELISA wash buffer (PBS that contained 0.05% Tween-20 and 0.01% thimerosal) and blocked for 1 hour with ELISA diluent (PBS that contained 0.05% Tween-20, 0.5% BSA, and 0.01% thimerosal). Subsequently, 50 μL of sample, positive control, or negative control and 50 μL of biotin-rhuMAb HER2 were added to appropriate wells and allowed to incubate for 1 hour at room temperature (RT). The titer of the positive control was determined by an initial 1:100 dilution of the sample followed by serial 1:2 dilutions. The plates were washed in ELISA wash buffer, then 100 μL of PBS that contained 2.2 mmol/L of orthophenylene diamine (OPD) and 0.012% hydrogen peroxide was added to each well. The colorimetric reaction was quenched with 100 μL of 4.5 mol/L of sulfuric acid and absorbance was measured at 492-nm wavelength in an automated plate reader. Intra-assay and interassay variability averaged 2.6% and 12.1%, respectively.

Detection of p185$^{HER2/neu}$ ECD in Serum

The method for detection of shed HER2 ECD levels in serum is an ELISA-based assay and has been described in detail elsewhere.[23] Briefly, the ELISA uses pairs of anti-HER2 monoclonal antibodies (Genentech, Inc) that recognize mutually exclusive determinants of the ECD of p185$^{HER2/neu}$. Wells were coated overnight at 4°C with MAb 7F3, which does not compete with rhuMAb HER2 for shed HER2 ECD binding. Assay standards (recombinant, p185$^{HER2/neu}$ ECD) and patient samples were added to appropriate wells and allowed to incubate for 2 hours. After a wash step, secondary antibody was added (MAb 4D5 to detect free shed HER2 ECD and MAb 2C4 to detect total shed HER2 ECD) for 2 hours. The bound conjugate was detected with OPD substrate and the resulting absorbance was measured at a 490-nm wavelength. The range of the assay is 2.75 to 1,800 ng/mL in serum or plasma.

RESULTS

Patient Characteristics

Thirty-nine patients were enrolled onto the study, and their characteristics are listed in Table 1. Patients ranged in age from 29 to 75 years. Eighty-six percent of the patients had a KPS of 90% or greater. High levels of HER2/*neu* overexpression (3+) were observed in 82% of the patients. Twenty-four of 37 patients (65%) who had measurements of serum shed HER2/*neu* ECD performed before treatment had levels greater than 2.75 ng/mL (the lower limit of detection in the ELISA assay). Only one third of the patients for whom hormone receptor data were available were either estrogen receptor– or progesterone receptor–positive, consistent with previous studies that showed an inverse correlation between HER2/*neu* overexpression and hormone receptor expression.[24] Twenty-seven of the 39 patients (69%) were postmenopausal at diagnosis, and a majority of the patients had a high disease burden, with 18 of 39 patients (46%) who had three or more sites of metastatic disease. This patient population had been heavily pretreated before study entry, with 35 of 39 patients (90%) in whom two or more prior chemotherapeutic regimens had failed for metastatic disease. In addition, all patients had to show resistance to standard chemotherapy as evidenced by tumor progression while receiving chemotherapy treatment to be eligible for this trial.

Table 1. Patient Characteristics

Characteristic	No.	%
Age, years		
Mean	50	
Range	29-75	
Karnofsky performance status, % (n = 37)		
100	22	59
90	10	27
80	4	11
70	1	3
Level of HER2/*neu* overexpression		
2+	7	18
3+	32	82
Detection of shed HER2 ECD (n = 37)		
Receptor status		
Estrogen receptor–positive (n = 37)	13	35
Progesterone receptor–positive (n = 36)		
Menopausal status		
Premenopausal	10	26
Postmenopausal	27	69
Perimenopausal	2	5
No. of metastatic sites		
1	7	18
2	14	36
≥ 3	18	46
Sites of metastasis		
Lung	19	49
Lymph node	19	49
Bone	18	46
Chest wall/skin	17	44
Liver	14	36
Breast	4	10
Ovary	1	2.5
Eye	1	2.5
No. of prior chemotherapy regimens for metastatic disease		
1	4	10
2	18	46
≥ 3	17	44
Prior hormonal therapy	21	54
Prior radiotherapy	27	69

NOTE. N = 39.

Toxicity

Toxicity data are listed in Table 2. During the main study, 105 cycles of CDDP were administered in combination with 314 weekly IV doses of rhuMAb HER2. During the maintenance phase, an additional 81 cycles of CDDP and 434 doses of rhuMAb HER2 were administered. Thirty-seven patients had KPS assessed at baseline and at least once during the course of the study. The KPS remained unchanged in 19 patients (the majority of whom had

Table 2. Grade 3 or 4 Clinical Adverse Events, Irrespective of Causality

Event	Grade 3		Grade 4	
	No.	%	No.	%
Main study (n = 39)				
Accidental injury	1	3	0	0
Back pain	1	3	0	0
Infection	2	5	0	0
Dyspnea	2	5	0	0
Anorexia	1	3	0	0
Nausea and/or vomiting	7	18	0	0
Increased AST	1	3	0	0
Increased alkaline phosphatase	1	3	0	0
Hyperbilirubinemia	3	8	1	3
Nephrotoxicity	0	0	1	3
Asthenia	5	13	0	0
Hypertonia	1	3	0	0
Leukopenia	2	5	0	0
Anemia	3	8	0	0
Thrombocytopenia	4	10	0	0
Maintenance phase (n = 19)				
Hyperglycemia	1	5	0	0
Sepsis	0	0	1	5
Cardiomyopathy	1	5	0	0
Nausea and/or vomiting	2	10	1	5
Hyperbilirubinemia	1	5	0	0
Peripheral neuropathy	2	10	0	0
Leukopenia	1	5	0	0
Anemia	1	5	0	0
Thrombocytopenia	4	21	0	0

KPS > 80%), improved in two patients, and decreased in 16 patients. Four patients experienced weight loss in excess of 10% of their baseline body weight during the study. Four patients experienced fever greater than 38°C during rhuMAb HER2 infusion or at the postinfusion measurement. There was no significant difference in blood pressure between pretreatment and posttreatment measurements across all treatment days. During the main study, 22 of 39 patients (56%) experienced at least one episode of grade III or IV toxicity. The most frequent grade III toxicities observed were nausea and/or vomiting in seven patients (18%), asthenia in five patients (13%), thrombocytopenia in four patients (10%), anemia in three patients (8%), and leukopenia in two patients (5%). One episode of reversible grade IV nephrotoxicity was registered during the main study, and this patient's renal function recovered after 9 days. One patient developed grade IV hyperbilirubinemia during the main study. This event was believed to be disease-related rather than treatment-related. During the maintenance phase, 10 of 19 patients (53%) experienced grade III or IV toxicity. The most frequent grade III toxicities were thrombocytopenia, four patients (21%); nausea and/or vomiting, two patients (10%); and peripheral neuropathy, two patients (19%). Two patients experienced grade IV toxicity during the maintenance phase, one patient with sepsis and another with gastrointestinal toxicity. Grade III/IV toxicity reported as possibly related to rhuMAb HER2 was infrequent and was reported in six of 39 patients (15%). These events consisted of grade III cytopenia in three patients, grade III nausea or anorexia in two patients, grade III asthenia in one patient, and grade III hyperbilirubinemia in one patient. In most of these cases, we were not able to dissociate toxicity possibly related to rhuMAb HER2 from toxicity likely caused by CDDP or the patient's underlying disease. There was no report of grade IV toxicity attributable to rhuMAb HER2 administration. We observed no evidence of increased toxicity in those tissues that are known to express $p185^{HER2}$, ie, lung, gastrointestinal tract, CNS, or skin, nor was there any toxicity at the IV injection site. Three patients discontinued the main study treatment because of toxicity or intercurrent medical illness (two with nephrotoxicity and one with hepatic failure), and one patient discontinued the maintenance phase as a result of cardiomyopathy. The latter patient had a cumulative anthracycline dose of 420 mg/m^2 and had also received prior chest-wall irradiation. This patient also had a history of concurrent hypertension and diabetes. Four patients died before day 70; however, in each case, patients had been removed from the study because of disease progression before death. In summary, the toxicities observed were consistent with those previously reported for CDDP alone in a heavily pretreated population of breast cancer patients (Table 3).

Response and Response Duration

Tumor response was evaluated on day 70 during the main study and every 10 weeks during the maintenance phase protocol. Patients with symptoms or suspected progressive disease could have tumor assessment at any time during the course of the study. Thirty-seven of the 39 patients enrolled (95%) were assessable for tumor response (Table 4). Assessable patients were defined as those who met all eligibility criteria, received at least one dose of therapy, and underwent response evaluation other than at baseline. Patient deaths before tumor evaluation were considered assessable (progressive disease). Two patients were not assessable for tumor response because of adverse events before tumor assessment. During the main study, the median number of doses of rhuMAb HER2 per patient was nine (range, one to nine doses). The median number of doses of CDDP per patient was three, and the average administered dose was 74 mg/m^2. Three patients missed one dose of CDDP or received dose reduction as dictated by the study guidelines. Nineteen patients (49%) entered the maintenance phase protocol, in

Table 3. Studies of Single-Agent Cisplatin in Patients With Previously Treated Metastatic Breast Cancer

Investigator	Dosage	No.	CR	PR	Response Duration	Nephrotoxicity* (%)	Neurotoxicity†	Gastrointestinal Toxicity	Leukopenia‡ (%)	Thrombocytopenia
Bull et al[38] (1978)	70 mg/m² every 3 weeks	16	0	2	21-85 days	8	36% Hearing loss	97% Severe nausea	44	47% < 50,000/μL > 7 days
Samal et al[39] (1978)	15 mg/m²/d × 5 every 4 weeks or 120 mg/m² every 4 weeks	23	0	0	NA	0	9% Tinnitus 4% Ataxia/lethargy	70% Nausea and vomiting	29-50	50%-57% < 100,000/μL
Yap et al[35] (1978)	100 mg/m² every 3-4 weeks or 20 mg/m² × 5 every 4 weeks	26	0	0	NA	21-33	8% Tinnitus 4% Hearing loss	17%-57% Moderate to severe nausea and vomiting	NR	NR
Ostrow et al[36] (1980)	100 mg/m² every 3-4 weeks	17	1	0	3 months	35	29% Hearing loss 6% Optic neuritis 6% Peripheral neuropathy	100% Nausea and vomiting, frequently severe	33	6% < 20,000/μL
Forastiere et al[37] (1982)§	60 mg/m² every 3 weeks or 120 mg/m² every 3-4 weeks	37	0	5	1-6 months	13.5	66% Tinnitus 33% Hearing loss (5% tinnitus/hearing loss with 60 mg/m²)	100% Nausea nd vomiting	3-7	1%-2% < 50,000/μL
Total		119	1	7	21 days-6 months	0-35	5%-66% Tinnitus/hearing loss 0%-6% Peripheral neuropathy	17%-97% Nausea and vomiting frequently severe	3-50	1%-47% < 50,000/μL
Overall response rate 7% (95% CI, 2 to 11)										

Abbreviations: CR, complete response; PR, partial response; NA, not available; NR, not reported.
*Creatinine level > 2 mg/dL.
†High-frequency hearing loss > 20 dB.
‡WBC count < 3,000/μL.
§All 5 responses in this study occurred in the 120-mg/m² arm.

which the median number of rhuMAb HER2 doses per patient was 17 (range, three to 75 doses), and the median number of CDDP doses per patient was four (range, one to 10 doses), with an average CDDP dose of 65 mg/m². Ten patients required dose reductions of CDDP during the maintenance phase. The maximum number of rhuMAb HER2 doses received by any patient was 84.

Of the 37 patients assessable for tumor response, eight patients had a partial response documented during the main study, and one additional patient had a partial response that occurred during the maintenance phase. There were no complete responses in this study. Three patients met the criteria for minor response and six patients had stable disease during the main study. Disease progression was observed in 19 patients. The overall objective response rate among assessable patients (main study plus maintenance phase) was 24% (nine of 37 patients), all of whom had partial responses (95% confidence interval [CI], 12.4 to 41.6). The overall response rate for all patients (intent-to-treat population) was 23% (nine of 39 patients; 95% CI, 11.7 to 39.7). The sites of response were lung (n = 5), lymph node (n = 2), chest wall/skin (n = 6), and liver (n = 1). The median response duration was 5.3 months (range, 1.6 to 18 months). The characteristics of the responders are listed in Table 5. Pretreatment clinical variables were evaluated to determine if any variable was predictive of clinical response. Neither age, KPS, body weight, degree of HER2/*neu* overexpression, prior treatment, number or sites of metastases, hormone receptor status, or pretreatment shed HER2/*neu* ECD serum concentration correlated with clinical response. Furthermore, we found no association between fever during the rhuMAb HER2 loading dose and clinical response, nor did febrile reaction to rhuMAb HER2 corre-

Table 4. Response Data for Main Study Plus Maintenance Phase Among 37 Assessable Patients

Response	No. of Patients	%
Complete response	0	0
Partial response	9	24.3
Overall response	9	24.3
Minor response	3	8
Stable disease	6	16
Disease progression	19	51.3

Table 5. Characteristics of Patients Who Responded to CDDP Plus rhuMAb HER2

Patient Age (years)	No. of Prior Chemotherapeutic Regimens for Metastatic Disease	HER2 Expression	Site(s) of Objective Response	Maximum Shed HER2/*neu* ECD (μg/mL)	Response Duration (months)	Time to Tumor Progression (months)
39	2	3+	Chest wall, supraclavicular	0.201	7.7	10.0
63	1	3+	Chest wall	0.056	5.3	7.5
45	2	2+	Lung	0.019	4.0	6.7
39	5	3+	Liver	2.21	1.6	4.4
68	2	3+	Lung	0.048	9.4	11.5
41	1	2+	Lung, supraclavicular	0.004	2.7	8.4
37	1	3+	Chest wall, lung	0.004	6.7	9.1
50	1	3+	Chest wall	None detected	18	20.5
43	0	3+	Chest wall, lung	0.062	4.7	7.0

NOTE. All nine patients had objective partial responses.

late with pretreatment shed HER2/*neu* ECD concentration in serum.

rhuMAb HER2 Pharmacokinetics

To determine the effect of concomitant CDDP administration on rhuMAb HER2 pharmacokinetics, we compared the mean pharmacokinetic parameters measured in the current study with those in patients from a previously reported phase II clinical trial of rhuMAb HER2 alone in patients with HER2/*neu*-overexpressing advanced breast cancer (Table 6).[25] These data show that there is no significant difference in rhuMAb HER2 pharmacokinetics with the coadministration of CDDP when compared with treatment with antibody alone.

In addition, there was an inverse relationship between rhuMAb HER2 serum half-life and serum shed HER2 ECD of 0.5 μg/mL or greater. Indeed, patients with any measurable shed HER2/*neu* ECD serum level, compared with patients without measurable circulating ECD, had lower mean trough rhuMAb HER2 concentrations (18.7 *v* 43.6 μg/mL; $P = .0001$) across all time points (n = 443 observations: Fig 1). Among the subset of patients with measurable shed HER2/*neu* ECD levels, an inverse log-linear relationship between rhuMAb HER2 trough and shed HER2/*neu* ECD serum levels ($R = 0.79$; $P = .0001$) was seen (Fig 2). Although shed HER2/*neu* ECD may interfere with the quantitation of rhuMAb HER2, significant loss of quantitation of trough rhuMAb HER2 concentration was not observed unless the ratio of rhuMAb HER2 to shed HER2/*neu* ECD concentration was less than 10:1. Therefore, the observed relationship between rhuMAb HER2 serum concentration and serum shed HER2/*neu* ECD cannot be explained solely on the basis of assay interference.

*Correlation of Shed HER2/*neu *ECD Levels and Clinical Response*

The relationship between the maximum serum shed HER2/*neu* ECD concentration and level of HER2/*neu* expression (2 + *v* 3 +), as well as disease burden (number of metastatic sites), is shown in Fig 3. These data suggest a relationship between the maximum observed shed HER2/*neu* serum concentration with both degree of HER2/*neu* overexpression and disease burden, although these correlations did not reach statistical significance, perhaps because of the small numbers of patients in each of these subgroups. Clinical pretreatment variables, which included level of HER2/*neu* overexpression, number of metastatic sites, and serum shed HER2/*neu* ECD, were not predictive of clinical outcome; however, the difference in serum shed HER2/*neu* ECD concentration (day 70 posttreatment *v* day 0 pretreatment) was significantly associated with clinical outcome (disease progression *v* stable or responsive disease; $P = .008$, Fisher's exact test). Patients with disease progression showed a significant increase in serum shed HER2/*neu* ECD over

Table 6. Mean Pharmacokinetic Parameters

Study	Half-Life (days)	Shed HER-2 ECD (μg/mL)	Max Cpeak (μg/mL)	Max Ctrough (μg/mL)	Mean Css (4 μg/mL)	No. of Patients Max Ctrough < 10 μg/mL*
rhuMAb HER2 alone	9.2 ± 5.3 (n = 39)	< 0.5	113 ± 35 (n = 46)	54 ± 32 (n = 45)	52.3 ± 23.6 (n = 45)	7/40
	2.9 ± 3.2 (n = 6)	≥ 0.5				
rhuMAb HER2 + CDDP	11.0 ± 4.4 (n = 30)	< 0.5	121 ± 84 (n = 37)	85 ± 18.1 (n = 37)	50.1 ± 21.6 (n = 37)	12/37
	4.0 ± 2.6 (n = 7)	≥ 0.5				

Abbreviations: Max Cpeak, maximum concentration peak; Max Ctrough, maximum concentration trough; Css, mean concentration steady state.
*Number of patients with at least one minimum rhuMAb HER2 through concentration less than 10 μg/mL.

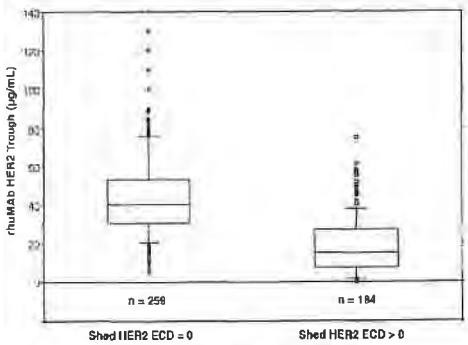

Fig 1. Comparison of serum $(rhuMAb\ HER2)_{trough}$ for all patients at all time points. Patients with detectable levels of shed HER2/*neu* ECD had lower mean $(rhuMAb\ HER2)_{trough}$ (18.7 µg/mL *v* 43.6 µg/mL, $P = .0001$) compared with patients without detectable serum shed HER2/*neu* ECD.

time (paired two-tail *t* test, $P = .0006$; Fig 4A). Patients with stable or responsive disease had a significant decrease in serum HER2/*neu* ECD after CDDP plus rhuMAb HER2 therapy ($P = .004$; Fig 4B); however, in these patients, a decrease in serum shed HER2/*neu* ECD concentration was not sufficient to discriminate between patients with stable disease and those with objective clinical responses, because five of six patients with stable disease had a decrease in shed HER2/*neu* ECD that was not associated with a measurable objective clinical response.

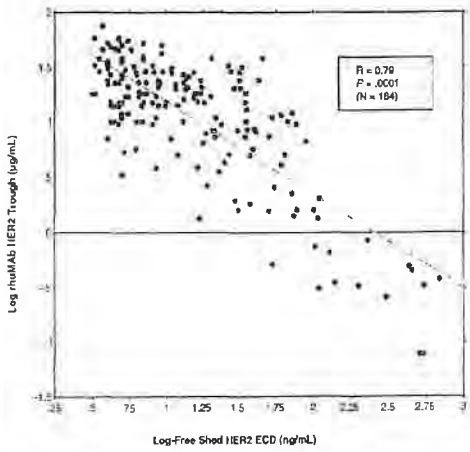

Fig 2. Measurement of serum trough rhuMAb HER2 concentration among the subset of patients with detectable levels of shed HER2/*neu* ECD. These data demonstrate an inverse relationship between serum shed HER2/*neu* ECD and rhuMAb HER2 trough concentration ($P = .0001$).

Human Antihumanized Antibody Responses

Seven hundred forty-eight doses of rhuMAb HER2 were administered in this study. There have been no cases in which measurable antibodies to rhuMAb HER2 were detected despite repeated weekly IV exposure, in some cases for many months. In addition, there were no reports of severe allergic reactions, such as bronchospasm, hypotension, urticaria, or eosinophilia after rhuMAb HER2 administration.

DISCUSSION

Therapeutic antitumor antibodies have long been proposed as an attractive approach to cancer therapy. However, despite many years of research, this approach to cancer therapy has not gained wide use in clinical practice for many reasons, which include (1) the lack of antitumor efficacy of antibodies in human clinical trials, (2) the lack of antibody specificity for tumor targets, (3) the induction of immune responses against nonhuman antibodies that preclude the use of multiple doses, and (4) the technical limitations to large-scale production and purification of monoclonal antibodies. Amplification and overexpression of the HER2/*neu* proto-oncogene occurs in 25% to 30% of breast cancers that provide a tumor-selective target for antibodies directed against $p185^{HER2/neu}$. Previous studies showed that a murine monoclonal antibody, 4D5, directed against the ECD of the HER2/*neu* protein, has growth inhibitory effects on malignant cells that overexpress this receptor.[26] This anti-HER2 antibody has been humanized so that it is less capable of eliciting either human antimurine or antihumanized antibody responses. Humanized antibodies may also be capable of affecting antitumor immune responses. We have previously shown the clinical efficacy of rhuMAb HER2 alone in a phase II clinical study,[25] and the combination of rhuMAb HER2 with CDDP had significant antitumor efficacy against HER2/*neu*-overexpressing breast cancers in a phase I clinical trial.

The mechanism of action of CDDP involves the formation of an equated cisplatin species that can react bifunctionally with DNA, which results in inter- and intrastrand cisplatin-DNA adducts that lead to inhibition of DNA synthesis.[27-30] One mechanism of resistance to CDDP involves removal of cisplatin-DNA adducts through DNA excision-repair processes.[31,32] Experimental data suggest that this DNA repair activity can be significantly decreased by the binding of ligands (EGF) to or antibodies against cell-surface type I receptor tyrosine kinases, such as the EGFR or $p185^{HER2/neu}$.[19,33,34] This phenomenon translates into a two-log increase in CDDP killing of cells that is specific to those cells that overexpress the HER2 receptor.[19] In preclinical in

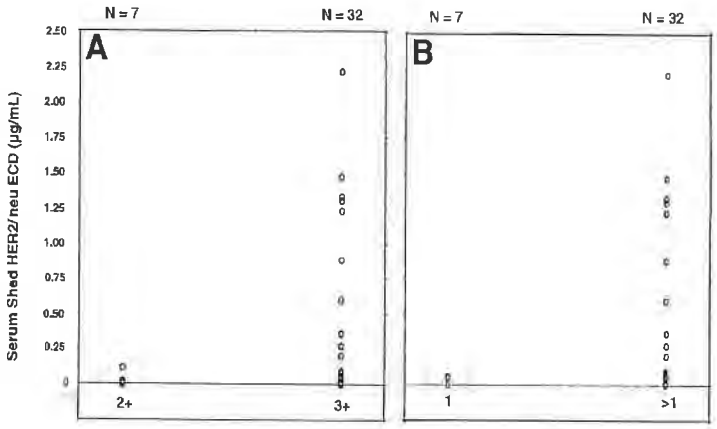

Fig 3. Maximum total serum shed HER2/*neu* ECD levels according to level of HER2/*neu* overexpression (A) and the number of metastatic sites (B). These data suggest a possible association between shed HER2/*neu* ECD, both with degree of HER2/*neu* overexpression and with number of metastatic sites.

vivo breast xenograft models, this resulted in the cure of some animals.

To assess the response of previously treated patients with metastatic breast cancer to CDDP alone, we have reviewed the literature and compared that experience to the results in this study. To date, there have been five reports on the use of CDDP as a single agent in previously treated patients with advanced breast cancer.[35-39] These studies are listed in Table 3. Of 119 patients treated with a variety of CDDP administration schedules, only one complete response and seven partial responses were seen for an overall objective response rate of 7% (95% CI, 2 to 11). The duration of responses ranged from 21 days to 6 months, and no responses were seen in patients treated with doses of less than 70 mg/m^2.

In the current trial, we administered rhuMAb HER2 in combination with CDDP to 39 patients with advanced breast cancer who had disease progression while receiving chemotherapy before study entry. We observed nine objective partial responses (eight during the main study and one during the maintenance program) among 37 patients who were assessable for response for an overall objective response rate of 24% (95% CI, 12.4 to 41.6). The sites of response were diverse (lung, liver, lymph node, and chest wall/skin), and the response duration ranged from 1.6 to 18 months, with a median response duration of 5 3 months. Clinical response data in a trial run in parallel to this study, which used weekly IV administration of rhuMAb HER2 as a single agent in patients with metastatic breast cancer with

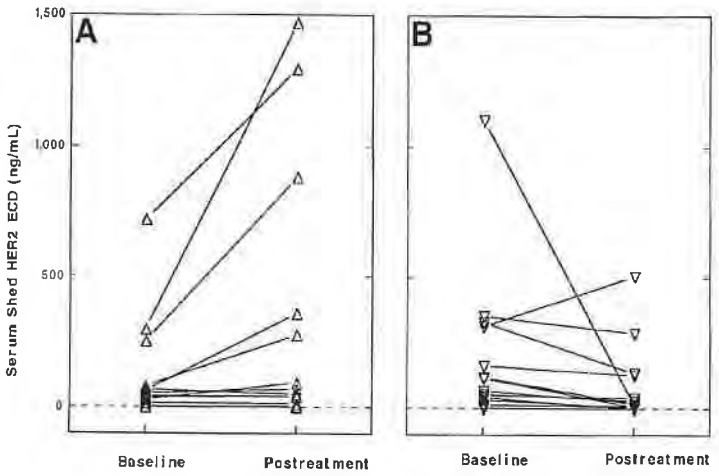

Fig 4. (A) Shed HER2/*neu* ECD at baseline (pretreatment) and day 70 (posttreatment) for patients with disease progression demonstrating a significant increase in shed HER2/*neu* ECD ($P = .006$). (B) For patients with stable or responsive disease, there was a significant decrease in shed HER2/*neu* ECD ($P = .004$).

the same dose and dosing schedule used in the current study, showed an objective response rate of 12% (95% CI, 4 to 26), with mild to moderate fever and chills reported as the predominate toxicity.[25]

There are at least four potential explanations for the observed objective clinical responses seen in the current trial. First, some of the patients may have responded to CDDP alone; however, the response rate observed in the current study is substantially higher than those reported previously for single-agent CDDP, especially considering the relatively modest CDDP dose administered and the requirement for demonstration of resistance to ongoing treatment with cytotoxic drugs in patients enrolled onto this study. Furthermore, overexpression of HER2/*neu* has been associated with CDDP resistance in previous studies.[40-43] It is, therefore, less likely that the observed objective response rate resulted from a unique sensitivity to CDDP among patients with HER2/*neu*-overexpressing breast cancer. Second, it is also possible that some of the clinical responses observed were caused by the action of rhuMAb HER2 alone. However, in the previously published parallel trial, the objective response rate to rhuMAb HER2 alone was approximately one half (12%) that seen in this study. On binding to $p185^{HER2/neu}$, rhuMAb HER2 causes downregulation of $p185^{HER2/neu}$ expression and disrupts the formation of HER2/HER3 and HER2/HER4 heterodimers. These events are accompanied by a decrease in cell proliferation.[45,46] Because this effect is not cytotoxic, administration of rhuMAb HER2 alone may not be expected to result in objective clinical responses; however, the rhuMAb HER2 antibody used in this study has been engineered to elicit antibody-dependent cellular cytotoxicity by host natural-killer cells, macrophages, and neutrophils, and this may have resulted in some of the observed objective clinical responses.[47] A third mechanism to account for clinical responses with CDDP plus rhuMAb HER2 might simply be the additive effects from each agent alone. Finally, it is possible that downregulation and/or inactivation of HER2/*neu* receptor activity by rhuMAb HER2 may result in increased response to CDDP by decreasing cellular repair of cisplatin-induced DNA adducts, thus effecting the clinical responses seen with this treatment. This latter hypothesis is supported by data that indicate such a mechanism is operative in HER2/*neu*-overexpressing breast cancer cells when treated with anti-HER2/*neu* antibodies.[19]

The toxicity observed in this study essentially parallels that reported previously for single-agent CDDP in a similar patient population in which treatment was frequently accompanied by gastrointestinal, renal, neurologic, and hematologic toxicity (Table 3). There was no evidence that rhuMAb HER2 enhanced the toxicity of CDDP. Moreover, the toxicity data from the current clinical trial are consistent with preclinical data that indicate that the enhanced cytotoxicity of CDDP mediated by rhuMAb HER2 is restricted to cells that contain HER2/*neu* amplification/overexpression.[19] This clinical observation validates the specificity proposed in the REC model and shows that the increased CDDP killing effects are unique to cells that contain the HER2/*neu* alteration. The only observed toxicity unique to rhuMAb HER2 infusion was mild to moderate fever and chills in a minority of patients, usually during or just after the rhuMAb HER2 loading dose. This phenomenon was also noted in earlier phase I and II clinical trials with this drug.[25] We found no correlation between febrile reaction to rhuMAb HER2 and serum HER2/*neu* ECD, nor was there any correlation between fever and clinical response.

Coadministration of CDDP with rhuMAb HER2 had no measurable effect on the mean pharmacokinetic parameters of IV administered rhuMAb HER2 compared with values obtained with weekly IV administration of rhuMAb HER2 as a single agent (Table 6). Furthermore, in light of the distinct mechanisms of elimination for these two agents (clearance of IgG through the reticuloendothelial system and predominantly renal elimination of CDDP), as well as the absence of any increase in toxicity attributable to CDDP, we believe it is unlikely that rhuMAb HER2 impacts significantly on CDDP pharmacokinetics, although the pharmacology of CDDP was not directly evaluated in this study.

Twenty-four of 37 patients (65%) who had measurements of serum shed HER2/*neu* ECD performed before treatment had detectable serum levels in a sensitive ELISA assay. As noted in the previous study of single-agent rhuMAb HER2, serum HER2/*neu* ECD was associated with rhuMAb HER2 pharmacokinetics (Table 6; Figs 1 and 2). There are three potential mechanisms that may account for this observation. First, rhuMAb HER2 may bind to HER2/*neu* ECD, which results in an antigen/antibody complex formation that results in a more rapid clearance of rhuMAb HER2 from serum. Second, the serum shed HER2/*neu* ECD may merely be a marker of high disease burden that reflects an increased rhuMAb HER2 binding by tumor cells that results in shortened rhuMAb HER2 serum half-life. This hypothesis is supported by the observation of a significant inverse correlation between serum rhuMAb HER2 concentration and xenograft volume, which is independent of serum shed HER2/*neu* ECD in athymic mice that bear HER2/*neu*-overexpressing breast carcinoma xenografts.[48] The third possibility is that there may have been interference in the assay used to measure serum rhuMAb HER2 caused by the presence of serum HER2/*neu* ECD, which resulted in

artifactually low rhuMAb HER2 measurements in the patients with high levels of shed HER2/*neu* ECD. Significant loss of quantitation of rhuMAb HER2, however, is not observed unless the ratio of serum rhuMAb HER2 to shed HER2/*neu* ECD is less than 10:1. This occurred in only a small number of samples in this study. The extent to which any or all of these mechanisms account for the observed pharmacokinetic relationships between shed HER2/*neu* ECD and rhuMAb HER2 concentrations cannot be determined from the current data set and requires further study. Pretreatment serum HER2/*neu* ECD levels did not correlate with clinical response to CDDP plus rhuMAb HER2 therapy. This apparent lack of correlation between pretreatment shed HER2/*neu* ECD and clinical response may be caused by lack of statistical power to detect such a difference, because there were only 24 patients with measurable levels of shed HER2/*neu* ECD before treatment. An important aspect of the current data is the demonstration that measurable serum HER2/*neu* ECD does not preclude clinical response to CDDP plus rhuMAb HER2 because eight of the nine responders had measurable shed HER2/*neu* ECD during the course of this study, which included one patient with a very high level (2.21 μg/mL).

We were unable to identify any clinical pretreatment variables that correlated with clinical outcome, which included age, KPS, body weight, degree of HER2/*neu* overexpression, prior treatment, number or sites of metastases, hormone-receptor status, or pretreatment shed HER2/*neu* ECD serum concentration. Although pretreatment serum HER2/*neu* ECD did not correlate with response to rhuMAb HER2 plus CDDP, we found that trends in shed HER2/*neu* ECD over time did correlate with overall clinical outcome, specifically with disease progression. Nine of 13 patients with progressive disease showed an increase in shed HER2/*neu* ECD during treatment, and six of six patients with objective partial response and measurable serum levels showed a decrease in shed HER2/*neu* ECD after treatment with the combination. A significant decrease in shed HER2/*neu* ECD, however, was also evident in five of six patients with stable disease, as well as in one patient with disease progression, which indicated a decrease in HER2/*neu* ECD concentration alone was not sufficient to discriminate between stable disease and objective clinical response in this small data set. These data further suggest that serial measurement of serum HER2/*neu* ECD may have limited use as a predictive factor for objective clinical response, but it may be a useful marker of treatment failure in patients who continue to show increases in serum HER2/*neu* ECD during treatment.

This is the first report of a therapeutic strategy that uses a combination of chemotherapy plus anti-HER2/*neu* antibody. The REC approach shows activity in patients with breast cancer with prior clinical resistance to chemotherapy, and the objective response rate of approximately 24% after treatment with CDDP plus rhuMAb HER2 holds promise that the REC strategy that used rhuMAb HER2 with cytotoxic drugs may be a viable therapeutic approach that warrants further study. We have tested the combination of rhuMAb HER2 with other chemotherapeutic agents known to be active in breast cancer in preclinical models both in vitro and in vivo. These studies showed evidence for an additive or synergistic efficacy of rhuMAb HER2 in combination with alkylating agents, epipodophyllotoxins, taxanes, vinca alkaloids, anthracyclines, and some antimetabolites[49,50] (R.J. Pietras et al, unpublished data, May 1997). These preclinical studies formed the basis for an ongoing phase III clinical trial that evaluates combinations of rhuMAb HER2 with doxorubicin plus cyclophosphamide or paclitaxel in previously untreated patients with HER2/*neu*-overexpressing metastatic breast cancer. The results from this trial will shed further light on the clinical utility of the combined HER2/*neu* antibody/chemotherapy approach.

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EXHIBIT 6

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE
----------x
GENENTECH, INC. and CITY OF HOPE,
Plaintiffs,
-against- Case No.
-cv-924-CFC 1:18
AMGEN INC.,
Defendant.
----------x

DEPOSITION OF LARRY NORTON, M.D.
New York, New York
June 17, 2019

Reported By:
ERIC J. FINZ

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HOWARD BRODSKY, Videographer

THE VIDEOGRAPHER: Good morning. Here begins the video recorded deposition of Dr. Larry Norton, M.D., taken by the defendant, in the matter of Genentech Incorporated and City of Hope, the plaintiffs, versus Amgen Incorporated, defendant, Civil Action No. 1:18-cv-924-CFC, in the United States District Court, for the District of Delaware. 10:40:51 10:40:52 10:40:54 10:40:57 10:40:59 10:41:00 10:41:03 10:41:06 10:41:09 10:41:13 10:41:15

This deposition is proceeding at Memorial Sloan Kettering Cancer Center, Breast and Imaging Center, 300 East 66th Street, 9th floor, New York, New York 10065, on Monday, June 17, 2019, at approximately 10:41. 10:41:16 10:41:19 10:41:23 10:41:25 10:41:30 10:41:34 10:41:37

My name is Howard Brodsky and I am the legal video specialist in association with Veritext Legal Solutions, with offices located in New York, New York. The court reporter is Eric Finz, in association with Vertex. 10:41:39 10:41:42 10:41:44 10:41:47 10:41:48 10:41:51 10:41:54

Will counsel please state their appearances for the record. 10:41:57 10:41:58

MS. ARORA: Priya Arora of Cooley representing defendant Amgen. With me is my colleague Michelle Rhyu of Cooley. 10:42:00 10:42:02 10:42:04 10:42:05

MS. PIROZZOLO: Lisa Pirozzolo from WilmerHale, representing the plaintiffs. And with me is my colleague Stephanie Lin. 10:42:07 10:42:09 10:42:12 10:42:14

MR. KRASNOO: Ethan Krasnoo from Reavis Page Jump, representing the witness and Memorial Sloan Kettering. And my colleague is on the telephone, Charlene Choi, in-house counsel at Memorial Sloan Kettering. 10:42:16 10:42:18 10:42:21 10:42:25 10:42:26 10:42:27 10:42:31

THE VIDEOGRAPHER: Thank you very much. 10:42:31 10:42:32

MS. FETSCH: Good morning. 10:42:35

THE VIDEOGRAPHER: Will the court reporter please swear in the witness. 10:42:36 10:42:37 10:42:38

MS. PIROZZOLO: There is one other lawyer on the phone. 10:42:38 10:42:39

THE VIDEOGRAPHER: I'm sorry. Will other remote counsel please state their appearances for the record. 10:42:40 10:42:42 10:42:45 10:42:48

MS. FETSCH: Katrina Fetsch from White & Case, representing defendant Samsung Bioepis. 10:42:52 10:42:52 10:42:54

MR. KRASNOO: And I'll just say that another colleague, in-house counsel from Memorial Sloan Kettering, Rebecca Kim-Kriesberg, may also be joining us shortly in person. 10:42:59 10:43:00 10:43:01 10:43:05 10:43:06 10:43:09

THE VIDEOGRAPHER: Thank you very much. 10:43:10 10:43:10

Will the court reporter please swear in the witness. 10:43:11 10:43:12

LARRY NORTON, M.D., having been first duly sworn by the Notary Public (Eric J. Finz), was examined and testified as follows: 10:43:13 10:43:13 10:43:13

EXAMINATION BY 10:43:20

MS. ARORA: 10:43:20

Q. Good morning. Thank you very much for taking the time to be with us 10:43:21 10:43:26

Q. So by late '90s you think you would have administered the three-weekly Herceptin? 12:04:32 12:04:35 12:04:37

MR. KRASNOO: Objection. 12:04:38

MS. PIROZZOLO: Objection. 12:04:38

A. It always depends on whether we were giving a drug along with it. If the patient is coming in for a weekly injection anyway, then we may want to go with the weekly. If the patient is just getting trastuzumab, when chemotherapy finishes, to finish a year of trastuzumab it's more convenient for the patient to get every three weeks. That's the way we would use it in that particular setting. 12:04:38 12:04:40 12:04:42 12:04:44 12:04:46 12:04:49 12:04:52 12:04:54 12:04:56 12:04:58

Q. So just with the factor of patient convenience, an oncologist would be willing to give the three-weekly dosage of Herceptin and a chemotherapy in the interest of patient convenience? 12:04:59 12:05:01 12:05:03 12:05:05 12:05:08

MR. KRASNOO: Objection. 12:05:10

MS. PIROZZOLO: Objection. 12:05:11

Q. By late '90s? 12:05:11

MR. KRASNOO: Objection. 12:05:14

MS. PIROZZOLO: Objection. 12:05:15

A. I don't think it was an obvious. I think it was an individual decision to be made for the individual patient's life and individual patient's comfort. I mean, you know, we had established, at some period of time we had established the pharmacokinetics of trastuzumab was such that you could achieve the blood levels that you wanted, whether you gave it every week or you gave it every three weeks. 12:05:15 12:05:17 12:05:19 12:05:20 12:05:22 12:05:24 12:05:26 12:05:28 12:05:29 12:05:32 12:05:33

Q. But the factor driving that would be patient convenience; right? 12:05:34 12:05:36

A. That would be one factor, yes. It would be a factor, yes. 12:05:37 12:05:39

Q. Okay. So just if you can go back to the 1998 time frame when the drug was actually approved. Did you make any recommendation regarding testing it for three-weekly Herceptin dosage? 12:05:40 12:05:56 12:05:59 12:06:06 12:06:09

A. Yes. 12:06:11

Q. And what kind of recommendation was that? 12:06:13 12:06:14

A. Well, I mean, I think it was -- and you've alluded to some of the 12:06:16 12:06:19

work already that had been done that suggested that, is that if we could achieve adequate levels to block HER2 in the majority of patients by giving it every three weeks, it would be valuable to establish that as an option for the treating oncologist. And therefore it should be studied. 12:06:21 12:06:23 12:06:24 12:06:27 12:06:29 12:06:32 12:06:33 12:06:35

Q. Do you remember discussing that with someone after that pivotal phase III trial? 12:06:37 12:06:37 12:06:40

MS. PIROZZOLO: Objection. 12:06:41

A. After the pivotal phase III trial? I remember discussing it all during that period. 12:06:42 12:06:45 12:06:47

Q. Oh, okay. 12:06:47

A. Since we were aware of the pharmacokinetics of the drug and the half-life and other factors. I remember having a lot of discussion about this. 12:06:48 12:06:48 12:06:49 12:06:51

Q. Do you remember who you discussed it with? 12:06:52 12:06:53

A. I remember -- I remember that it was an active area of discussion. And I do remember specifically having this 12:06:55 12:07:01 12:07:04

discussion with Steve Shak, at -- during the coffee break at an advisory board meeting, where it was during the coffee break and he asked me what I thought of it. And I thought that it was a reasonable thing to study, both on the basis of work that we had done previously, but also on the basis of the mathematical -- the mathematical analysis of the pharmacokinetics of the drug. I remember that very specifically. 12:07:07 12:07:12 12:07:15 12:07:18 12:07:21 12:07:25 12:07:27 12:07:29 12:07:32 12:07:35 12:07:38

Q. And just to be clear, we are talking about the three-weekly dosage of Herceptin; right? 12:07:38 12:07:38 12:07:40

A. Yeah, the transfer from weekly to every three weeks was something that was discussed. The mathematics suggested that it would be a reasonable thing to study. 12:07:40 12:07:42 12:07:45 12:07:48 12:07:49

Q. Do you know what advisory board meeting that was? 12:07:50 12:07:52

A. No. It was not in New York, that's all I remember. I don't remember what -- it's all a blur, I don't remember what city it was in. I remember where I 12:07:52 12:07:54 12:07:56 12:07:59

Q. And that's where you discussed the three-weekly Herceptin dosage? 12:26:56 12:26:57

A. Again, the exact timing is not something I can vouch for. 12:27:00 12:27:02

Q. That's fine. 12:27:03

A. But roughly during this exciting late '90s period that we were making these discoveries. 12:27:04 12:27:05 12:27:08

Q. So around December of '97, '98, was the time when you had that advisory board meeting that we had discussed earlier? 12:27:08 12:27:11 12:27:13 12:27:14

MS. PIROZZOLO: Objection. 12:27:16

A. Again, it's supposition on my part. I don't remember the dates. And I don't have any records of those dates, it's all lost in time. 12:27:16 12:27:18 12:27:20 12:27:22

Q. But it was something that was being discussed around this time. Right? 12:27:24 12:27:26

MS. PIROZZOLO: Objection. 12:27:28

MR. KRASNOO: Objection. 12:27:29

A. To the best of my recollection, during this period of time is when there was a discussion about could there possibly be a better schedule 12:27:30 12:27:33 12:27:36 12:27:38

of Herceptin, for sure. 12:27:41

Q. Okay. 12:27:42

A. And I do recall having a discussion with Steve where I thought it was very reasonable to go to a three week regimen for mathematical pharmacokinetic reasons. And in fact, I scribbled some numbers on a napkin, which has long been discarded I'm sure. So, and showed why it was rational to do a three-week schedule. 12:27:43 12:27:44 12:27:49 12:27:53 12:27:57 12:28:00 12:28:04 12:28:08 12:28:10

Q. So you remember noting down some information on a napkin, as you referred to? 12:28:10 12:28:12 12:28:15

A. Um-hum. I showed some calculations, some pharmacokinetic calculations. 12:28:15 12:28:18 12:28:20

Q. Okay. Do you know who Brian Leyland-Jones is? 12:28:21 12:28:23

A. Yes, a colleague of mine. 12:28:24

Q. Who is he? 12:28:25

A. He's a medical oncologist, clinical investigator. 12:28:26 12:28:28

Q. Does he specialize in pharmacokinetics? 12:28:29 12:28:30

A. Yes. We were all involved in the writing of the paper and the analysis of the results, absolutely. 12:31:47 12:31:49 12:31:51

Q. Okay. And there would have been discussions regarding dosing regimens, regarding mathematical calculations? 12:31:52 12:31:55 12:31:57 12:32:00

MR. KRASNOO: Objection. 12:32:02

A. It's inconceivable to me that if I was involved in the discussion that it wouldn't have talked about dosing regimens and mathematics, yes, because that's what I do. 12:32:02 12:32:05 12:32:06 12:32:08 12:32:10

Q. When you -- you said that there were some mathematical calculations that you presented to Steve Shak. 12:32:10 12:32:12 12:32:15

A. Um-hum. 12:32:18

Q. Can you describe what mathematical information that would have been? 12:32:19 12:32:21 12:32:22

MS. PIROZZOLO: Objection. 12:32:23

A. Yeah, I mean, very -- I mean, I remember very precisely. Is because paclitaxel is -- trastuzumab is a very interesting drug because it's half-life 12:32:24 12:32:27 12:32:29 12:32:32

is dependent on the doses administered, 12:32:34
right. And that's even in the package 12:32:36
insert, the FDA package insert. So if 12:32:38
you give a higher dose it lasts longer in 12:32:40
the blood basically. 12:32:43
So that by going to a higher 12:32:44
dose, you're going to have a blood level 12:32:46
that's going to last longer, all right, 12:32:48
which, you know, makes it rational to 12:32:49
think that an every three-week regimen at 12:32:51
a higher dose level is going to give you 12:32:54
adequate blood levels. 12:32:56
The question is what is, you 12:32:57
know, what is the actual mathematical 12:32:59
likelihood of that. And so understanding 12:33:02
the -- understanding the relationship 12:33:04
between dose and half-life can be plugged 12:33:06
into a first order kinetic equation. And 12:33:09
if you do those calculations, you could 12:33:13
show that the end of three weeks you 12:33:15
would have -- you should have an adequate 12:33:17
blood level compared to if you gave a 12:33:19
lower dose every week. 12:33:21
And just the way the math 12:33:22
works out, it's very linear, so that 2 12:33:24

milligrams every week and, you know, 6 milligrams every three weeks will get you in the ballpark of the appropriate blood level. That's just the way the math works out. And that's what I showed. 12:33:27 12:33:30 12:33:32 12:33:35 12:33:37

Q. And you explained that to Steve Shak? 12:33:38 12:33:39

A. Yeah, yeah. 12:33:40

Q. And what was his reaction? 12:33:41

MS. PIROZZOLO: Objection. 12:33:42

A. I do not remember him -- I don't remember his reaction. But this conversation was, you know, it was literally like Steve, it's probably okay to give it every three weeks because of blah, blah, blah, blah, blah, blah, and as you see it comes up to numbers. Just like that. Still one must test it, obviously. But from a kinetics point of view it's, you know, that would be -- it would be a rational thing to test. 12:33:43 12:33:45 12:33:46 12:33:48 12:33:51 12:33:53 12:33:56 12:33:58 12:34:03 12:34:05 12:34:08

Q. And was there something that he discussed about how they would approach testing it? 12:34:10 12:34:13 12:34:16

MR. KRASNOO: Objection. 12:34:18

C E R T I F I C A T E

STATE OF NEW YORK)
: ss.
COUNTY OF NEW YORK)

I, ERIC J. FINZ, a Shorthand Reporter and Notary Public within and for the State of New York, do hereby certify:

That LARRY NORTON, the witness whose deposition is hereinbefore set forth, was duly sworn by me and that such deposition is a true record of the testimony given by the witness.

I further certify that I am not related to any of the parties to this action by blood or marriage, and that I am in no way interested in the outcome of this matter.

IN WITNESS WHEREOF, I have hereunto set my hand this 20th day of June, 2019.

ERIC J. FINZ

EXHIBIT 7

ONCOLOGY

Oncology 1999;57(suppl 1):21–26

Optimizing Chemotherapy for Patients with Advanced Breast Cancer

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Key Words

Breast cancer · Metastatic · Combination chemotherapy · Clinical trials · Xeloda® (capecitabine)

Abstract

Chemotherapy is offered to almost all patients with metastatic breast cancer. Optimization of treatment has four major goals: (1) To improve access to chemotherapy. Orally active chemotherapy is an attractive option for those patients when access to hospital is limited by financial considerations, long journeys or patient reluctance. In the past, only alkylating agents (cyclophosphamide, chlorambucil, melphalan) could be administered orally. The activity (first- and second-line) of Xeloda® (capecitabine) with limited side effects and the development of oral vinorelbine and anthracyclines should improve access to chemotherapy and also concentrate further interest on treatment with long-term administration of cytotoxic agents. (2) To improve response rates and duration in first-line treatment. Response rates have been increased by the use of combinations of taxoids and anthracyclines and/or alkylating agents and/or fluoropyrimidines (>60–70% with complete remission in 10–15% of patients). There is increasing interest in sequential use of active agents or combinations at their optimal doses. Nevertheless, such 'induction regimen' fail to prolong response duration (rarely longer than 9–12 months). The use of less-toxic maintenance chemotherapy regimens increases response duration and disease-free survival. Such maintenance regimens could be used on an outpatient basis and will be further simplified by the availability of active oral agents such as the novel fluoropyrimidine Xeloda. (3) To increase cure rates. This can only be considered with first-line treatment in selected patients (long disease-free interval, minimal number of visceral sites and ability to tolerate high-dose chemotherapy). The completed studies with high-dose chemotherapy and hematopoietic stem cell support have, in fact, shown only a minimal effect on cure rates. Incorporation of very active agents such as taxoids and use of multicycle high-dose therapy may improve these results. (4) To offer alternative active regimens in second and subsequent metastatic progression. Taxoids, vinorelbine and, more recently, Xeloda all achieve a 20–40% response rate in these situations. The reintroduction of agents previously used for adjuvant or first-line therapy can also be considered.

Introduction

Although screening and early diagnosis as well as systemic adjuvant therapy are increasing disease-free and overall survival of patients with localized breast cancer [1, 2], 20–40% will ultimately experience metastatic progression of disease. Furthermore, 4–10% of patients already have metastatic breast cancer (MBC) at presentation [3]. Thus, it is crucial that treatment of patients with MBC is optimized in terms of both outcome and availability. Chemotherapy has a particularly important role as even patients who initially benefit from hormonal therapy will ultimately develop resistance and require chemotherapy.

Optimization of treatment for MBC has four major goals:

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- To allow patients better access to chemotherapy.
- To improve the response rates and progression-free survival (PFS) achieved by initial (first-line) chemotherapy [4].
- To improve cure rates. It has been shown consistently that 3–7% of patients with MBC experience long disease-free survival (DFS) and eventually cure. New approaches, including high-dose chemotherapy and/or the use of new active cytotoxic agents, are attempting to improve on these figures.
- To offer patients alternative active regimens once they fail first-line chemotherapy.

Obviously these approaches also aim to improve patients' quality of life (QoL), but they should also be cost-effective. Allowing more patients to receive chemotherapy has implications for healthcare budgets.

Improving Access to Chemotherapy

The use of chemotherapy in patients with breast cancer (in both adjuvant and metastatic settings) is increasing rapidly. However, chemotherapy is probably not offered equally to all patients. This is partly because of limited resources, but also because of personal choice of the patients who may be reluctant to receive chemotherapy either in hospital or even on an outpatient basis.

The increasing use of day-hospital and outpatient clinics already offers a number of patients better access to chemotherapy. The improvements achieved in symptomatic care in cancer patients (permanent central venous access, oral antiemetics, control of neutropenia and anemia with hematopoietic growth factors) have played a major role in shortening the duration of hospital-based treatments. At the same time, intravenous combinations of cytotoxics are most often given as short infusions every 3 weeks. However, transportation to and from the hospital is a major constraint, as the mean journey length may be one hour (personal data) and worsen some side effects, e.g. drug-induced emesis.

The development of home-care is still limited in Europe: general practitioners (GPs) have limited practical experience in treating breast cancer patients [5]. The development in some urban areas of home-care networks involving oncologist(s), GPs and trained nurses has already permitted some chemotherapy regimens, including long-lasting intravenous infusions, to be given at home.

A major advance in this area is the development of orally active cytotoxics and cytotoxic regimens with alkylating agents such as cyclophosphamide, melphalan, chlorambucil and hexamethylmelamine. A number of oral fluoropyrimidines are in development. Xeloda (capecitabine), an oral fluoropyrimidine, is active in anthracycline- and taxoid-resistant breast cancer [6–8] and allows the design of oral combination regimens, which need further investigation. Other oral agents are currently being studied including vinorelbine [9, 10] and idarubicin [11], while there is active research into orally active taxoids, topoisomerase I and II inhibitors and other fluoropyrimidine derivatives.

Thus, one can be confident that access to cancer care will improve rapidly as the range of drugs develops and this will contribute to the optimization of chemotherapy for breast cancer in the future.

Chemotherapy for First Metastatic Progression

The use of chemotherapy for first metastatic progression aims either to achieve a cure or, in a palliative setting, to improve response rate and PFS. The choice depends on evaluation of prognostic factors as summarized in table 1 [12].

Conventional-Dose First-Line Chemotherapy to Improve Response Rate and Survival

Conventional-dose regimens remain the most widely used and validated approach in first-line chemotherapy. However, increasing use of adjuvant chemotherapy has complicated initial chemotherapy for metastatic disease. Patients who have received anthracycline-based adjuvant treatment may have reduced chemosensitivity to these agents at metastatic relapse.

The activity of currently available cytotoxic agents in terms of response rates is shown in table 2. Doxorubicin, epirubicin, paclitaxel and docetaxel are the most active agents. While activity (and hematologic toxicity) of paclitaxel appears similar to that of doxorubicin [13], the activity of docetaxel was superior [14]. Interestingly, Xeloda has activity at least equivalent to that of CMF when used first-line [7] and somewhat superior to paclitaxel when used as a single agent in second-line chemotherapy, even in patients who have failed anthracyclines [8].

Conventional chemotherapy regimens consistently achieve response rates of 43–64% and PFS from 5–11 months (table 3). A number of trials are attempting to improve those results, mostly by defining new and more active combinations of agents. Combination of the most active agents, anthracyclines and taxoids, is a promising approach in patients who have not received previous

Table 1. Prognostic parameters during first metastatic progression of breast cancer [12]

Parameter	Good prognostic factors	Poor prognostic factors
Disease-free interval	>12–18 months	<12–18 months
Previous adjuvant chemotherapy	No anthracyclines	Anthracycline-based treatment
Visceral sites	Absent	Present
Number of metastatic sites	Limited (<3)	Multiple
Performance status	0–1	2
Lactic dehydrogenase (LDH)	Normal	Elevated

Table 2. Response rates with active cytotoxic agents in metastatic breast cancer

Agent	Dose (mg/m²)	RR (first-line), %	RR (second-line), %
Doxorubicin	50–75	38–52	15–29
Epirubicin	50–120	32–48	15–25
Mitoxantrone	12–14	28–40	15–25
Pirarubicin	45	30–50	>20
Paclitaxel	175	30–45	20–35
Docetaxel	100	50–65	40–55
Vinorelbine	30/7d	40–60	>20
Cyclophosphamide	400–1,000	34	22
Fluorouracil	600	34	15
Xeloda	2,510/dx14	25[1]	36
5-FU[2]	250/d	20–40	20–25
Methotrexate	60	34	–
Ifosfamide	4–6 g/m²	30–40	–
Altretamine	150/dx7–1	27	2
Melphalan	2–6/dx5–30	23	4

RR = Response rate.
[1] Increased to 30% (F. Hoffmann-La Roche Ltd, data on file).
[2] Administered by continuous i.v. infusion.

anthracyclines during adjuvant therapy. Paclitaxel/anthracycline combinations [15] have been used in a variety of doses and schedules with simultaneous or sequential administration. High overall response rates (>60%) and complete response (CR) rates (>5%) were achieved at the expense of frequent neutropenia (>70%) and increased cardiotoxicity with doxorubicin. Similarly, docetaxel/anthracycline combinations in either simultaneous or sequential regimens have been studied [16]; they achieve high response rates (>70%) with neutropenia as a dose-limiting toxicity. These combinations can be used rarely for more than 6–8 cycles and it is not clear that they prolong response duration. Three drug regimens incorporating fluoropyrimidines or cyclophosphamide are currently being studied. Sequential regimens (taxoids followed by doxorubicin/epirubicin) are also being investigated. For those patients who have received anthracycline-based adjuvant therapy previously, new treatment options are being studied.

Table 3. Activity of some conventional first-line chemotherapy regimens in advanced breast cancer

Regimen	Adjuvant chemotherapy, %	RR %	PFS months
CMF and related	0	45–62	5–8
FAC and related	0	43–60	8–11
FU-VNB	63	64	10

RR = Response rate; PFS = progression-free survival; CMF = cyclophosphamide/methotrexate/5-FU; FAC = 5-FU/doxorubicin/cyclophosphamide; FU-VNB = 5-FU/vinorelbine.

Maintenance Chemotherapy

Although classical and newer induction regimens regularly achieve high response rates, they have not been associated with major changes in response duration. Emerging evidence argues that prolonging the duration of chemo-

therapy increases response duration and PFS up to 18 months compared with only 9–12 months when no maintenance treatment is used [17–22], although there is no clear impact on OS. Such maintenance chemotherapy should not use agents with cumulative toxicity and should use combinations associated with minimal toxicity and constraints.

When designing maintenance regimens, anthracyclines and taxoids are often avoided and reliance placed upon 5-FU-based regimens such as CMF (cyclophosphamide/methotrexate/5-FU), although some groups have used FEC (5-FU/epirubicin/cyclophosphamide) for 18 months, which was made possible by the availability of cardioprotective agents. The availability of orally active fluoropyrimidines, such as Xeloda, is attractive. They may replace 5-FU and could lead to the design of completely oral regimens (Xeloda/cyclophosphamide and/or other oral alkylating agents).

First-Line Chemotherapy with Curative Intent

The use of potentially curative first-line chemotherapy became a possibility when the initial results from studies of high-dose chemotherapy with autologous stem cell rescue (HDC/ASCR) suggested that this approach could increase cure rate in patients with MBC. HDC/ASCR is based on the hypothesis that high-dose chemotherapy will overcome drug resistance and eradicate metastatic disease, thus increasing the proportion of women who are 'cured'. However, with more data available, particularly from randomized studies [23], it has became clear that firstly, the impact of high-dose chemotherapy will be less than initially expected, and secondly, careful selection of the patients who will benefit from such an approach is needed.

In one completed randomized study, HDC/ASCR was given as consolidation therapy to patients achieving a major response with induction chemotherapy and was compared with patients given no maintenance treatment [24]. In a second study, a tandem intensification regimen of cyclophosphamide, mitoxantrone and etoposide was compared with a standard-dose, non-conventional induction chemotherapy regimen [25]. In both trials, high-dose chemotherapy achieved longer DFS (9 months), but this was not clearly superior to DFS achieved with validated standard combination regimens. However, only those patients with favorable prognostic parameters appeared to benefit from high-dose chemotherapy [26].

A number of high-dose strategies are currently being investigated including tandem intensification as consolidation therapy, intensive sequential chemotherapy with stem cell support and/or incorporation of other active agents such as taxoids into high-dose chemotherapy regimens. However, more time is needed before the impact of those approaches can be assessed. Dose-dense chemotherapy uses high doses of active agents and short intervals between doses in repeating cycles, but does not require stem cell support [27]. Available data suggest that such regimens achieve high response rates (60–80%) and prolonged response duration and DFS (12–18 months) while better preserving QoL of the patients and being less expensive than high-dose chemotherapy [28–32]. New regimens incorporating paclitaxel or docetaxel are being studied, as are sequential regimens.

Second-Line and Subsequent Chemotherapy

Almost all patients who have received first-line chemotherapy for their metastatic progression will relapse or progress and thus require subsequent treatment. Until recently, second-line chemotherapy received little consideration, although a number of cytotoxic agents have recognized activity in this situation (table 2). Patients who failed or are resistant to taxoids and anthracyclines have received even less attention, although recent data suggest that this may be of further clinical benefit to the patient. In this situation, continuous or prolonged exposure to agents that have been previously used has achieved responses. In a series of studies, continuous infusion of 5-FU as a single agent achieved an overall response rate of 29% [33]. Many of these patients were heavily pretreated and response rates of up to 54% were reported in individual studies. Combination chemotherapy with continuous 5-FU achieved response rates up to 89% but with the occurrence of hand-foot syndrome and myelosupression. However, there are few if any data to suggest a role for modulation of 5-FU by folinic acid in this situation.

Second-Line Chemotherapy

A number of agents have established activity in this situation after failure of first-line chemotherapy regimens including anthracycline-based regimens. In particular, the activity of the taxoids has led to recognition by the regulatory bodies of the need for second-line chemotherapy. Xeloda, as an intermittent regimen of 2,510 mg/m^2/day for 2 weeks followed by one week of rest, has achieved a response rate of 36% in this situation in patients failing anthracyclines [8], and appears to be an excellent treatment option.

Whether combination regimens should be used rather than single agents at their optimal dose is still unclear. For

example, docetaxel was more active and less toxic than 5-FU and vinorelbine (response rates 33 and 26%, respectively) [34]. The sequence of taxoids followed by doxorubicin at progression (or vice versa) achieved response rates >30% [35]. However, a number of studies suggested that carefully designed combination chemotherapy regimens can achieve responses in up to 50% of the patients and disease stabilization in 15–30% [36–42].

Third and Subsequent Lines of Chemotherapy

This topic is almost never addressed in the published literature, although chemotherapy is offered routinely to patients whose disease progresses after second-line chemotherapy, with an estimated response rate of 30–40%. There are data to suggest that these patients achieve a fairly constant response rate to an agent not previously received irrespective of the number of previous treatment regimens [43]. McLachlan and Tannock have shown that third-line regimens improved status and symptoms [44]. 5-FU given as a continuous low-dose infusion is most frequently advocated for third-line use. In addition, Xeloda has been shown to be active in patients who have previously received anthracyclines and are resistant to paclitaxel [6]. Fluoropyrimidines not only have interesting activity with responses in 20–30% of the patients, but also a very favorable safety profile in these heavily pretreated patients.

It is also possible to administer agents originally used in the adjuvant setting or during first-line chemotherapy. We have reintroduced anthracyclines (with the cardioprotectant, dexrazoxane) in such patients with acceptable side effects and an interesting activity (clinical benefit in 40–60% of the patients with median duration 5–9 months) (personal data).

Conclusions

The treatment of patients with metastatic breast cancer is evolving constantly. One of the key issues is the availability of a larger number of active agents leading to the design of a variety of active regimens to deal with the diversity of situations encountered: age range, previous adjuvant therapy, previous hormonal therapy, number and sites of metastatic localizations. Whilst progress has been slow, one can now assume that a response will be achieved in more than 60% of patients with first metastatic progression, and that DFS in those patients can be increased to 16–18 months. Second-line regimens will achieve 30–50% responses and PFS in the range of 6–8 months. Subsequent regimens may achieve 20–40% response rates and, perhaps more importantly, a clinical benefit in 40–60% of those patients. Overall, median overall survival for metastatic patients may reach 36–40 months [45].

Of interest is the fact that these improvements in response and survival are not achieved with a detrimental effect on QoL. Improved symptomatic care, control of side effects of chemotherapy, and availability of orally active agents such as Xeloda, all contribute to the improvement in QoL in patients receiving long-term chemotherapy.

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EXHIBIT 8

THE CHEMOTHERAPY SOURCE BOOK

Michael C. Perry

Editor

Compliments

of

THE CHEMOTHERAPY SOURCE BOOK

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24
Enzymes and Random Synthetics

Alan P. Lyss, M.D.

This chapter will discuss an important but diverse group of antineoplastics which do not share common mechanisms of action, biochemical structures, pharmacokinetics, or clinical spectra of activity or toxicity. Each agent will be considered individually.

I. L-Asparaginase

Nomenclature

Generic name: L-asparaginase
Sources: Escherichia coli L-asparaginase (EC 3.5.1.1, NSC-109229)
Erwinia carotovora L-asparaginase (NSC-106977)
Commercial name: Elspar
Chemical name: L-asparaginase

Antitumor Activity: The single clinical indication for usage of L-asparaginase is in the induction therapy of acute lymphoblastic leukemia (ALL). As a single agent, complete remission (CR) rates of 50 to 60% have been observed in ALL patients (1, 2). Despite high CR rates, remissions tend to be short-lived when L-asparaginase is continued as a single agent, with median remission durations of only 1 to 8 months. Therefore, this agent is usually used in combination with other antileukemic drugs (3). Among patients who experience a relapse, 30 to 50% of prior L-asparaginase responders will obtain a CR to reinduction therapy (2).

L-asparaginase has minor activity against other hematologic malignancies (e.g., acute nonlymphocytic leukemia, blast crisis of chronic myelogenous leukemia) and no activity against a variety of solid tumors (2).

Mechanism of Action: In 1953 it was appreciated that guinea pig serum had antitumor activity which was especially marked in the therapy of ALL and which was shown to reside in the enzyme L-asparaginase. The discovery of *E. coli* L-asparaginase resulted in availability of large quantities of the enzyme and allowed the mechanism of action, pharmacology, and clinical usefulness of L-asparaginase to be studied further (1).

L-asparaginase hydrolyzes L-asparagine to aspartic acid and ammonia, which results in a cellular deficiency of L-asparagine. Normal human cells and resistant tumor cells have high levels of asparagine synthetase, which allows them to synthesize additional L-asparaginase endogenously. Sensitive tumor cells lack asparagine synthetase and, therefore, require exogenous sources of L-asparagine. Treatment of sensitive tumors with L-asparaginase results in rapid inhibition of protein synthesis and delayed inhibition of DNA and RNA synthesis. As sensitive tumor cells become resistant to the drug, they develop high levels of asparagine synthetase and, therefore, the capability to synthesize L-asparagine from endogenous sources. Unfortunately, various measurements of in vitro effects of asparagine deprivation and in vivo measurement of asparagine synthetase and L-asparagine levels have failed to predict ultimate clinical response to therapy with L-asparaginase (2, 4).

Pharmacology: The volume of distribution for L-asparaginase is slightly greater than plasma volume and there is indirect evidence that the drug may be sequestered within organs such as the liver. L-asparaginase does not cross the blood-brain barrier, although responses in central nervous system leukemia have been described (2). The half-life after intravenous injection of *E. coli* L-asparaginase is 8 to 30

hours (1). Plasma levels after i.m. injection are approximately 10 to 50% of those achieved after i.v. administration (2, 5). L-asparaginase may be detectable in plasma for 3 weeks after high doses. In hypersensitive individuals plasma clearance may be greatly accelerated (5).

Availability and Storage: L-asparaginase is supplied in 10 ml sterile vials which contain 10,000 IU of asparaginase and 80 mg mannitol in a white, lyophilized powder or plug with no preservatives. The drug may be given either i.v. or i.m. Vials should be stored at 2° to 8°C.

Preparation and Use: For i.v. use, each vial should be reconstituted with 5 ml of sterile water for injection or sodium chloride injection. This solution should be stored at 2° to 8°C and should be given within an 8-hour period following reconstitution.

For I.M. use, each vial should be reconstituted with 2 ml sodium chloride injection and should be administered within 8 hours and only if clear. The reconstituted drug should be stored at 2° to 8°C until time of administration.

Administration: When administered by i.v. infusion, the reconstituted vials should be diluted with isotonic solutions of sodium chloride injection or 5% dextrose injection. If a small number of gelatinous fiber-like particles are noted after standing, filtration through a 5.0 micron filter during administration will remove the particles with no loss in potency. When administered i.v., the drug should be given over no less than 30 minutes through the side arm of an infusion of 0.9% normal saline solution (NS) or D5W.

For i.m. administration, the volume given at a single injection site should be limited to 2 ml. If more than 2 ml is required, a greater number of injection sites should be used.

Dosage: The usual doses of L-asparaginase in the induction therapy of ALL are 6000 IU/m^2 every other day for 3 to 4 weeks or daily doses of 1,000 to 20,000 IU/m^2 for 10 to 20 days (5). As noted above, L-asparaginase is almost never utilized as a single agent for induction of remission in these patients and is most commonly used in regimens that employ vincristine, prednisone, and other agents.

Side Effects and Toxicities: In general, the toxic effects of L-asparaginase are due to hypersensitivity reactions and to the inhibitory effects of the drug on protein synthesis. This agent is not cytotoxic to bone marrow stem cells, oral and gastrointestinal tract mucosa, or hair follicles (2). Side effects are generally worse in adults than in children (3), may be dose-related (2, 6), and are usually reversible after discontinuation of therapy (2).

Hypersensitivity phenomena, including fever, dermatoses (especially urticaria), dyspnea, hypotension, agitation, and epigastric pain, are seen in 25% of patients and may not occur with continuation of therapy (2). Anaphylaxis occurs in approximately 10% of patients and is more common with i.v. than with i.m. administration and with intermittent (weekly or monthly) than with continuous (daily or thrice weekly) schedules. Skin testing is not helpful in determining risk of anaphylaxis, but the appearance of passive hemagglutinating antibody titers plus rapid plasma clearance of L-asparaginase may have prognostic utility (2). Patients who have received prior *E. coli* L-asparaginase should be treated with *Erwinia* L-asparaginase if reinduction courses are required after an initial relapse (3).

General malaise, anorexia, mild nausea, and vomiting are commonly observed during therapy and may result in weight loss in 25% of patients. Liver enzyme abnormalities are common, may correlate with hepatic steatosis upon histologic examination of the liver, and are transient, disappearing after treatment has been discontinued (1). Severe hepatotoxicity is seen in less than 5% of cases. Similarly, mild changes in renal function are common (34%) but result in acute renal failure (apart from renal failure due to rapid tumor lysis) in only 1% of cases (3).

Effects of L-asparaginase on the pancreas are protean. Nonketotic hyperglycemia has been observed and has been associated with decreased levels of insulin. The hyperglycemia is easily controlled with exogenous insulin and disappears after therapy with L-asparaginase has been discontinued (1). Hypoamylasemia is common and is associated with clinical evidence of pancreatitis in 7% of patients (1, 3). Pancreatic toxicity is dose-related and necessitates cessation of therapy (1).

L-asparaginase causes predictable and very common effects on hemostatic factors, with reductions in fibrinogen and other clotting factors (factors IX and XI, antithrombin III, proteins C and S, histidine-rich glycoprotein, and α-2 macroglobulin) and fibrinolytic enzymes (plasminogen and α-2 antiplasmin) in 60 to 100% of treated patients (3, 6). Clinical

bleeding is observed in less than 1% of patients, however, despite continuation of therapy with L-asparaginase.

Thrombotic and hemorrhagic events most commonly affect the CNS and are approximately equally frequent, with cortical infarction, capsular infarction, intracerebral hemorrhage, hemorrhagic infarction, and cerebral venous and dural sinus thrombosis described (6). Symptoms may include headache, alterations in mental status, seizures, hemiparesis, and/or vomiting. Therapy for thrombotic and hemorrhagic events has been variable, but the outcome of CNS events has been generally good, with 63% of pediatric patients recovering from neurologic deficits and only 12% dying of neurologic causes (6). Second neurologic events have not been described in patients who required additional therapy with L-asparaginase, although some patients were preventatively treated with fresh frozen plasma infusions on the days of L-asparaginase therapy when retreatment was instituted (6).

CNS symptoms which were unassociated with cerebrovascular complications of L-asparaginase have been noted and include hallucinations, "fugue states," and inappropriate behavior. Diffuse slowing on electroencephalography has been described. These CNS effects have improved with discontinuation of therapy (1).

2. Dacarbazine

Nomenclature

Generic name: Dacarbazine (DTIC, DIC, NSC-45388)

Commercial name: DTIC-Dome

Chemical name: 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide

Antitumor Activity Dacarbazine is a chemotherapeutic agent with activity against malignant melanoma, soft tissue sarcomas, Hodgkin's disease, and malignant abdominal neuroendocrine tumors (APUDomas) but no other human neoplasms. It is among the most active single agents against malignant melanoma, with response rates from 8 to 34% (7). A dose-response relationship against melanoma has not been clearly demonstrated and augmentation of dacarbazine activity by use in combinations is controversial.

As a single agent dacarbazine has produced temporary remissions in 56% of Hodgkin's disease patients (7). In soft tissue sarcoma trials, dacarbazine has had an 18% objective response rate, with responses of brief duration (8). Synergism of dacarbazine with doxorubicin was suggested in preclinical studies (7) and, therefore, the most common usage of dacarbazine in Hodgkin's disease and sarcoma patients is in combination with doxorubicin and other agents. Dacarbazine plus doxorubicin has been superior to doxorubicin alone in terms of response rates and overall survival in soft tissue sarcoma patients in nonrandomized trials (8).

In a series of 14 patients with APUDomas of a variety of histological types, 7 dacarbazine-treated patients experienced objective responses of 1 to 10 years duration (9).

Mechanism of Action: At least in part, dacarbazine produces its antineoplastic effect as an alkylating agent. Demethylation in the liver leads to generation of a methyl diazonium cation, which then results in production of an active methyl cation and nitrogen and in the methylation of nucleic acids (5). In addition, the methyltriazinoimidazole carboxamide metabolite inhibits the incorporation of purine nucleosides into DNA. Therefore, dacarbazine has effects on several phases of the cell cycle; its antineoplastic effects show little evidence of schedule dependency (5).

Pharmacology: Dacarbazine is metabolically activated by the hepatic microsomal enzyme system and disappears from plasma with a terminal half-life of 41 minutes (5). It is well-absorbed when given p.o., but higher peak concentrations are achieved by I.v. administration, the usual route of delivery. Up to 50% may be recovered unchanged in the urine.

Availability and Storage: Dacarbazine is supplied in 10 ml vials containing 100 mg and in 20 ml vials containing 200 mg of active drug as an ivory-colored solid, with anhydrous citric acid and mannitol. The reconstituted solution may be stored at 4°C for up to 72 hours or at ambient temperature for up to 8 hours.

Preparation and Use: The 100 mg vial of dacarbazine should be reconstituted with 9.9 ml and the 200 mg vial with 19.7 ml of sterile water for injection. Prior to infusion this solution may be further diluted with D5W or 0.9% sodium chloride. The diluted solution may be stored for up to 24 hours at 4°C and for up to 8 hours at ambient conditions of light and temperature.

Administration: When small volumes of da-

carbazine are administered by the rapid i.v. push technique, pain along the vein of injection may be observed. Infusions of diluted drug, in 100 to 200 ml of D5W or in 0.95 NS given over 15 to 30 minutes, are better tolerated and allow close monitoring for extravasation, which can cause pain and skin necrosis (10).

Dosage: Dacarbazine has been given in a variety of treatment schedules. The most common regimens in contemporary clinical use employ either 250 mg/m^2/day for 5 days or 850 mg/m^2 on 1 day. Courses can be repeated every 21 days, depending on prior treatment history, concomitant antineoplastic therapy, and patient tolerance. Historically, dosages of 150 mg/m^2/day for 10 days every 4 weeks have been employed but confer no advantage over high doses over abbreviated periods of time (5, 10).

Side Effects and Toxicities: The dose-limiting side effect of dacarbazine is moderate myelosuppression, with greatest depression of WBC and platelet counts occurring 21 to 25 days after therapy (10). Nausea is most severe on the first day of multiple-day infusions and may be lessened by lowering the dose on the first day and increasing it gradually thereafter (5). A flu-like syndrome of fever, myalgia, and malaise may occur approximately 1 week after therapy and is generally mild and self-limited. Enhancement of doxorubicin cardiotoxicity and fulminant hepatic veno-occlusive disease have been reported (5).

3. Hexamethylmelamine

Nomenclature

Generic name: Hexamethylmelamine (HMM, HXM, NSC-13875)

Commercial name: Altretamine

Chemical name: 2,4,6-tris (dimethylamine)-S-triazine

Antitumor Activity: Although hexamethylmelamine (HMM) may have minor activity against breast cancer, endometrial carcinoma, lymphoma, bilharzial bladder cancer, head and neck tumors, and colon carcinoma, it is clinically important only with respect to its contribution to the therapy of ovarian adenocarcinoma and bronchogenic lung cancer.

Among 198 patients in the medical literature who received HMM as a single agent for epithelial ovarian tumors, the objective response rate was 21% and was highly dependent on the extent of prior therapy. In previously untreated ovarian cancer patients, up to 32% of patients may experience temporary remissions (11). HMM is most commonly employed in combination with other agents, especially cyclophosphamide, doxorubicin, and cisplatin, in the initial chemotherapy of ovarian adenocarcinoma. Its additive contribution to these multidrug regimens is controversial (12).

HMM has reproducible activity against bronchogenic carcinoma (11). In single agent trials in non-small-cell lung cancer patients, HMM produced 4 to 33% objective responses (mean, 12%) of limited duration. In small cell lung cancer responses have been observed in 17 to 42% of patients (mean, 32%), with a CR rate of approximately 8%. Combination chemotherapy trials in non-small-cell and in small cell lung cancer have not determined the relative contribution of HMM to polychemotherapy regimens which include other effective agents (11).

Mechanism of Action: HMM is a substituted melamine which is structurally similar to triethylenemelamine (10). It was originally thought to exert its antitumor activity as an alkylator, but two lines of evidence suggest that that is not the case. First of all, failure of the nitrobenzyl pyridine test, an in vitro test of alkylating agent activity, suggests that, in its native state, HMM lacks alkylating agent activity (13). Second, HMM is active against human tumors that are alkylator-resistant (5, 10).

HMM inhibits incorporation of precursors into DNA and RNA, but the mechanism is unknown (10, 13). The active intermediate has not been identified.

Pharmacology: Because of limited aqueous solubility, HMM is given p.o. only. It is rapidly absorbed from the gastrointestinal tract, with 62% appearing in the liver within 24 hours and 95% within 72 hours (13). It is rapidly demethylated by the mixed function oxidase system in hepatic microsomes to a variety of active methylmelamine derivatives and formaldehyde (5). Plasma half-life of the parent compound ranges from 2.9 to 10.2 hours and displays interpatient variability (5, 11, 13). CSF concentration is 6% of the plasma concentration.

Availability and Storage: HMM is now commercially available. It is supplied in 50- and 100-mg capsules, which are stored at am-

bient temperature in tightly-sealed bottles containing a desiccant. When stored in this fashion, intact capsules remain stable for at least 2 years (10).

Administration: HMM is given p.o., in divided doses, with meals and after premedication with antiemetics. Concurrent administration with pyridoxine has been attempted in an effort to limit peripheral neurotoxicity. No benefit from pyridoxine has been convincingly demonstrated (10).

Dosage: Most treatment regimens employ HMM at a dose of 4 to 12 mg/kg body weight for 14 to 21 days, with cycles repeated at 28- to 42-day intervals. More prolonged (e.g., up to 90 consecutive days of therapy) treatment durations have been associated with a high rate of neurotoxicity and gastrointestinal intolerance (13). HMM is most often incorporated in regimens which utilize combinations of cytotoxic drugs.

Side Effects and Toxicities: In contrast to many other chemotherapeutic agents, myelotoxicity from HMM is usually mild, consisting of leukopenia and thrombocytopenia at 3 to 4 weeks. Recovery from nadir counts usually occurs within 1 week of discontinuation of therapy (10).

Gastrointestinal and peripheral nervous system toxicity are dose-limiting for HMM. Gastrointestinal intolerance is manifested by anorexia, nausea, diarrhea, and abdominal cramps. Nausea usually begins several days after initiation of therapy. These side effects are dose- and duration-related and may be lessened by administration of HMM in divided doses with, or 1 to 2 hours after, meals.

Neurotoxicity occurs in 20% of patients and is characterized by paraesthesias, muscle weakness, ataxia, static tremors, and hyperreflexia. CNS symptoms such as agitation, hallucinations, depression, extrapyramidal effects, and seizures have been described. These toxicities are reversible and generally diminish in severity rapidly after therapy is withdrawn. Neurologic effects are more common with continuous, low-dose regimens than with pulse-dosing schedules (5, 10, 13).

4. Hydroxyurea

Nomenclature

Generic name: Hydroxyurea (NSC-32065)

Commercial name: Hydrea

Antitumor Activity: Hydroxyurea is most commonly used in the treatment of the acute blastic and chronic phases of chronic myelogenous leukemia and in the management of other hematologic conditions such as essential thrombocythemia, polycythemia vera, hypereosinophilia, and hyperleukocytosis due to acute leukemia (14). It causes rapid, but transient, reductions in high leukocyte and platelet counts.

In blastic crises of chronic myelogenous leukemia hydroxyurea is generally used as initial therapy, while definitive treatment is being planned or undertaken. A recent report suggested that a combination of hydroxyurea and plicamycin may induce a second chronic phase in a proportion of patients with myeloid blast crisis (15). In essential thrombocythemia, polycythemia vera, the chronic phase of chronic myelogenous leukemia, and hypereosinophilia, it may be used to facilitate chronic control of elevated blood counts.

Hydroxyurea has shown activity against renal cell cancer, malignant melanoma, ovarian cancer, head and neck tumors, and prostate cancer, in which it is generally combined with other agents or with radiation therapy (10). Its independent contribution to the therapy of these solid neoplasms is uncertain.

Mechanism of Action: The exact mechanisms of action of hydroxyurea have not been established. It is a potent and immediate inhibitor of the enzyme ribonucleotide reductase, causing inhibition of DNA synthesis, without inhibiting RNA or protein synthesis. This effect can be reversed, in vitro, by removal of the drug or by adding deoxyribonucleotides (16). It is cell-cycle specific for the S phase and may hold other cells in the G_1 phase of the cell cycle (10). Hydroxyurea may prevent DNA repair mechanisms from operating, but this is controversial.

Pharmacology: Hydroxyurea is well-absorbed when given p.o., achieving peak serum concentrations within 1 to 2 hours (16). It readily penetrates the blood-brain barrier, achieving peak CSF levels within 3 hours (10). Serum half-life is approximately 3.5 hours (16), with 70 to 80% recovered unchanged in the urine within 12 hours. Approximately 50% of an oral dose is metabolized in the liver, excreted in the urine as urea, and eliminated in the respiratory tract as carbon dioxide (10). Negligible amounts remain in the body beyond 24 hours.

Availability and Storage: Hydroxyurea is commercially available as 500 mg capsules for oral

use, which should be stored at room temperature in a tightly sealed container including a desiccant.

Administration: Hydroxyurea is administered orally in divided doses or as a single daily dose. For patients who are unable or unwilling to swallow capsules, the content of each capsule may be added to a glass of water and administered orally or through an enteral feeding tube.

Dosage: The dose of hydroxyurea is based on lean body or actual weight, whichever is less. The usual daily dose in patients with leukemia is 20 to 50 mg/kg until the WBC count falls to less than 50,000, after which the dose is adjusted downward or therapy is temporarily discontinued (17). In the majority of patients symptoms and elevated blast counts can be controlled with 1 to 3 gm/day. Similar initial doses have been used in patients with other hematologic conditions (e.g., thrombocythemia), with dosages modified once normal counts are achieved (14).

In the treatment of the solid tumors in which hydroxyurea has activity, dosage guidelines are not well-established, but 20 to 30 mg/kg daily or 80 mg/kg every third day have been employed (10). The intermittent approach may offer some reduction in toxicity, but therapy should be interrupted if the WBC count falls below 2500/mm^3 or the platelet count below 100,000/mm^3.

Side Effects and Toxicities: The major toxic effect of hydroxyurea is dose-related myelosuppression. Leukopenia is predictable, with onset at a median of 10 days. Myeloblast counts, however, may fall much more rapidly. Significant anemia and thrombocytopenia are less common and occur after approximately 10 days. Megaloblastosis, which is unrelated to deficiency of folic acid or vitamin B_{12}, is common with chronic administration (10, 14).

Nausea, diarrhea, constipation, and stomatitis may occur in patients who receive prolonged, high-dose therapy. Central nervous system toxicity and azotemia have also been reported (10).

5. Mitotane

Nomenclature

Generic name: Mitotane (o,p'-DDD, NSC-38721)

Commercial name: Lysodren

Chemical name: 1,1-dichloro-2-(*O*-chlorophenyl-2-(*p*-chlorophenyl)-ethane

Antitumor Activity: The single clinical indication for therapy with mitotane is in the therapy of inoperable, recurrent, or metastatic carcinoma of the adrenal cortex. Adjuvant use of the drug has been suggested, based on retrospective data indicating that treated patients lived longer than historical controls (18). Remissions have been reported in biochemically functioning and in nonfunctional tumors.

Among 115 inoperable adrenal carcinoma patients treated between 1965 and 1969, Lubitz et al. reported an objective response rate of 61% and a biochemical (i.e., reduction in 17-ketosteroid and 17-hydroxysteroid urinary excretion) response rate of 89% with a median remission duration of 6 months (19). Others have found lower objective and biochemical response rates but have confirmed the activity of mitotane in this rare tumor (18, 20).

Boven et al. reported a sustained CR, confirmed by laparotomy, in a patient with metastatic adrenal cortical carcinoma who was free of cancer 2 years after mitotane had been discontinued (21). However, CRs are rare in other series (18).

Mechanism of Action: In therapeutic doses, mitotane causes adrenal cortical atrophy, with cytotoxic effects on mitochondria of adrenal cortex cells in the zona fasciculata and in the zona reticularis. The drug blocks adrenal steroid 11-β-hydroxylation and, therefore, decreases production of cortisol. Additional biochemical effects include glucose-6-phosphate dehydrogenase inhibition and reduced triphosphopyridine synthesis or incorporation in adrenal cell metabolism (10, 21).

Some authors have documented altered peripheral cortisol and androgen metabolism (10) and have suggested that the doses of replacement corticosteroids may have to be increased with long-term use of mitotane (21).

Pharmacology: Since oral absorption is variable, pharmacokinetic studies have failed to show either dose-response or dose-toxicity relationships. It has been suggested that therapy be adjusted by measurement of plasma levels of mitotane (10, 21) or be increased until toxicity is observed (20).

Up to 60% is eliminated unchanged in the stool and 10 to 25% appears in the urine. The large remaining fraction is stored in body fat and may be released into the bloodstream for weeks or months after discontinuation of therapy (10). With usual daily dosing, plasma lev-

els steadily increase. The minimum effective plasma level is 10 μg/ml, but the optimal plasma level is uncertain (20, 21).

Availability and Storage: Mitotane is supplied in 500 mg scored tablets that may be stored at ambient temperature.

Administration: Mitotane is given p.o. Since the drug concentrates in fat, it should not be taken with meals that are high in fat content (10). Since mitotane causes predictable deficiencies in glucocorticoids, all patients should receive concomitant steroid replacement, e.g., cortisone acetate 25 mg every morning and 12.5 mg every evening (20). As noted above, some patients require increased dosages of glucocorticoid replacement with long-term mitotane therapy (21). Glucocorticoid replacement should continue for at least 1 month after discontinuation of therapy, with continued replacement if adrenal function remains deficient beyond that time (18). Some patients will require mineralocorticoid replacement as well (20).

Dosage: The usual initial dosage of mitotane is 2 to 6 gm/day in 3 to 4 divided doses, with dosage incrementally increased to 9 to 10 gm/day until toxicity or therapeutic blood levels (at least 10 μg/ml and, ideally, greater than 14 μg/ml) are documented (10, 20, 22). Maximum doses of 18 to 19 gm/day have been reported (10). Biochemical responses occur first and require a minimum of 4 weeks of therapy (18). Treatment is generally continued for prolonged periods, but failure to achieve response by 3 months usually (90% of cases) implies ultimate treatment failure.

As noted above, concurrent therapy with glucocorticosteroids and, often, mineralocorticoids, is required.

Side Effects and Toxicities: Adverse reactions to mitotane include gastrointestinal disturbances, especially anorexia, nausea, vomiting, and diarrhea, which occur in most patients at daily dosages of 8 to 10 gm. CNS toxicities of lethargy, severe depression, sedation, vertigo, ataxia, visual disturbances, or lightheadedness occur in 40 to 60% of patients and are generally reversible with dose adjustment (10, 18, 20). Repeated neurological assessment is recommended when therapy is prolonged. Allergic rashes have been reported in up to 15% of patients (22). Other toxicities (e.g., hemorrhagic cystitis, proteinuria, hematuria, hypertension, orthostasis, myalgias, leukopenia, liver enzyme abnormalities, or fever) are rare (10).

6. Procarbazine

Nomenclature

Generic name: Procarbazine (NSC-77213)

Commercial name: Matulane

Chemical name: *N*-isopropyl-(2-methyl-hydrazino)-*p*-toluamide hydrochloride

Antitumor Activity: Although procarbazine has activity against polycythemia vera, malignant melanoma, medulloblastoma, bronchogenic cancer, and multiple myeloma, it is most commonly employed in combination chemotherapy regimens for Hodgkin's disease and non-Hodgkin's lymphoma.

As a single agent in previously untreated advanced Hodgkin's disease patients, procarbazine has shown reponse rates of 53 to 69% with CR rates as high as 37% (23). Although randomized studies to assess its relative contribution to combination regimens such as the mechlorethamine, vincristine, procarbazine, and prednisone (MOPP) regimen have not been performed, there are fewer CRs to regimens that lack procarbazine than in regimens that include it. For example, Luce et al. found that the combination of an alkylating agent, a vinca alkaloid, and prednisone (without procarbazine) produced 36% CRs, with a median remission duration of 19 weeks. This is contrasted with procarbazine-containing combinations with an alkylating agent, a vinca alkaloid, and prednisone, which have shown CR rates of 71 to 81% and median remission durations of 29 to 42 months (23). A pilot study of carmustine (BCNU), vincristine, and prednisone with or without procarbazine suggested that procarbazine adds independent activity to the regimen (24).

In non-Hodgkin's lymphoma, single-agent trials of procarbazine have shown response rates of 36 to 40%. Nonrandomized comparisons of three-drug regimens which lack procarbazine to four-drug regimens which include it have suggested superiority for the four-drug combination (23).

Procarbazine produces responses in 18% of bronchogenic carcinoma patients, with small cell cancer the most responsive (response rate, 67%) histologic subtype. Even in small cell cancer, however, the independent contribution of procarbazine in combination regimens is controversial 923).

Mechanism of Action: The precise mechanism of the antineoplastic effect of procarbazine is uncertain. Metabolism of the drug by the hepatic microsomal enzyme system produces

highly reactive free radicals including hydrogen peroxide, formaldehyde, and hydroxide radicals that may alkylate and methylate DNA (10). These effects resemble those of ionizing radiation and alkylating chemotherapeutic agents, although there is lack of cross-resistance with classical alkylators (10, 23). The cytotoxic effect of procarbazine is manifest in the S phase of the cell cycle and studies have shown that it interferes with mitosis by prolonging interphase (10). In vitro studies have demonstrated its potential to induce chromosomal breakage, which contributes to its marked teratogenic and carcinogenic potential.

Pharmacology: Procarbazine is rapidly absorbed from the gastrointestinal tract and quickly equilibrates between the blood and CSF, with peak CSF levels attained in 30 to 90 minutes (10). It has a plasma half-life of 7 to 10 minutes and is rapidly metabolized, with up to 70% of the metabolites recovered in the urine within 24 hours. The remainder is exchanged in expired air as methane and carbon dioxide (23).

Availability and Storage: Procarbazine is commercially available in 50 mg capsules, which may be stored at ambient temperature for at least 2 years, so long as contact of the drug with moisture is assiduously avoided (10). An intravenous preparation is available for investigational purposes, but stability has been problematic.

Administration: Procarbazine is given orally, in three to four divided doses per day. Some investigators recommend that initial daily doses be low and that the daily dose be incrementally increased in order to minimize the nausea that may occur when treatment is initiated.

Because of disulfiram (Antabuse)-like reactions, patients should be counseled against using ethanol while taking procarbazine. In addition, sympathomimetic drugs (e.g., ephedrine), antihistamines, tricyclic antidepressants, and heavy intake of foods which are high in tyramine content (e.g., ripe cheese, bananas, etc.) should be avoided because procarbazine exhibits some monoamine oxidase inhibitory activity. Potent hypnotics (e.g., barbiturates) should be avoided because procarbazine has mild hypnotic effects and may depress microsomal inactivation of other agents (5).

Dosage: Procarbazine is usually given in dosages of 50 to 200 mg/m^2/day (rounded to the nearest 50 mg) for 10 to 20 days. Continuous daily therapy is generally avoided because of concerns about drug-induced carcinogenesis with long-term exposure. Dosage should be based on the patient's actual body weight, except in cases of morbid obesity or large volumes of ascites or peripheral edema. When renal or hepatic function is compromised, dosage should be reduced substantially.

Side Effects and Toxicities: The dose-limiting toxicity of procarbazine is myelosuppression, which may be slow in onset (median nadir at approximately 4 weeks) and slow to resolve, with resolution complete by 6 weeks (10).

Gastrointestinal effects such as nausea, anorexia, and diarrhea can be minimized by initiating therapy with low (e.g., 50 mg/day) doses and increasing daily doses sequentially until the target daily dose is achieved. Tolerance to these effects generally occurs with continued administration (23).

Hypersensitivity reactions such as a maculopapular rash, pulmonary infiltrates, and fever are not uncommon and may be minimized by concurrent administration with corticosteroids. Continued administration of procarbazine with corticosteroids does not usually lead to progressive cutaneous reactions, exfoliative dermatitis, or anaphylaxis (5).

CNS effects such as nightmares, depression, insomnia, nervousness, and hallucinations may occur in as many as 30% of patients and may limit tolerance to continued therapy, even at low doses (10). Other neurotoxic reactions such as paresthesias, ataxia, dizziness, and headache have been described. These effects may be reversible despite continuation of therapy (23).

Azospermia and cessation of menses are frequent with high-dose procarbazine and may be irreversible, even after treatment has been discontinued (10). As described above, patients should be counseled about the potential for teratogenicity and carcinogenicity. Other toxic reactions (e.g., ophthalmic effects, abnormal liver enzymes, etc.) are rare.

7. Cisplatin

Nomenclature

Generic name: Cisplatin (NSC-119875)
Commercial name: Platinol
Chemical name: cis-Diamminedichloroplatinum II

Antitumor Activity: The antineoplastic effect of cisplatin was discovered serendipitously during an experiment to test the effect of electric fields on growing cells (25). When *E. coli* were grown in a culture apparatus containing

platinum electrodes, bacterial growth proceeded, but cell division was inhibited, so that the *E. coli* grew into very long filaments. This effect was mediated by cis-dichlorodiammineplatinum II, an electrolysis product of the platinum electrode in the presence of ammonium chloride. Cisplatin has been tested extensively against human tumor cell lines in vitro and in clinical trials and has proven to be one of the most active antineoplastic agents in clinical use, with a broad spectrum of antitumor activity.

The contribution of cisplatin to the treatment of testicular cancer represents the most important role of the agent to date. Among patients with metastatic testicular cancer, cisplatin-containing combinations result in CRs in over 80% of patients; 70% of all such patients are cured by these regimens (26). Essentially all patients with early testicular cancer, stages I and II, are cured by adjuvant therapy with cisplatin-based regimens.

Responses are also obtained when single-agent cisplatin is used to treat other genitourinary tumors, such as bladder cancer, prostatic carcinoma, penile cancer, cervical carcinoma, endometrial cancer, and ovarian cancer. High CR rates and long survival have been reported for cisplatin-containing combination regimens in transitional cell carcinoma of the bladder (27) and ovarian cancer (26). However, the advantage of combination therapy over single agent cisplatin has been questioned for the other genitourinary neoplasms.

Cisplatin is among the most active single agents against non-small-cell bronchogenic carcinoma, although responses are seen in only 10 to 15% of treated patients. As a single agent in small cell lung cancer patients, cisplatin produces responses in 5 to 30% of treated patients, but synergy with etoposide and other agents has been suggested by in vitro and clinical studies (26). Cisplatin/etoposide combination therapy is highly active in limited-stage and extensive-stage small cell lung cancer and is useful as initial therapy, as salvage therapy for patients who are refractory to other combinations, and as late consolidation treatment for limited-stage patients who have had remissions induced by other agents (28).

Cisplatin is highly active against metastatic osteogenic sarcoma, with single agent response rates of 20 to 25%, and has been incorporated successfully into neoadjuvant and adjuvant regimens in combination with doxorubicin (26). It is less active in soft tissue sarcoma patients but may have value in patients with uterine mixed mesodermal sarcomas since 18% of previously treated patients have shown responses to single agent cisplatin (26).

Refractory non-Hodgkin's lymphoma (20%), Hodgkin's disease (35%), and epidermoid cancers of the esophagus and head and neck regions have shown responses to single agent cisplatin and to cisplatin-based combination regimens. Cisplatin also has activity against gastric adenocarcinoma, cloacogenic cancer of the anus, thymic carcinoma, pediatric brain tumors, neuroblastoma, adenocarcinoma of the breast, and malignant melanoma. Its value in comparison to other agents in the therapy of these tumors is the subject of ongoing inquiry (26).

Mechanism of Action: Cisplatin was the first heavy metal compound to be studied extensively and to achieve therapeutic usefulness as an antineoplastic. Electrical neutrality is apparently required, in order to facilitate passage through cell membranes (10). The drug is unstable in media which are low in chloride content. Because of the 30-fold difference in intracellular versus extracellular chloride content, it has been suggested that cisplatin is activated intracellularly by generation of a positively charged, aquated complex which has the activity of a bifunctional alkylating agent (5, 10, 26). It binds directly to DNA, inhibiting its synthesis by altering the DNA template via the formation of intrastrand cross-links. The cytotoxic effects of cisplatin lack cell-cycle dependency.

Synergy of cisplatin with antimetabolites and with etoposide has been demonstrated in vitro and in clinical trials. While the mechanism responsible for synergy has not been fully explained, enhancement of cisplatin's affinity for DNA binding sites, alterations in the mode of DNA damage, and disruption in the DNA repair process have been suggested (26).

Pharmacology: Cisplatin is cleared rapidly from plasma during the first 2 hours after intravenous injection, but clearance proceeds much more slowly thereafter due to binding to plasma proteins and erythrocytes (5, 26). When given intravenous push (IVP), cisplatin demonstrates triphasic kinetics with half-lives of 0.3 hour, 1.0 hour, and 24 hours. Clearance is considerably prolonged in patients with renal insufficiency or ascites. It penetrates poorly across the blood-brain barrier but does enter the CSF

in small amounts after intravenous administration. The clinical relevance of the low concentrations achieved in CSF is uncertain. Cisplatin is excreted primarily in the urine, with 23 to 70% recovered in the urine within 24 hours and 90% recovered within 5 days (10, 26).

The pharmacokinetics of intraperitoneally administered cisplatin have been studied extensively (29). When given by infusion through an indwelling intraperitoneal catheter, cisplatin concentration in tissues lined by peritoneum is augmented by 2.5- to 8-fold and the drug is cleared by first-order kinetics. The area under the concentration × time curve is increased upwards of 30-fold, the peak peritoneal concentration of unbound cisplatin is increased by 20-fold, the dose of cisplatin that can be given safely is substantially increased, and the concentration of active cisplatin in the plasma is not decreased by intraperitoneal administration, in comparison to intravenous cisplatin.

Availability and Storage: Cisplatin is available in vials containing 10 mg or 50 mg of drug as a white, lyophilized powder with mannitol and sodium chloride plus hydrochloric acid. It should be refrigerated at 4°C and protected from light during storage. When stored in this way it is stable for at least 2 years. When stored at ambient temperature it is stable for at least 12 months from the date of manufacture (10). The reconstituted solution is stable for 20 hours at room temperature. Protection from light is recommended, in order to prevent decomposition. Once reconstituted it should not be refrigerated, in order to avoid formation of a precipitate.

Preparation and Use: The 10 mg and 50 mg vials of cisplatin should be diluted with 10 ml and 50 ml, respectively, with sterile water for injection, USP. The resultant solution should be clear and colorless and will contain cisplatin at a concentration of 1 mg/ml.

Needles or intravenous sets containing aluminum parts should not be used for preparation or administration of cisplatin. Aluminum reacts with cisplatin, causing precipitate formation and loss of potency.

Administration: Cisplatin is generally administered by intravenous infusion, although intraperitoneal administration has been recently investigated (26, 29). Since the relative value and role of intraperitoneal cisplatin is the subject of ongoing inquiry, only intravenous administration will be described in this section.

Cisplatin was originally given by the intravenous push technique, but nephrotoxicity was common and dose-limiting. Nephrotoxicity was reduced or eliminated by techniques which facilitated high urine flow. Prehydration of patients with normal saline infusions for several hours prior to administration of the drug is uniformly recommended. In addition, the reconstituted cisplatin should be diluted further in 0.9% NS to a concentration of 0.1 to 1 mg of drug/ml of saline. In addition, mannitol 25 to 50 mg may be added to the infusion bag or furosemide 10 to 40 mg may be given concomitantly with the initiation of cisplatin infusion to facilitate further urine flow. Finally, the drug should be infused over 1 to 24 hours in order to minimize nausea and vomiting and nephrotoxicity.

Dosage: Cisplatin has been used in a variety of dosages and schedules but is generally given in doses of 40 to 120 mg/m^2 in a single daily dose or in doses of 20 to 33 mg/m^2/day for 3 to 5 days. Treatment may be repeated at intervals of 3 to 4 weeks, depending on patient tolerance and bone marrow reserve. Higher doses, e.g., 40 mg/m^2/day for 5 days or up to 270 mg/m^2 on 1 day, have been administered when "protecting" agents such as 3% NS, sodium thiosulfate, or the investigational agent WR-2721 are administered concurrently (29).

Side Effects and Toxicities: Although the nephrotoxicity of cisplatin was originally felt to be dose-limiting, renal toxicity has been preventable when adequate hydration is provided and when the precautionary measures described above are taken. More recently, the gastrointestinal, neuropathic, and myelosuppressive effects of cisplatin have proven to be dose-limiting. The toxic effects of cisplatin may be categorized as acute, subacute, and chronic.

Acute toxicities: Hypersensitivity phenomena have been described rarely with cisplatin therapy and consist of a spectrum from dermatologic reactions (facial flushing, angioneurotic edema, urticaria) to anaphylaxis. Hypersensitivity reactions are generally well-controlled with antihistamines and corticosteroids and, in severe cases, epinephrine, and may not preclude retreatment (10, 26).

Nausea and vomiting within 24 hours are seen in 100% of patients who receive cisplatin, unless combinations of antiemetic medications are aggressively utilized in a prophylactic manner. Both central (i.e.,

chemoreceptor trigger zone) and peripheral mechanisms have been proposed for the emetogenic effect of cisplatin. Cannabinoids, sedative-hypnotics, butyrophenones, dexamethasone, and metoclopramide are generally included in combination regimens to prevent this toxicity. Delayed nausea and vomiting (24 to 96 hours after cisplatin administration) has been described and may dictate continuation of antiemetics during the first week of therapy (30). Diarrhea and anorexia are other gastrointestinal side effects and should be managed symptomatically.

Subacute toxicities: Cisplatin is especially toxic for the S3 segment of the proximal renal tubule (29), but the exact mechanism of its nephrotoxicity is unknown. The detrimental effect on renal function usually peaks between the 10th and 20th days after therapy and may be augmented by concomitant therapy with aminoglycoside antibiotics and other nephrotoxic drugs (10, 26). These should be used with caution in patients who have received cisplatin.

As noted above, clinical evidence of nephrotoxicity can be avoided in most patients if aggressive hydration schedules are used and if proper attention to high urine flow is given. However, subclinical reduction in renal function (i.e., glomerular filtration rate and renal plasma flow) may occur in most patients who receive cisplatin, even if serum creatinine remains unchanged (26). In most cases, these nephrotoxic effects are temporary and reversible.

Hypomagnesemia occurs in approximately 60% of patients due to hypermagnesiuria, but symptoms and signs of neuromuscular irritability are uncommon (26). Prophylactic supplementation of magnesium levels will not prevent renal magnesium wasting. If hypocalcemia develops, it should be corrected only after correction of magnesium deficiency has been accomplished.

Cisplatin therapy is associated with at least temporary infertility in the vast majority of patients. Among males receiving cisplatin for testis cancer, almost all become azospermic within the first two cycles of therapy, but recovery of normal sperm morphology, motility, and sperm counts occurs in 40% within 1.5 to 2 years (26). More than 33% of testicular cancer patients who have received cisplatin therapy, but who did not have retroperitoneal lymph node dissections, have successfully fathered children (31).

Life-threatening myelotoxicity is unusual with standard-dose cisplatin chemotherapy and is generally transient, with nadir WBC and platelet depression at approximately 14 days and recovery at approximately 21 days after therapy (10). Effects of RBC production may be delayed and cumulative. Hemolytic anemia has rarely been described (26). Myelosuppression is more common and may be dose-limiting with high-dose cisplatin regimens (29).

Chronic toxicity: The neurotoxicity associated with cisplatin has been well-described. As nephrotoxicity has become less troublesome and less dose-limiting, neurotoxicity has emerged as a dose-limiting and potentially disabling complication (26, 29).

Cisplatin in standard doses has been associated with peripheral neuropathy that is usually manifest clinically as sensory paresthesias in a "stocking/glove" distribution. Decreased proprioceptive sensation and vibratory sensory loss may be demonstrated on neurologic examination and by formal testing of nerve conduction velocity. Segmental demyelination of peripheral nerves has been seen in histologic specimens. Although up to 40% of long-term survivors of testicular cancer may continue to complain of paresthesias, these sensory phenomena rarely limit the performance status of patients. Motor dysfunction has been rarely observed with standard-dose cisplatin therapy but is much more common in high-dose regimens. It may be age- and cumulative dose-related, occurring in children and young, previously untreated patients much less commonly than in older, previously treated individuals (26, 29, 31).

Clinical signs and symptoms of central nervous system toxicity from cisplatin are relatively uncommon. Ophthalmic toxicity (e.g., retrobulbar neuritis and papilledema), normal pressure hydrocephalus, and seizures have been described (10, 26). Damage to the organ of Corti may result in dose and age-related ototoxicity in up to 50% of patients, which may be manifest as high-frequency hearing loss and tinnitus (10, 26, 31, 32). Children and patients who experience clinical nephrotoxicity may be more

susceptible to ototoxicity from cisplatin (10,26). Vestibular toxicity, otalgia, and deafness are rarely reported.

Chronic vascular toxicity, especially Raynaud's phenomenon, has been reported with cisplatin. Although Raynaud's phenomenon clearly occurs in testis cancer patients who have been treated with regimens that lack cisplatin, it is at least two times more frequent in regimens that include it (31). Myocardial infarctions, coronary artery disease, and cerebrovascular accidents have been reported in survivors of testicular cancer who have received cisplatin, but the incidence of these severe complications and the cause-effect relationship to cisplatin therapy have been difficult to establish conclusively.

Because of the bifunctional alkylating agent activity of cisplatin, predisposition to the occurrence of second malignancies has been a concern. While acute leukemia and other tumors have been reported in long-term cancer survivors who have received cisplatin therapy, when one subtracts patients with extragonadal germ cell cancers (who have an increased risk of spontaneous development of acute leukemia) and patients who have received other alkylating agents or maintenance therapy, there is no increased risk of second tumors among cisplatin-treated patients.

8. Carboplatin

Nomenclature

Generic names: Carboplatin, CBDCA, JM-8, NSC 241 240

Commercial name: Paraplatin

Chemical name: *cis*-diammine [1,1-cyclobutane dicarboxylato-(2-)0,0'] platinum II

Antitumor Activity: Carboplatin was synthesized by Cleare and Rosenberg in 1981. It is the first analog of cisplatin to become commercially available and, in in vivo studies, has demonstrated comparable activity to cisplatin (33). Cross-resistance with cisplatin was demonstrated in three murine tumor cell lines (L1210, P388, and M5076) (34). Superior activity to cisplatin was shown against P246 epidermoid carcinoma. An increased rate of nuclear protein phosphorylation of tumor cells versus normal cells in comparison with cisplatin, as well as the lack of nephrotoxicity in animals, were factors which led to clinical testing of this agent (33).

Clinical comparisons of carboplatin and cisplatin as single agents or in combination have yielded comparable results. Carboplatin activity was first demonstrated in previously treated patients with advanced ovarian cancer, in whom it produced response rates of 14 to 60%, with the higher rates noted in patients who had not received prior cisplatin. When analysis was restricted to patients whose tumor progressed during cisplatin therapy, carboplatin produced responses of short duration in only 5% (35). Impressive CR rates have been reported in studies in which higher than conventional doses of carboplatin were employed. For example, Ozols et al. gave 800 mg/m^2 of carboplatin by 48-hour continuous intravenous infusion and observed CRs in 13% of patients; median survival of CR patients was in excess of 21 months (36).

Based on these and other studies, carboplatin has been tested as first-line therapy for ovarian cancer and has produced clinical CR rates of 9 to 62% and overall response rates of 45 to 85%, with a clear dose-response relationship (37). In randomized studies that have compared carboplatin and cisplatin in newly diagnosed, advanced ovarian cancer, comparable activity has been noted. However, large differences in favor of carboplatin were seen with respect to the incidence of renal toxicity, ototoxicity, peripheral neuropathy, gastrointestinal toxicity, and overall willingness to continue with treatment (37). Only myelosuppression was observed more frequently among carboplatin-treated patients. Similarly, phase III randomized trials of carboplatin versus cisplatin in combination with other drugs in de novo advanced ovarian cancer have shown comparable clinical CR rates (14 to 44%), overall response rates (48 to 90%), median response duration, and survival (38).

Carboplatin has demonstrated activity against small cell lung cancer, in which it has been predominantly tested in previously untreated patients with extensive disease. In that setting, single agent response rates of 10 to 79%, of brief duration (6 to 18 weeks), have been observed (35). In combination with etoposide 29 to 60% CR rates and 12 to 13% CR rates have been seen in limited-disease and extensive-disease patients, respectively. Once again, these remissions have not been durable (34).

Preliminary data suggest that carboplatin has activity similar to cisplatin in non-small-cell lung cancer, head and neck cancer, and in genitourinary cancer patients, particularly in those patients with testicular, bladder, and cervical

chemoreceptor trigger zone) and peripheral mechanisms have been proposed for the emetogenic effect of cisplatin. Cannabinoids, sedative-hypnotics, butyrophenones, dexamethasone, and metoclopramide are generally included in combination regimens to prevent this toxicity. Delayed nausea and vomiting (24 to 96 hours after cisplatin administration) has been described and may dictate continuation of antiemetics during the first week of therapy (30). Diarrhea and anorexia are other gastrointestinal side effects and should be managed symptomatically.

Subacute toxicities: Cisplatin is especially toxic for the S3 segment of the proximal renal tubule (29), but the exact mechanism of its nephrotoxicity is unknown. The detrimental effect on renal function usually peaks between the 10th and 20th days after therapy and may be augmented by concomitant therapy with aminoglycoside antibiotics and other nephrotoxic drugs (10, 26). These should be used with caution in patients who have received cisplatin.

As noted above, clinical evidence of nephrotoxicity can be avoided in most patients if aggressive hydration schedules are used and if proper attention to high urine flow is given. However, subclinical reduction in renal function (i.e., glomerular filtration rate and renal plasma flow) may occur in most patients who receive cisplatin, even if serum creatinine remains unchanged (26). In most cases, these nephrotoxic effects are temporary and reversible.

Hypomagnesemia occurs in approximately 60% of patients due to hypermagnesiuria, but symptoms and signs of neuromuscular irritability are uncommon (26). Prophylactic supplementation of magnesium levels will not prevent renal magnesium wasting. If hypocalcemia develops, it should be corrected only after correction of magnesium deficiency has been accomplished.

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Preliminary data suggest that carboplatin has activity similar to cisplatin in non-small-cell lung cancer, head and neck cancer, and in genitourinary cancer patients, particularly in those patients with testicular, bladder, and cervical

tumors. Activity has also been observed in pediatric brain tumors (medulloblastoma). Carboplatin has no activity in colon cancer and in previously treated breast cancer patients (34).

Mechanism of Action: Carboplatin and cisplatin share the same mechanism of cytotoxicity (34). Carboplatin binds to DNA in a similar, but slower, aquation reaction and cross-links DNA, resulting in non-cell cycle-dependent tumor cell lysis. Cross-resistance with cisplatin has been demonstrated in murine tumor cell lines.

Pharmacology: The reduced reactivity of carboplatin in comparison with cisplatin results in slower binding to plasma proteins. Therefore, within 4 hours of administration, a greater percentage of ultrafilterable platinum is present in the urine. The 65% urinary excretion of carboplatin is approximately double that of cisplatin (34, 37). The free-platinum half-life in serum, however, is 6 hours, in contrast to less than 1 hour for the parent compound. The acute lethal dose is 10-fold greater (37).

Both carboplatin and cisplatin are cleared by the kidneys. Elimination of the former is almost completely by glomerular filtration. As a result, there is little concentration of carboplatin at the renal tubular level, which may account for its diminished nephrotoxic potential (34). On the other hand, reduced renal function increases the serum half-life of carboplatin and results in increased myelotoxicity. Therefore, the dose must be adjusted in patients with renal dysfunction. Egorin et al. have developed a formula for dosage of carboplatin which is based on the pretreatment platelet count, creatinine clearance, and body surface area (39).

Availability and Storage: Carboplatin is commercially supplied as a sterile lyophilized powder in single-dose vials containing 50 mg, 150 mg, or 450 mg of the drug, in equal parts by weight with mannitol. Unopened vials are stable for as long as 3 years when stored at temperatures of 15° to 30°C and when protected from light.

Preparation and Use: Immediately prior to use, each vial of carboplatin should be reconstituted with either sterile water for injection, USP, 5% dextrose in water, or sodium chloride injection, USP, in sufficient volume to produce a carboplatin concentration of 10 mg/ml. Carboplatin can then be further diluted to concentrations as low as 0.5 mg/ml with 5% dextrose or sodium chloride.

As is the case with cisplatin, aluminum reacts with carboplatin, causing precipitate formation and loss of potency. Needles or intravenous sets containing aluminum parts should not be used in its preparation or administration. The diluted drug should be visually inspected for particulate matter and discoloration prior to administration.

When prepared as directed, carboplatin solutions are stable at ambient temperature for 8 hours. Since no antibacterial preservative is included in the formulation, it is recommended that carboplatin solutions be discarded 8 hours after dilution.

Administration: Carboplatin is usually administered by i.v. infusion over 15 minutes or longer. There is no need for pretreatment or posttreatment hydration or forced diuresis. Intraperitoneal administration has been used in investigational settings. The intraperitoneal route does not modify the maximum dose that can be administered or the pattern of toxicity encountered (34). Therefore, intraperitoneal therapy will not be further described in this section.

Carboplatin, as a single agent, has been effective and well-tolerated at a dosage of 400 to 500 mg/m^2 on day 1 every 28 days in solid tumor patients with good performance status and bone marrow reserve and 240 to 320 mg/m^2 for patients with diminished marrow reserve. Pediatric patients tolerate higher dosages (34). In general, it is not recommended that intermittent courses be repeated until the granulocyte count is at least 2,000/mm^3 and the platelet count is in excess of 100,000/mm^3. Dosages in excess of 900 mg/m^2 intravenously have been given in some studies but generally by continuous i.v. infusion over 48 hours (37).

As described above, patients with impaired kidney function (creatinine clearance values below 60 cc/min) are at increased risk for severe myelosuppression from carboplatin. Reduced initial dosages should be based on the Egorin formula (39) and subsequent doses should be adjusted by patient tolerance and by the degree of myelosuppression observed.

Side Effects and Toxicities: The dose-limiting toxicity of carboplatin is reversible myelosuppression. The nadir platelet count usually occurs 21 days after administration; recovery is usually complete by 28 days. The granulocyte nadir occurs a few days later and is usually less severe than the impact of the drug on the platelet count, although some patients, partic-

ularly those who have been heavily treated with other agents or radiation therapy, require 5 to 6 weeks to recover normal hematologic parameters. Reductions in hemoglobin are mild and are rarely dose-limiting (34).

Nonhematologic side effects of carboplatin have generally been mild. Nephrotoxicity is uncommon, especially considering that carboplatin is not usually given with pretreatment hydration or forced diuresis (33, 34). Emesis is noted by only 50% of patients, generally within 6 hours, and is seldom problematic beyond 12 hours of drug administration. It can generally be controlled with mild antiemetic medications. Peripheral neuropathy and ototoxicity are generally reported only by patients who had experienced these toxicities during prior cisplatin therapy (34, 37). These nonhematologic toxicities (and liver dysfunction) may become more substantial if carboplatin is given in marrow transplantation studies and with hematopoietins (35). Alopecia and rash are other adverse effects that have been described infrequently. Currently, the lack of severe nonhematologic effects favor carboplatin over cisplatin in almost all comparative studies.

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54
Chemotherapy of Breast Cancer

Carl G. Kardinal, M.D., F.A.C.P.

Until recently, systemic cytotoxic or hormonal therapy was restricted to breast cancer patients with advanced metastatic disease. Now, with a better understanding of breast cancer biology, systemic therapy has been integrated into the multidisciplinary treatment of the patient with newly diagnosed disease. The recognition that breast cancer is frequently a systemic disease at diagnosis is a major conceptual change that has revolutionized our approach to this all-too-common malignancy. This conceptual change has provided the theoretical framework not only for systemic adjuvant therapy but also for breast-conserving surgery. Appropriately administered systemic therapy, in conjunction with conservative surgery and radiation, has improved the survival of breast cancer patients when compared to radical surgery alone.

BREAST CANCER BIOLOGY

Current concepts of breast cancer can be traced to Henri Francois Le Dran (1685–1770), a French surgeon. He proposed that breast cancer was a local disease that spread to regional lymph nodes and that the only chance for cure was early surgery (1). The concept that breast cancer was a localized disease that spread in an orderly fashion dominated cancer theory for the next 200 years. This hypothesis was carried to its logical conclusion by William Stewart Halsted, who developed the radical mastectomy in 1890. Radical cancer surgery based upon anatomic considerations was unchallenged for over 75 years (2).

Bernard Fisher noted that the Halstedian theory of en bloc resection was based upon Virchow's proposal that regional lymph nodes (RLN) are effective filters and barriers to tumor spread. Fisher was able to demonstrate in the laboratory that RLN were ineffective barriers to the passage of tumor cells (3, 4). He then proposed an *alternative hypothesis* of breast cancer biology and proceeded to verify the hypothesis through the clinical trials of the National Surgical Adjuvant Breast Project (NSABP) (2, 5–7) (Table 54.1).

Early NSABP trials confirmed that the natural history of breast cancer was directly related to the number of axillary nodes involved. This has proved to be the single most important prognostic variable (Table 54.2, Fig. 54.1) (8–10). The prognostic value of axillary nodal involvement and the apparent lack of therapeutic value of axillary dissection confirmed clinically that RLN are ineffective tumor cell filters.

Between August 1971 and August 1974, the NSABP conducted a clinical trial involving 1765 patients with clinical stage I and II breast cancer, comparing the standard Halsted radical mastectomy to a total (simple) mastectomy with or without radiation. By stage, there was no difference in disease-free or overall survival in any of the treatment groups (11).

The logical extension of this trial is that even more conservative breast-sparing operations should be equivalent to total mastectomy or a modified radical mastectomy (MRM). Between April 1976 and January 1984, the NSABP randomized 1855 women to an MRM or a segmental mastectomy (lumpectomy) with an axillary dissection (12). The data from this trial confirmed that women with primary breast tumors of 4 cm or less who were treated with a lumpectomy, axillary dissection, and primary breast irradiation had disease-free and overall survivals equivalent to those of women treated with

Table 54.1. Two Divergent Hypotheses of Tumor Biology[a]

Halstedian Hypothesis	Alternative Hypothesis
Tumors spread in an orderly defined manner based upon mechanical considerations	There is no orderly pattern of tumor cell dissemination
Tumor cells traverse lymphatics to lymph nodes by direct extension, supporting en bloc dissection	Tumor cells traverse lymphatics by embolization, challenging the merit of en bloc dissection
The positive lymph node is an indicator of tumor spread and is the instigator of disease	The positive lymph node is an indicator of a host-tumor relationship which permits development of metastases rather than the instigator of distant disease
Regional lymph nodes are barriers to the passage of tumor cells	Regional lymph nodes are ineffective barriers to tumor cell spread
Regional lymph nodes are of anatomic importance	Regional lymph nodes are of biologic importance
The bloodstream is of little significance as a route of tumor dissemination	The bloodstream is of considerable importance in tumor dissemination
A tumor is autonomous from its host	Complex host-tumor interrelationships affect every facet of the disease
Operable breast cancer is a local-regional disease	Operable breast cancer is a systemic disease
The extent and nuances of operation are the dominant factors influencing patient outcome	Variations in local-regional therapy are unlikely to substantially affect survival
No consideration is given to tumor multicentricity	Multicentric foci of tumor are not of necessity a precursor of clinical overt cancer

[a]From Fisher B. Cancer surgery: a commentary. Cancer Treat Rep 1984;68(1):31–41.

Table 54.2. Treatment Failure after Standard Radical Mastectomy[a]

		Percent Treatment Failures			
	Number	18 Months	3 Years	5 Years	10 Years
All Patients	370	19		39.7	49.5
Negative nodes	198	6		17.7	24.1
Positive nodes	172	35		71.0	76.1
Premenopausal					
Negative nodes	52	6	17	21.2	25.5
Positive nodes	60	50	61	70.0	75.3
1–3 nodes positive	24	13		45.8	56.6
≥4 nodes positive	36	64	82	86.1	88.9
Postmenopausal					
Negative nodes	146	6	15	16.4	23.6
Positive nodes	112	22	50	62.5	76.0
1–3 nodes positive	58	16	37	51.7	67.9
≥4 nodes positive	54	48	62	74.1	84.3

[a]Data from Fisher B, Redmon RS, Wolman RS, Slack NH, Medoff JE, Noer RJ. Surgical adjuvant chemotherapy in cancer of the breast: results of a decade of cooperative investigation. Ann Surg 1968;168:337–356; Fisher B, Slack N, Katrych D, Wolmark N. Ten- year follow-up results of patients with carcinoma of the breast in a cooperative clinical trial evaluating surgical adjuvant chemotherapy. Surg Gynecol Obstet 1975;140:528–534; and Fisher B, Bauer M, Wickerham L, Redmond CK, Fisher ER. Relation of number of positive axillary nodes to the prognosis of patients with primary breast cancer. An NSABP update. Cancer 1983;52:1551–1557.

MRM. Thus, the surgical principles of the alternative hypothesis are valid.

The other major principles of the alternative hypothesis—that there is no orderly pattern of tumor cell dissemination, that tumor cells traverse lymphatics by embolization, and that operable breast cancer is a systemic disease because of the presence of micrometastases at diagnosis—have also been tested. The now numerous trials of systemic adjuvant chemotherapy and endocrine therapy have confirmed these principles by demonstrating improved disease-free and absolute survival in systemically treated cases (13).

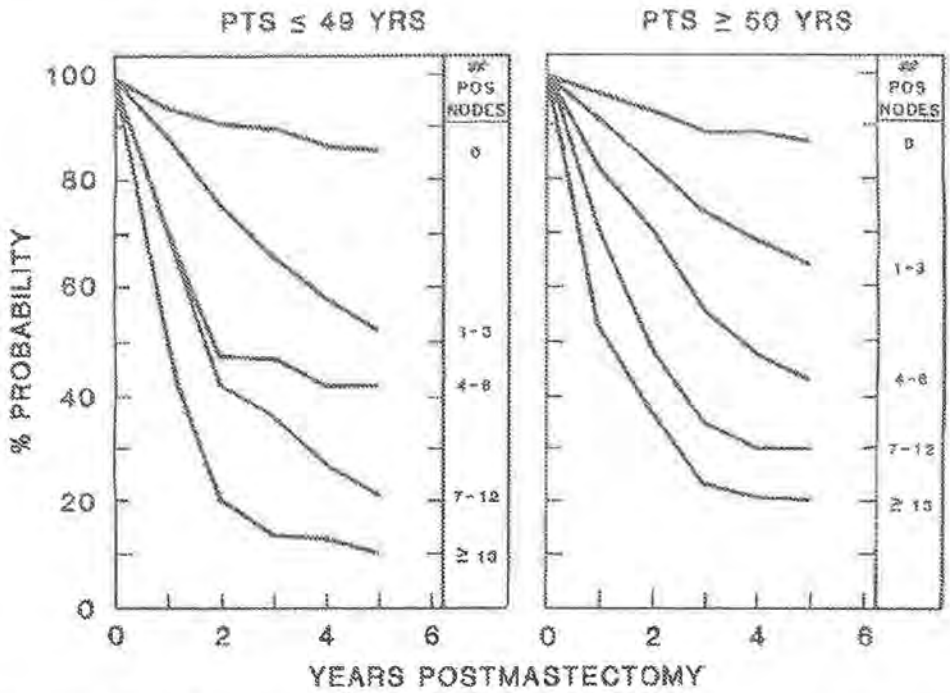

Figure 54.1. Disease-free survival (% probability of remaining disease-free) relative to age and number of positive axillary nodes for patients (PTS) ≤49 years and ≥50 years. (From Fisher B, Bauer M, Wickerham L, Redmond CK, Fisher ER. Relation of number of positive axillary nodes to the prognosis of patients with primary breast cancer: an NSABP update. Cancer 1983;52:1551–1557.)

Table 54.3. Relationship Between Estrogen Receptor Status of Breast Cancer and Objective Response to Endocrine Therapy[a]

ER+	ER−
(Responses/Total)	*(Responses/Total)*
522/977 (53%)	36/567 (6%)

[a]Modified from Wittliff JL. Steroid-hormone receptors in breast cancer. Cancer 1984;53:630–643.

Table 54.4. Relationship Between Estrogen Receptor Status and Response to Additive and Ablative Endocrine Therapy[a]

	ER+ (Responses/Total)	ER− (Responses/Total)
Additive hormone treatment	59/105 (56%)	12/109 (11%)
Ablative endocrine therapy	59/107 (55%)	8/94 (8%)
Total	118/212 (56%)	20/203 (10%)

[a]Modified from Wittliff JL. Steroid-hormone receptors in breast cancer. Cancer 1984;53:630–643.

Hormone Receptors

Breast cancer biology was further illuminated in the early 1970s by the description of the *estrogen receptor* and its relationship to response to endocrine therapy and to prognosis (14–18). Jensen and associates (14), McGuire and associates (17, 18), Wittliff (15), and DeSombre and associates (19) established the relationship between responsiveness to additive or ablative forms of hormonal manipulation and the presence of the cytosol-binding protein for estrogen (Table 54.3). By restricting hormonal manipulation of patients whose tumors are estrogen receptor (ER) positive (ER+ ≥10 fmol/mg protein), response rates to endocrine therapy can be increased from 25% in unselected cases to 55%. Interestingly, the response rates to additive (56%) and ablative (55%) hormonal therapy in ER+ cases are identical (Table 54.4). This reflects the fact that the most commonly used forms of hormonal therapy basically do one thing: they block the production or the action of estrogen. It has also been recognized that re-

Table 54.5. Quantitation of ER and Response to Endocrine Therapy[a]

ER (fmol/mg)	Primary Cancer	Metastatic Breast
0–10	9%	8%
10–50	50%	40%
100	83%	61%

[a]Data from Allegra JC. Rational approaches to the hormonal treatment of breast cancer. Semin Oncol 1983;10(suppl 4):25–30; Osborne CK, Yochmowitz MG, Knight WA, McGuire WL. The value of estrogen and progesterone receptors in the treatment of breast cancer. Cancer 1980;46:2884–2888.

Table 54.6. Relationship Between Estrogen (ER) and Progesterone (PR) Receptor Status and Response to Endocrine Therapy[a]

ER+ PR+	ER+ PR−	ER− PR−	ER− PR+
135/174 (78%)	55/164 (34%)	16/165 (10%)	5/11 (45%)

[a]Modified from Wittliff JL. Steroid-hormone receptors in breast cancer. Cancer 1984;53:630–643.

sponsiveness to hormonal therapy is directly related to the quantitative amount of ER present, as illustrated in Table 54.5 (20, 21).

The synthesis of progesterone receptor (PR) is dependent upon an intact cellular hormonal system (22). The presence of PR in addition to ER further predicts hormonal responsiveness. ER + PR + tumors yield a 78% rate of response to hormonal therapy (Table 54.6).

The ER and PR content of a breast cancer is an important prognostic indicator as well as an indicator of response to endocrine therapy (23–29). Receptors correlate with cellular turnover rates, nuclear grade, and degree of histologic differentiation (30, 31). Receptors also correlate with disease-free interval (the time from diagnosis to documented recurrence), with receptor-positive cases having a significantly longer disease-free interval than receptor-negative cases (Fig. 54.2). The prolonged disease-free interval of ER + patients correlates well with the earlier clinical observation that patients with disease-free intervals of 2 years or longer are more likely to respond to hormonal therapy than patients with shorter disease-free intervals.

ENDOCRINE THERAPY

The basis for the endocrine therapy of breast cancer is summarized in Figure 54.3 (32). *Estrogen* is the major stimulus for the growth of hormone-dependent breast cancer, and most forms of endocrine therapy for breast cancer are directed toward inhibiting, ablating, or otherwise interfering with estrogen activity. The ovary is the principal site of estrogen synthesis, but estrogen is also synthesized by the adrenal gland, adipose tissue, and even by mammary tumors themselves (33, 34).

Prolactin is the next most important hormone in breast development and function. Prolactin acts synergistically with the growth hormone to promote ductal development (35). Highly specific receptors for prolactin have been demonstrated in human mammary carcinoma (36, 37). However, the actual role of prolactin in human breast cancers is as yet undefined.

Progesterone has no effect on the normal breast unless there is concomitant estrogen stimulation. Under these conditions, progesterone interacts with prolactin to promote lobuloalveolar developments (38).

Enthusiasm regarding the endocrine therapy of breast cancer has been cyclic. Oophorectomy was introduced at the turn of the 20th century but rapidly fell out of favor since it was not a curative procedure. There was a resurgence of interest in endocrine therapy from the 1940s through the 1960s, when several major breakthroughs occurred: (*a*) the full range of ablative therapy (oophorectomy, adrenalectomy, and hypophysectomy) was developed, and (*b*) additive hormonal therapy was initiated with high dose estrogens, androgens, and progestins.

With the introduction of combination chemotherapy in the late 1960s and 1970s, endocrine therapy of breast cancer again fell out of favor (39, 40). Endocrine therapy was unreliable, yielding only a 20 to 30% objective response, and 6 to 8 weeks were required for the response to occur. With chemotherapy, objective responses occurred in 60 to 70% of patients treated, and the onset of action was relatively rapid.

In the 1980s, however, there was a strong resurgence of interest in endocrine therapy of breast cancer. Receptors for estrogen and progesterone and their role in predicting response to hormonal manipulation had been documented (Tables 54.4–54.6). Endocrine therapy could now be more specific, and higher response rates in selected cases could be anticipated. The introduction of newer hormonal agents such as tamoxifen, megestrol acetate, and aminoglutethimide rendered the more toxic androgens and estrogens to secondary roles. The major surgical ablative procedures of adrena-

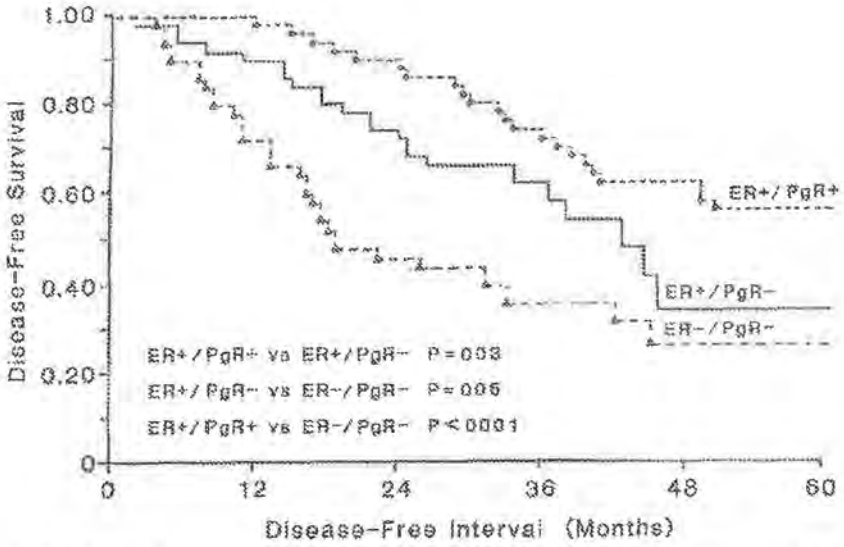

Figure 54.2. Relationship between estrogen and progesterone receptor status and disease-free survival. Patients who were ER+ PgR+ ($N = 104$) had a significantly longer disease-free survival than patients who were ER+ PgR− ($N = 39$), or patients who were ER− PgR− ($N = 40$). (From Clark GM, McGuire WL, Hubay CA, Pearson OH, Marshall JS. Progesterone receptors as a prognostic factor in stage II breast cancer. N Engl J Med 1983;309:1343–1347.)

lectomy and hypophysectomy were replaced by better additive agents. Luteinizing hormone-releasing hormone (LHRH) agonists were synthesized and are now being introduced into clinical practice (41). The era of hormonal therapy spawned in the 1980s will continue to mature in the 1990s (42).

Tamoxifen

Tamoxifen (Nolvadex) is currently the treatment of choice for postmenopausal women with hormonally responsive breast cancer. Tamoxifen has achieved this status because of its effectiveness (a 76% response rate in ER+ PR+ cases) and because it is essentially devoid of serious side effects (43). Tamoxifen binds reversibly with the estrogen receptor, forming an inert complex that blocks estrogen-mediated protein synthesis. A now classic Mayo Clinic trial compared diethylstilbesterol (DES), the previous hormonal agent of choice, to tamoxifen. This study confirmed an equivalent response rate and duration, but the toxicity of tamoxifen was considerably less than that of DES (44). Following this publication, DES was relegated to a secondary role, and tamoxifen emerged as the primary agent for the initial treatment of hormonally responsive breast cancer. The response rates to tamoxifen in ER+, ER+ PR+, ER+ PR−, and ER− PR− cases are essentially the same as those noted in Tables 54.3–54.6. However, Voegl et al. (45) reported a 25% response to tamoxifen in highly selected patients with "receptor-poor" metastatic breast cancer. They attributed this to false-negative receptor results and urged that receptor values be integrated with classical clinical and histopathologic variables. For example, patients with disease-free intervals of greater than 2 years tend to be hormonally responsive.

Tamoxifen is a nonsteroidal antiestrogen structurally related to DES and is weakly estrogenic in castrated rats (46). This weak estrogenic activity is probably why tamoxifen appears to protect against rather than promote the development of *osteoporosis* (47).

Tamoxifen is cytostatic rather than cytocidal and acts as a cell cycle inhibitor, with cells accumulating in the G_0 and G_1 phases (48, 49). In studies in rat mammary carcinoma, normal cell cycling returns when the drug has been cleared from the system (50). The cytostatic action of tamoxifen and the potential reversibility of its

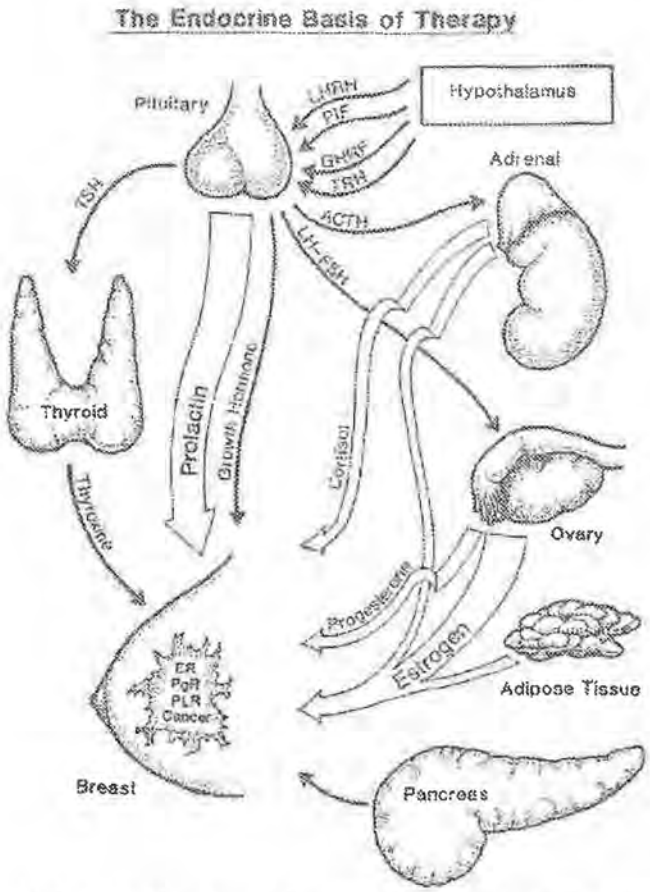

Figure 54.3. The endocrine basis of breast cancer therapy. Estrogen and prolactin are the dominant mitogens to normal breast tissue. However, the role of prolactin in human breast cancer is not yet established. Ductal growth is promoted by estrogen in the presence of growth hormone; lobular development is promoted by prolactin and progesterone. Estrogen is synthesized not only in the ovary but also in adrenal and adipose tissue. The roles of cortisol, thyroxine, and insulin are permissive rather than regulatory. LHRH, Luteinizing hormone-releasing hormone; PIF, prolactin inhibitory factor; GHRF, growth hormone releasing factor; TRH, thyrotropin releasing hormone; ACTH, adrenocorticotropic hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone; TSH, thyroid stimulating hormone; ER, estrogen receptor; PgR, progesterone receptor; PLR, prolactin receptor. (From Kardinal CG. Endocrine therapy of breast cancer. In: Donegan WL, Spratt JS, eds. Cancer of the Breast. 3rd ed. Philadelphia: WB Saunders, 1988:501–540.)

effect have prompted prolonged use of this drug, especially in the adjuvant setting (51–53).

The toxicity of tamoxifen is usually minimal but headaches, hot flashes, or both occur occasionally. Reduced levels of antithrombin III have been reported, but this is of questionable clinical significance (54, 55). Of more concern is the reported development of endometrial cancer in women receiving long-term tamoxifen (56, 57). The actual incidence is as yet undetermined. No increased risk of endometrial cancer has been observed in the large controlled NSABP tamoxifen trials.

TAMOXIFEN IN PREMENOPAUSAL WOMEN

The response rates and durations of response associated with oophorectomy and tamoxifen in premenopausal women (58–61) are equivalent. In addition, a prior response to ta-

moxifen may be a good predictor of response to subsequent oophorectomy (62, 63). This implies that tamoxifen should be used as primary therapy and oophorectomy should be restricted to patients who have responded previously to tamoxifen. However, this finding has not been confirmed by other investigators (64, 65). Tamoxifen is emerging as the primary treatment of choice in premenopausal women as well as in postmenopausal women with hormonally responsive metastatic breast cancer.

TAMOXIFEN DOSE

In *postmenopausal* patients, tamoxifen does not have a significant dose-response curve. Responses to the standard dose of 10 mg twice daily are equivalent to responses to 20 mg twice daily, and doses up to 90 mg daily are no more effective. In *premenopausal* women, the usual recommended dose is still 10 mg twice daily; however, many of the studies cited previously have utilized 20 mg twice daily, and doses up to 120 mg daily have been evaluated (66). The higher doses are more effective in suppressing menses but are not more effective in inducing an antitumor response.

TAMOXIFEN FLARE

Tamoxifen flare characterized by increased bone or soft tissue pain and occasionally hypercalcemia occurs in approximately 10% of cases (67). When a flare occurs, it develops in the first few weeks of therapy. Contrary to its manifestations, which may resemble progression, a flare generally heralds a response to treatment. Flares should be treated with analgesics or other symptomatic therapy, and full-dose tamoxifen should be continued. Brooks and Lippman (68) have proposed that the flare occurs because it requires several weeks for tamoxifen to reach therapeutic levels, and at lower considerations the drug may be estrogenic and stimulatory.

Hormonal flares are by no means unique to tamoxifen and were described as "induced hypercalcemia" with high-dose DES by Hall et al. in 1963 (69). The same significance was noted; that is, hormonally induced hypercalcemia may indicate that the tumor has retained its hormonal responsiveness and that hormonal treatment should be continued. Hypercalcemia and pain flares have also been reported with megestrol acetate and, again, may herald a response (70).

TAMOXIFEN AS PRIMARY THERAPY

Tamoxifen has been evaluated as primary therapy for breast cancer in *elderly women* (71–73). A 27% complete remission rate and a 61% total objective response rate can be anticipated. Overall survival is the same as in elderly women treated with mastectomy. However, the local recurrence rate in tamoxifen-treated patients is higher. Surgery should be performed if at all possible despite the age of the patient; however, in elderly patients who are poor surgical candidates because of intercurrent medical disease, tamoxifen alone may provide worthwhile palliation.

The integration of tamoxifen into the *adjuvant therapy* of breast cancer is discussed in the section on the adjuvant treatment of stage I and stage II breast cancer.

Progestins

The progestational agents megestrol acetate (Megace) and medroxyprogesterone acetate (MPA, Provera) appear to have activity equivalent to that of tamoxifen in advanced, hormonally responsive breast cancer in postmenopausal women (74–76). However, progestins are generally relegated to a secondary role since the duration of response to initial tamoxifen is slightly greater than for megestrol. Patients initially treated with tamoxifen who after treatment failure are crossed over to megestrol or MPA have a response rate of only 14 to 22%. Patients treated initially with MPA or megestrol who are subsequently treated with tamoxifen respond only 5 to 23% of the time. Clearly, better second-line hormonal therapy is needed.

Progestins have a direct cytotoxic action on human breast cancer cells in long-term tissue culture but probably act in vivo as antiestrogenic compounds that inhibit estrogen-induced protein synthesis (77).

The side effects of progestins are minimal but are slightly greater than those of tamoxifen. Progestins have a mild glucocorticoid action, and weight gain is a frequent problem. Johnson et al. (78) noted a 5% or greater increase in weight in 25% of breast cancer patients treated with progestins.

The standard dosage for megestrol acetate is 160 mg daily as a single or divided dose and 500 mg three times weekly for MPA (79). Unlike tamoxifen, progestins may have a steep dose-response curve. MPA in doses of 1000 mg daily

has been reported to yield a significantly higher response than 500 mg three times weekly (80). In preliminary studies, megestrol acetate in doses of 480, 800, 1280, and 1600 mg daily appears to be associated with higher responses as well as with responses in patients refractory to the standard 160-mg daily dose (81). The Cancer and Leukemia Group B is currently conducting a prospective randomized trial of megestrol at three dosage levels: 160 mg daily, 800 mg daily, and 1600 daily. This study should confirm the existence of a dose-response curve for progestins in metastatic breast cancer.

Aminoglutethimide

Aminoglutethimide (AG) blocks the aromatization of androstenedione to estrone. This action effectively blocks estrogen production in adrenal tissue, adipose tissue, and breast cancer tissue (33, 34). Samojlik and colleagues (82) have noted that in postmenopausal women with metastatic breast cancer treated with AG, plasma estrone levels declined 72%, and the urinary excretion of estrone fell 85% over a 12-week period. AG appears to be equally effective in inducing suppression of estrogen synthesis in postmenopausal and premenopausal women who have had a prior oophorectomy. AG has assumed a major role in the treatment of premenopausal women with hormonally responsive breast cancer. AG has essentially replaced the need for surgical adrenalectomy and hypophysectomy in the treatment of advanced breast cancer (83).

AG may be of particular benefit in relieving pain in patients with bone metastases (84, 85). Because the side effects of AG are greater than those of tamoxifen, megestrol acetate, or oophorectomy, AG has been relegated to a tertiary role in hormonal therapy. Acute toxicities occurring in the first 6 weeks include lethargy in 38%, drug rash in 33%, orthostatic hypotension with dizziness in 20%, ataxia in 10%, and drug fever in 2.5% of patients treated. After 6 weeks, even with continuous therapy, the side effects tend to decrease (83). Severe hematologic toxicity with leukopenia, thrombocytopenia, or even pancytopenia may occur in up to 4% of patients receiving AG (44).

The standard dose of AG is 1000 mg daily given in divided dosages of 250 mg every 6 hours. Hydrocortisone, 20 mg to 30 mg orally in the morning, should be administered to replace endogenous corticoids. Lower doses of 500 mg daily of AG, both with and without hydrocortisone, have been reported to produce responses equivalent to 1000 mg daily, with fewer side effects (86, 87).

A new aromatase inhibitor, *4-hydroxyandrostenedione*, is currently entering clinical trials. The response rates appear to be equivalent to those of AG, with fewer side effects (88–90).

Estrogens

Prior to the introduction of tamoxifen, diethylstilbesterol (DES), a synthetic estrogen, was the hormonal treatment of choice in postmenopausal women with advanced breast cancer (44, 91). The response rate to DES in patients with ER+ tumors is 63%. The mechanism by which estrogens act on metastatic breast cancer is unknown. Tumor cells that contain ER bind estrogens with greater affinity and specificity. High-dose estrogen is actually antiestrogenic.

The most commonly used estrogen is DES 5 mg three times daily. Other estrogen preparations that have been used are ethinyl estradiol, 3 mg daily, and conjugated equine estrogens, 30 mg daily.

Nausea is a common early side effect of DES. This may be avoided by increasing the dose in a stepwise fashion, that is, starting with 5 mg daily for 5 to 7 days, increasing to 5 mg twice daily for an additional 5 to 7 days, and then giving the full dosage of 5 mg three times daily. Increased nipple, aerolar, and axillary pigmentation is frequent. Fluid retention occurs in about one-third of cases and may aggravate or even precipitate congestive heart failure. The use of high-dose estrogens may be associated with thromboembolic phenomena.

Breakthrough or withdrawal uterine bleeding in postmenopausal women on estrogen therapy occurs in 40% of patients. This is usually of little clinical significance and responds to cessation of treatment or abates spontaneously with continued therapy but, if it persists, it may require further investigation.

Patients who respond to estrogen therapy initially but in whom the disease progresses later may respond to the sudden withdrawal of estrogens. *Estrogen rebound regression* was originally described by Escher in 1949 and occurs in up to 32% of estrogen responders (92, 93). The duration of rebound regression is usually 3 to 10 months, but Nestro and colleagues (94) re-

ported that the median duration is in excess of 18 months. *Tamoxifen withdrawal responses* have also been reported (95).

Androgens

Androgens were the first additive hormonal agents to prove useful in the treatment of metastatic breast cancer (96, 97). Androgens exert an antiestrogenic effect by complex interactions with three receptors: ER, PR, and androgen receptor (AR).

Androgens exert desirable subjective, hematopoietic, and anabolic effects (91). Patients treated with androgens may experience an increased sense of well-being, pain relief, increased appetite, and weight gain.

The side effects of androgens are predominantly those associated with the physiologic effects of male hormones, that is, virilization with frontal baldness, plethora, acne, hirsutism, fluid retention and, less commonly, an increased libido and clitoral hypertrophy. The virilizing effects vary with the androgenic preparation used and occur in more than 30% of patients treated with testosterone propionate and in 35 to 40% of patients treated with fluoxymesterone. The virilizing and therapeutic effects of androgens appear to be inseparable. Androgens with a 17 α methyl substitution, such as fluoxymesterone and methyltestosterone, may cause reversible cholestatic jaundice and, rarely, a multifocal hepatocellular necrosis termed peliosis hepatis (98, 99). Large areas of cystic hemorrhagic necrosis of peliosis hepatis may cause an abnormal liver scan that can be confused with metastases.

Fluoxymesterone (Halotestin) is the androgen of choice. The dosage for fluoxymesterone is 20 to 30 mg daily by mouth.

Combination Hormonal Therapy

Numerous combinations of hormonal agents have been evaluated in the treatment of metastatic breast cancer: fluoxymesterone plus ethinyl estradiol, DES plus testosterone propionate, tamoxifen plus megestrol acetate, tamoxifen plus medroxyprogesterone acetate, tamoxifen plus DES, tamoxifen plus prednisone, tamoxifen plus AG, tamoxifen plus fluoxymesterone, medroxyprogesterone acetate plus AG, and ethinyl estradiol plus medroxyprogesterone acetate. The results of all of these studies can be summarized with the statement that the combinations failed to demonstrate an advantage over the use of single-agent hormonal therapy. This is what would be anticipated because the mechanism of action of each of these agents is basically the same—inhibition of synthesis or action of estrogen.

New Agents for Hormonal Manipulation

LHRH AGONISTS

In animals, chronic treatment with supraphysiologic doses of luteinizing hormone-releasing hormone (LHRH) agonists causes: (*a*) decrease in gonadotropin (FSH and LH) excretion, (*b*) decrease in prolactin excretion, (*c*) decrease in plasma sex steroid concentration, (*d*) reduction in weight of secondary sexual organs, and (*e*) inhibition of the actions of the sex steroids at their target organs (100). LHRH analogues, therefore, act directly or indirectly on the pituitary, the gonads, and the target organs of the sex steroids. Schally and coworkers (41) have demonstrated significantly decreased tumor weight and volume in mouse rat mammary cancers treated with D-Trp^6-LH-RH (decapaptyl). Currently, the LHRH analogues, buserelin, goserelin, decapeptyl, and leuprolide, are being evaluated in clinical trials in advanced breast cancer.

Goserelin (Zoladex), an LHRH agonist, has been reported to yield a 45% objective response rate (10% complete response plus 35% partial response) in 134 premenopausal women with metastatic breast cancer (101). The highest response rates were seen in patients with local-regional metastases (62.5%) followed by osseous (46.7%), visceral (45%), and multiple sites (35.1%). Side effects included amenorrhea, vaginal spotting and, infrequently, headaches and sleep disturbances. Leuprolide was tested in 26 premenopausal women, with similar results (102).

CYTOTOXIC CHEMOTHERAPY OF BREAST CANCER

In 1969, Richard Cooper reported an 88% response (53 of 60 patients) to combination chemotherapy in hormone-resistant breast cancer (40). Cooper's regimen was CMFVP (cyclophosphamide, methotrexate, 5-fluorouracil, vincristine, prednisone). Following this report, clinical trials testing CMFVP and multiple variants of CMFVP were conducted (Table 54.7). Although none of these trials achieved a response rate of 88%, it was confirmed that in patients with metastatic breast

Table 54.7. Variations of the Cooper CMFVP Regimen in Advanced Carcinoma of the Breast[a]

Reference	Regimen	No. Evaluable	No. Responding	Response Rate (%)	Median Response Duration (mo.)
Cooper, 1969 (40)	CMFVP[b]	60	53	88	
Davis et al., 1974 (103)	CMFVP	74	31	42	7
CALGB 1974 (104)	CMFVP	82	41	50	6
Ramirez et al., 1975 (103)	CMFVP	48	30	62	
Brunner et al., 1975 (106)	CMFVP	91	45	49	8
WCSG 1975 (107)	$CMFPT_1$	60	35	58	11
Ramirez et al., 1975 (105)	CMFV	52	23	44	
Ahmann et al., 1975 (108)	CFVP	41	19	46	5
Canellos et al., 1976 (109)	CMFP	40	27	68	8
Ahmann et al., 1975 (108)	CFP	49	28	57	4
CALGB 1974 (104)	FVP	82	30	37	6
Brunner et al., 1975 (106)	CMV	46	15	33	8
Brunner et al., 1975 (106)	CMP	49	22	45	7
Canellos et al., 1976 (110)	CMF	93	49	53	6
Obis and Armentrout 1975 (111)	CMF	42	27	64	10
Creech et al., 1975 (112)	CMF	46	21	46	8
DeLena et al., 1975 (113)	CMF	41	27	66	4+
Total all Cooper variants		996	523	53	

[a]Modified from Kardinal CG. Chemotherapy. In: Donegan WL, Spratt JS, eds. Cancer of the breast. 2nd ed. Philadelphia: WB Saunders, 1979:405–447.
[b]C, cyclophosphamide; M, methotrexate; F, 5-fluorouracil; V, vincristine; P, prednisone; CALGB, Cancer and Leukemia Group B; WCSG, Western Cancer Study Group; T_1, triiodothyronine.

cancer, combinations of cytotoxic drugs could produce an objective response rate in the range of 60%, with 10 to 15% complete responses and a response duration of 8 to 12 months or more (103–113).

Combination chemotherapy was rapidly adopted as the treatment of choice for metastatic breast cancer because of its high predictable response rate and rapid onset of action. Conversely, hormonal therapy was relegated to a secondary or tertiary role since response rates were low and unpredictable and the onset of response might take 6 to 8 weeks. By the late 1970s, it was felt that with more fine-tuning of the pharmacokinetics of chemotherapeutic agents and the integration of drug pharmacokinetics with cell cycle kinetics, the potential for cytotoxic agents in breast cancer was almost limitless (114). The 1980s were foreseen as a period of great progress for breast cancer chemotherapy, but this did not happen. With few exceptions and only minor variations, the chemotherapeutic treatment of advanced breast cancer is the same today as it was in 1979.

SINGLE AGENTS IN BREAST CANCER

Breast cancer is responsive to all major classes of cytotoxic drugs: alkylating agents, antimetabolites, mitotic inhibitors, and the antitumor antibiotics. The available phase II data of single-

agent chemotherapy in advanced breast cancer are presented in Table 54.8. These data have been compiled from multiple phase II studies and should be interpreted as an indication of the actual response rate rather than as an absolute value. Presenting data in this manner entails many problems, since dosage levels or dosage schedules may be varied. More importantly, prior therapy and response criteria may not be specified (115, 116).

As can be seen in Table 54.8, there is often a marked difference in response rate for the same agent in patients who have been treated previously with chemotherapy and those who have never been treated: 0% versus 20% for ifosfamide, 6% versus 38% for cisplatin, 28% versus 52% for doxorubicin, and 13% versus 31% for mitoxantrone. This tremendous discrepancy in responses may well mean that an active drug might be overlooked if it is tested only in previously treated patients. For this reason, both the North Central Cancer Treatment Group and the Cancer and Leukemia Group B are now testing new phase II agents in chemotherapy-naive patients.

Although several single agents have activity in breast cancer, in current clinical practice they are generally incorporated into combination chemotherapeutic regimens. Most of the single agents will not be discussed individually; however, a few deserve special consideration, particularly the anthracycline analogues, including the hydroxyquinone, mitoxantrone, and the antimetabolite, 5-fluorouracil (5-FU). Renewed interest in 5-FU has been sparked by leucovorin modulation and by continuous infusion, an administration technique that has seemed to enhance activity.

Anthracycline Antibiotics

DOXORUBICIN (ADRIAMYCIN)

Adriamycin remains the most active single agent in the treatment of breast cancer. However, doxorubicin has a series of undesirable side effects such as cardiac toxicity, almost universal alopecia, and marked corrosiveness if the drug infiltrates the skin. This has prompted the search for a less toxic, equipotent analogue and for methods to reduce the cardiac toxicity of doxorubicin.

Utilizing the standard dosage schedule for doxorubicin of 50 to 75 mg/m^2 i.v. every 3 weeks, the incidence of cardiomyopathy increases dramatically once a cumulative dose of 550 mg/m^2 is exceeded. There have been three approaches to modifying the development of doxorubicin-induced cardiomyopathy: changing to a weekly dosage schedule, utilizing a continuous-infusion technique, and the addition of bispiperazinedione (ICRF-187).

Weekly doxorubicin has been studied extensively by Torti et al. (117). Endomyocardial biopsies were performed in 98 patients receiving 60 mg/m^2 doxorubicin every 3 weeks and in 27 patients receiving 20 mg/m^2 doxorubicin once weekly. At equivalent cumulative doses of doxorubicin, the weekly schedule was associated with significantly less anthracycline-induced cardiac damage, as confirmed by biopsy ($p = 0.002$). This response to weekly doxorubicin is equivalent to the every 3-week dose schedule (118).

Continuous-infusion doxorubicin in advanced breast cancer has been reported by the M.D. Anderson Cancer Center (119) to be associated with less cardiotoxicity. Investigators compared 48-hour and 96-hour continuous infusion to bolus intravenous administration. No difference in response rate was observed. At cumulative doses of 450 mg/m^2 or greater, the frequency of clinical congestive heart failure was 75% less in continuous-infusion groups ($p = 0.004$).

Bispiperazinedione (ICRF-187) has been reported to protect against doxorubicin-induced cardiac toxicity in women with advanced breast cancer (120). A group of 92 women was randomized to receive 5-fluorouracil plus doxorubicin plus cyclophosphamide (FDC) or FDC plus ICRF-187. At equivalent cumulative doses of doxorubicin, the group receiving ICRF-187 had significantly less cardiac toxicity ($p = 0.001$) and no alteration in antitumor effect.

A series of *doxorubicin analogues* are currently under study in clinical trials: epirubicin, idarubicin, 4'-deoxydoxorubicin, pirarubicin, and rubidisone. Among these, the agents studied most extensively in breast cancer have been epirubicin and idarubicin.

EPIRUBICIN

In a small series of previously untreated patients with breast cancer, *epirubicin* had a response rate of 67% (16 of 24 patients), which is equivalent to that of doxorubicin (121). The advantage of epirubicin is that it is associated with less cardiac toxicity than doxorubicin and with decreased hepatic clearance, which means that the drug can be used in patients with advanced liver metastases (122).

Table 54.8. Compiled Phase II Data of Single-Agent Chemotherapy in Advanced Breast Cancer[a]

Agent	No. Evaluable	CR[b]	PR	$\frac{CR+PR}{\text{No. evaluable}} \times 100$
Alkylating agents				
Chlorambucil	54	0	11	20%
Cyclophosphamide	189	0	60	32%
Ifosfamide (prior chemotherapy)	6	0	0	0
Ifosfamide (no prior chemotherapy)	20	0	4	20%
L-PAM (melphalan)	75	0	17	23%
Nitrogen mustard	92	0	32	35%
Prednimustine (prior chemotherapy)	32	0	10	31%
Prednimustine (no prior chemotherapy)	18	4	7	61%
Thiotepa	162	0	38	23%
Drugs with alkylating-like activity				
BCNU	76	0	16	21%
CCNU	155	0	18	12%
Methyl-CCNU	82	0	3	4%
Carboplatin	14	0	0	0
Cisplatin (prior chemotherapy)	80	1	4	6%
Cisplatin (no prior chemotherapy)	50	12	6	36%
Dibromodulcitol	104	1	7	8%
MGBG	96	1	6	7%
Streptonigrin	9	0	2	22%
Streptozotocin	19	0	0	0
Antimetabolites				
Arabinosyl cytosine	64	0	6	9%
5-Azacytidine	27	0	2	7%
Fludarabine	18	0	1	6%
5-Fluorouracil	1142	0	320	28%
5-FU (infusion)	166	2	43	27%
5-FU + leucovorin	228	6	62	30%
Ftorafur	31	2	16	58%
Methotrexate	259	0	87	34%
Triazinate	52	0	2	4%
Trimetrexate	40	0	3	8%
Mitotic inhibitors				
Vinblastine	95	0	19	20%
Vincristine	164	6	72	20%
Vindesine	141	1	28	21%
Navelbine (no prior chemotherapy)	24	4	9	54%
Etoposide (VP-16)	234	2	17	8%
Temposide (VM-26)	42	0	3	7%
Antitumor antibiotics				
Anthracyclines				
Doxorubicin (prior chemotherapy)	428	13	106	28%
Doxorubicin (no prior chemotherapy)	92	3	45	52%
4'Deoxydoxorubicin	27	0	1	4%
Epirubicin	140	6	43	34%
Idarubicin	130	6	42	37%
Menogaril	25	0	4	16%
Pirarubicin	71	4	18	31%
Rubidazone	88	1	4	6%
Hydroxyquinones				
Bisantrene	229	2	27	13%
Mitoxantrone (prior chemotherapy)	411	6	48	13%
Mitoxantrone (no prior chemotherapy)	217	13	55	31%

continues

Table 54.8. *Continued*

Agent	No. evaluable	CR[a]	PR	$\frac{CR+PR}{\text{No. evaluable}} \times 100$
Other				
Aclacinomycin A	22	0	0	0
Bleomycin	22	0	1	5%
Mitomycin C	70	0	29	41%
Miscellaneous				
α Interferon-recombinant	76	0	2	3%
Hexamethylmelamine	39	0	11	28%
Spirogermanium	103	1	2	3%

[a]Modified from data in Livingston RB, Carter SK. Single agents in cancer chemotherapy. New York: IFI Plenum, 1970, and in Anonymous: Compilation of phase II results with single antineoplastic agents (series). Cancer Treatment Symposia, Vol 4, 1985:4.
[b]CR, complete response; PR, partial response; BCNU, *N,N*-bis(2-chloroethyl)-*N*-nitrosourea; CCNU, *N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea; MGBG, methylglyoxal bis(guanylhydrazone).

IDARUBICIN (4-DEMETHOXYDAUNORUBICIN)

Idarubicin is less cardiotoxic than either doxorubicin or daunorubicin and has the advantage that it can be given orally (123, 124). The drug is active in metastatic breast cancer, but it is still too early to determine if idarubicin and doxorubicin are equivalent in terms of breast cancer responsiveness (125).

MITOXANTRONE

Mitoxantrone is a synthetic hydroxyquinone that is related structurally to doxorubicin. Mitoxantrone is an active drug in breast cancer as well as in acute leukemia and lymphoma (126). Clinically significant cardiac toxicity is infrequent but occurs in approximately 3% of patients receiving cumulative doses of 175 to 250 mg/m^2. Significant alopecia occurs in fewer than 10% of cases. Nausea and vomiting are uncommon. Mitoxantrone appears not to be a vesicant if it is infiltrated. The dose-limiting toxicity is myelosuppression, which may be prolonged. The current consensus is that mitoxantrone is an active drug in breast cancer and is less toxic than doxorubicin, but its response rate is approximately 10% less than that of doxorubicin (127–129). Mitoxantrone probably has a role in the treatment of frail elderly patients or patients with breast cancer who have an intercurrent medical illness (130, 131). The dosage of mitoxantrone as a single agent is 10 to 14 mg/m^2 i.v. every 3 weeks.

5-Fluorouracil

5-FU was synthesized in 1957 as an antitumor agent by Heidelberger and coworkers at the University of Wisconsin and was rapidly introduced into clinical practice (132). As a single agent, 5-FU became the most commonly used nonhormonal drug in the treatment of advanced breast cancer (133). With the introduction of combination chemotherapy in the 1970s, 5-FU was incorporated into most commonly used regimens, including Cooper's original CMFVP.

5-FU PLUS LEUCOVORIN

There is now renewed interest in 5-FU since it has been demonstrated that leucovorin (LV) can potentiate 5-FU cytotoxicity. Several clinical trials of 5-FU plus LV in previously treated patients with advanced breast cancer have now been reported. Swain et al. (134), using a 500-mg/m^2 dose of LV plus 5-FU, 375 mg/m^2 given daily for 5 consecutive days, noted a 24% objective response rate in 54 previously treated patients. Jabboury et al. (135), using LV 200 mg/m^2/day over 30 minutes plus 5-FU 200 mg/m^2/day by continuous infusion for 5 to 12 days, reported a 60% response (12 of 20 patients) in patients previously treated with chemotherapy. These results are promising and should be confirmed in larger clinical trials.

CONTINUOUS INFUSION OF 5-FU

The continuous infusion of 5-FU was introduced by Lokich et al. (136) and appears to have utility in previously treated patients with advanced breast cancer. Jabboury et al. (137) evaluated 5-FU, 250 mg/m^2/day, given by continuous infusion. The median duration of the infusion was 65 days, with a range of 19 to 508 days. Five of 32 patients responded. The main toxicities were stomatitis in 13 patients, the hand-foot syndrome (palmar-plantar erythrodysesthesia) in 6, and Coombs'-positive hemolytic

anemia in 2. Myelosuppression was uncommon. Using similar regimens, Hatfield et al. (138) reported a 28% response (7 of 25 patients) and Huan et al. (139) reported a 53% response (15 of 28 patients). 5-FU given by continuous infusion should be evaluated in previously untreated patients with metastatic breast cancer and should also be incorporated into combination chemotherapy regimens.

COMBINATION CHEMOTHERAPY

Since breast cancer is responsive to all of the major classes of chemotherapeutic agents, it is uniquely suited for combination chemotherapy. The use of combination chemotherapy has more than doubled the response rates of single agents. However, given the drugs currently available, the results for all commonly used combinations are more or less the same: a 60% objective response, with a 10 to 15% complete response and a response duration of 8 to 12 months. Since the results are essentially equivalent, it is difficult to make a strong case in favor of any given regimen.

A number of factors are predictive of a good response to chemotherapy: good performance status, a limited number of disease sites, response to prior hormonal therapy, and soft tissue-dominant metastases. Conversely, other factors are associated with a decreased probability of response: bone-dominant or liver-dominant metastases, prior chemotherapy, prior radiation therapy, and decreased lymphocyte counts. Menopausal status does not influence response to chemotherapy in patients with advanced breast cancer (140).

DNA flow cytometry and S-phase fraction have been reported to be predictive of response to cytotoxic drugs (141, 142). There seems to be a strong correlation between S-phase fraction and response to chemotherapy. All of 12 patients with S-phase fraction of 10% or more responded to treatment, and 6 of these responses were complete. DNA ploidy and histologic grade did not correlate with response.

FIRST-LINE CHEMOTHERAPY

First-line chemotherapeutic regimens for metastatic breast cancer fall into one of two overlapping categories: the Cooper CMFVP variants (Table 54.7) and the Adriamycin-(doxorubicin-) based combinations (142). Each has strong supporters, but it is difficult to define which represents "standard" or "state of the art" therapy. The dosage schedules for commonly used first-line chemotherapy regimens are outlined in Tables 54.9 (108, 143–148) and 54.10 (119, 149–155).

Table 54.9. First-Line Non-Adriamycin Chemotherapy Regimens for Metastatic Breast Cancer[a]

1. *CMFVP* (Cancer and Leukemia Group B) (143, 144)
   Cyclophosphamide 100 mg/m² p.o. days 1–14
   Methotrexate 40 mg/m² i.v. days 1 and 8
   5-FU 500 mg/m² i.v. days 1 and 8
   Vincristine 1 mg/m² i.v. days 1 and 8
   Prednisone 40 mg/m² p.o. days 1–14
   No treatment days 15–28; repeat on a 28-day cycle
2. *CMF* (National Cancer Institute of Milan) (145)
   Cyclophosphamide 100 mg/m² p.o. days 1–14
   Methotrexate 30–40[b] mg/m² i.v. days 1 and 8
   5-FU 400–600[b] mg/m² i.v. days 1 and 8
   No treatment days 15–28; repeat on a 28-day cycle.
3. *CMF* (National Cancer Institute of Milan) (146)
   Cyclophosphamide 400 mg/m² i.v. day 1
   Methotrexate 30 mg/m² i.v. days 1 and 8
   5-FU 400 mg/m² i.v. days 1 and 8
   Cycle repeated every 28 days.
4. *CMFP* (Eastern Cooperative Oncology Group) (147, 148)
   Cyclophosphamide 100 mg/m² p.o. days 1–14
   Methotrexate 30–40[b] mg/m² i.v. days 1 and 8
   5-FU 400–600[b] mg/m² i.v. days 1 and 8
   Prednisone 40 mg/m² p.o. days 1–14
5. *CF* (Mayo Clinic) (108, 147)
   Cyclophosphamide 150 mg/m² i.v. daily × 5
   5-FU 300 mg/m² i.v. daily × 5
   Prednisone 30 mg/day for 14 days, then 20 mg/day for 7 days, and 10 mg/day thereafter.
   5-day CF cycles repeated every 5 weeks.

[a] From Kardinal CG. Chemotherapy. In: Donegan WL, Spratt JS, eds. Cancer of the breast. 2nd ed. Philadelphia: WB Saunders, 1988:405–447.
[b] Lower dose level given to patients ≥65 years old.

CMF Versus CAF

CMF (cyclophosphamide, methotrexate, 5-fluorouracil) are the active components of the original five-drug Cooper CMFVP regimen. CMF, CMFVP, and other variants have been tested extensively against CAF (cyclophosphamide, Adriamycin, 5-fluorouracil) and CAFVP. The Cancer and Leukemia Group B (CALGB) has conducted two such trials (152, 156). In the first trial, CAFVP versus CMFVP was tested. The overall response rate for CAFVP was 71% com-

Table 54.10. First-Line Adriamycin (Doxorubicin)-Containing Regimens for Metastatic Breast Cancer[a,b]

1. AC (University of Arizona) (149)
   Adriamycin 40 mg/m² i.v. day 1
   Cyclophosphamide 200 mg/m² p.o. days 3–6
   Repeat cycle of 21 days.
2. AC (NSABP) (150, 151)
   Adriamycin 60 mg/m² day 1
   Cyclophosphamide 600 mg/m² day
   Repeat cycle every 21 days.
3. CAF (Cancer and Leukemia Group B) (152, 153)
   Cyclophosphamide 100 mg/m² p.o. days 1–14
   Adriamycin 25 mg/m² i.v. days 1 and 8
   5-FU 500 mg/m² i.v. days 1 and 8
   No treatment days 15–18; repeat cycle every 28 days.
4. CAF (Southeastern Cancer Study Group) (154, 155)
   Adriamycin 50 mg/m² i.v. day 1
   Cyclophosphamide 500 mg/m² i.v. day 1
   5-FU 500 mg/m² i.v. day 1
   Repeat cycle every 3 weeks.
5. FAC (M.D. Anderson Cancer Center) (119)
   5-FU 500 mg/m² i.v. days 1 and 8
   Adriamycin 50 mg/m² i.v. by continuous infusion over 48 to 96 hours
   Cyclophosphamide 500 mg/m² i.v. on day 1
   Repeat cycle every 28 days.

[a]From Kardinal CG. Chemotherapy. In: Donegan WL, Spratt JS, eds. Cancer of the breast. 2nd ed. Philadelphia: WB Saunders, 1979:405–447.
[b]The total cumulative dose of Adriamycin in all regimens should not exceed 450 mg/m².

pared to 50% for CMFVP ($p = 0.002$), but the response duration and survival were equivalent. Although this was a large study with 396 patients entered, only 302 were evaluable; 94 cases were ineligible or inevaluable. In addition, the CMFVP was given in two different dosage schedules, continuous and intermittent, which makes the data interpretation difficult.

In the second CALGB trial, CMF versus CAF versus CAFVP was tested. This trial also included the testing of nonspecific immunotherapy with the methanol extraction residue of bacillus Calmette-Guérin vaccine (MER). MER produced toxicity without apparent response or survival benefit and was dropped from the trial. The authors concluded that CAF and CAFVP are equivalent and that both are superior to CMF, but the response data presented do not support that conclusion.

The Southeastern Cancer Study Group tested CAF versus CMFVP (154). Again, this was a large study with a high rate of inevaluability (362 patients entered, 265 evaluable). The response rate for CAF was 55% and for CMFVP 40% ($p = 0.01$), but response duration and survival were the same.

In none of the above clinical trials was the response rate of CMF or CMFVP equal to the mean response rate of 52% reported in earlier trials (Table 54.7) or to the response rate of 53% for CMFP recently reported by the North Central Cancer Treatment Group (147). It must be concluded that CAF might have a slight advantage over CMF; however, the data are not compelling enough that CAF should be adopted as the standard therapy for metastatic breast cancer (137). More innovative therapeutic regimens are needed and must be tested in previously untreated patients if progress is to be made in the treatment of hormone-resistant metastatic breast cancer.

Adriamycin Analogues in Combination

EPIRUBICIN

Epirubicin (E) is being actively investigated in combination chemotherapy trials in previously untreated women with advanced breast cancer. FEC (5-FU 500 mg/m² days 1 and 8, epirubicin 50 mg/m² day 1, cyclophosphamide 500 mg/m² day 1) has been compared to FAC (Adriamycin 50 mg/m²). Activity was evaluated in 443 patients: 222 in the FEC arm and 221 in the FAC arm. The response rates were 53.6% for FEC and 56.5% for FAC. Response durations were equivalent. Myelosuppression and gastrointestinal toxicity were significantly less in the FEC-treated group. There were four episodes of cardiac toxicity in the FAC group and one in the FEC group (158). A similar response rate for FEC (46%) versus FAC (44%) was reported by Lopez et al. (159).

IDARUBICIN

Idarubicin has been tested in combination with 5-FU and cyclophosphamide in previously untreated women with metastatic breast cancer (160). Idarubicin, 15 mg/m² given by mouth on days 1, 2, and 3, was administered with 5-FU, 500 mg/m² given i.v. on days 1 and 8, and cyclophosphamide, 500 mg/m² given i.v. on day 1. An objective response was observed in 23 (5 complete responses plus 18 partial responses) of 42 evaluable patients (55%). The median duration of response was 8 months. Toxicity was mild. Left ventricular ejection fraction deter-

minations showed no detrimental change. Alopecia was minimal. Adriamycin analogues are, indeed, of interest and should be further evaluated in advanced breast cancer. These drugs should also be tested in the adjuvant setting.

MITOXANTRONE COMBINATIONS

Mitoxantrone (Novantrone = N) combinations have been evaluated by Bennett et al. (161) as first-line treatment in metastatic breast cancer. They compared CNF to CAF. Initial doses were 500 mg/m^2 of cyclophosphamide and 5-FU, with either 10 mg/m^2 of mitoxantrone or 30 mg/m^2 of doxorubicin. Each drug was administered intravenously on day 1 and repeated every 3 weeks. A total of 331 patients, 167 CNF and 164 CAF, were entered. The response rate for each of the treatment groups was disappointing and lower than would be anticipated from available single-agent data: 29% for the CNF group and 37% for the CAF group. There was no difference in median response duration or survival. Cardiotoxicity and alopecia were significantly less in the CNF group.

In a French trial, 142 patients were randomized to CNF or CAF. An objective response was obtained in 28 of 66 evaluable patients in the CAF group (42.4%) and 30 of 71 (42.2%) in the CNF group. Median response duration for CNF was 34+ weeks and for CAF was 37+ weeks (162). Mitoxantrone combinations may be somewhat less active than Adriamycin combinations but they are significantly less toxic.

CISPLATIN COMBINATIONS

Cisplatin as a single agent has significant activity in previously untreated patients with metastatic breast cancer (Table 54.8). Cisplatin combinations are also being tested as first-line and second-line therapy. Several clinical trials in previously untreated patients testing *CAF* (P = cisplatin), cisplatin plus etoposide, and MVAC (methotrexate, vinblastine, Adriamycin, and cisplatin) have been reported (163–165). Cisplatin combinations are active in metastatic breast cancer but not more active than the more commonly used, less toxic CMF and CAF regimens.

ALTERNATING NON-CROSS-RESISTANT CHEMOTHERAPY IN BREAST CANCER

Breast cancer provides an ideal setting for alternating non-cross-resistant chemotherapy (166, 167). Since doxorubicin is not cross-resistant with CMF, one might anticipate that alternating CMF combinations with doxorubicin combinations would result in increased response rates and response durations. A large series of these trials have been conducted and are summarized in Table 54.11 (148, 155, 168–175). These trials showed marginally significant improvement in overall response with no improvement in survival. Alternating non-cross-resistant chemotherapy in metastatic breast cancer has not lived up to the theoretical expectations.

SECOND-LINE CHEMOTHERAPY

Multiple regimens have activity in metastatic breast cancer in patients previously treated with CMF/CMFVP or CAF. If the patient has not received an anthracycline as part of the first-line chemotherapeutic regimen, clearly one should be incorporated into the second-line program since doxorubicin is the most active single agent in previously treated patients with breast cancer (Table 54.8). If the patient has already been treated with an anthracycline and is refractory to hormonal therapy, the choice is more difficult. Infusion chemotherapy with 5-FU or 5-FU plus leucovorin could be considered (see pp 960–961); infusion vinblastine, a mitomycin C-containing regimen, or possibly even a cisplatin-based combination could be used. None of these is clearly superior, responses are often brief, and complete remissions are rare. Although many patients may obtain temporary palliation with second-line chemotherapy regimens, quality of life issues must be considered in patients with advanced cancer (176, 177). Clearly more effective, less toxic therapy is needed. It cannot be too strongly emphasized that participation in clinical trials is the only means to that end.

Doxorubicin

Doxorubicin used as a single agent yields objective responses in 28% of patients previously treated with CMF/CMFVP. Doxorubicin has also been evaluated in a variety of combinations in previously treated patients. *VATH* (vinblastine, Adriamycin, thiotepa, Halotestin) was one of the earliest regimens tested (178, 179). Halotestin (fluoxymesterone) was incorporated into the VATH regimen as a bone marrow stimulant. However, as an androgen, it yields a 20% objective response in unselected patients with metastatic breast cancer. A 52% objective response in 19 patients refractory to

Table 54.11. Randomized Trials Comparing a Single Regimen with Alternating Non-Cross-Resistant Regimens

Regimen[a]	No. of Patients	Response Rate (%) PR[b] + CR	Response Rate (%) CR	Median Duration (mo) Response	Median Duration (mo) Survival	Reference
CFP versus	23	48	15	12	24	Ahmann et al., 1978 (169)
A/L-PAM	27	52	9	13	21	
CFP → A/L-PAM or A/L-PAM ⇔ CFP (2)	50	68[c]	10	16	22	
AC versus	26	50	12	10		Kennealey et al., 1978 (170)
AC ⇔ MF (1)	22	55	32	10		
CAF versus	46	63	13			Tormey et al., 1979 (171)
DAV ⇔ CMF (3)	48	71	10			
CFP versus	18	18	0	9	14	Nimelo et al., 1982 (172)
CA versus	42	42	2	12	18	
CFP ⇔ CA (1)	20	63[c]	0	11	19	
CMFP versus	135	59	14	11	19	Tormey et al., 1983 (148)
CMFP ⇔ AV (2)	176	58	14	10	16	
CAF versus	66	29	2	8	15	Vogel et al., 1984 (155)
CAF ⇔ CAMELEON (3)	91	17[c]	0	11	16	
CFP versus	41	46	7		18	Creagan et al., 1984 (173)
CAF ⇔ CFP (4)	45	49	4		11	
CMF ⇔ A ⇔ Mito C (1)	28	67	46	14		Cruciani et al., 1987 (174)
CAF versus	497	54	11		17	Aisner et al., 1988 (175)
VATH → CMFVP versus		58	11		17	
VATH ⇔ CMFVP (1)		52	12		17	

[a]*Modified from* Henderson IC, Hayes DF, Come S, Harris JR, Canellos G. New agents and new medical treatments for advanced breast cancer. Semin Oncol 1987;14:34–64.
[b]*Number cycles before first cross-over and/or number of cycles between each subsequent regimen alteration.*
[c]PR, partial response; CR, complete response; C, cyclophosphamide; A, doxorubicin; F, 5-fluorouracil; P, prednisone; L-PAM, L-phenylalanine mustard; M, methotrexate; D, dibromodulcitol; V, vincristine; CAMELEON, cytosine arabinoside, methotrexate, leucovorin rescue, vincristine; Mito C, Mitomycin C; VATH, vinblastine, adriamycin, thiotepa, halotestin.
[c]$p = 0.07$
[c]$p = 0.025$; $p = 0.08$ (other differences not statistically significant)

CMF/CMFVP was reported for the 5-day VATH regimen, and a 49% objective response was seen in 29 patients with the 1-day VATH regimens (Table 54.12)(179–182). However, in a larger clinical trial, the Eastern Cooperative Oncology Group reported a 38% response (32 of 84 patients) to VATH in previously treated patients with advanced breast cancer (183). VATH has also been tested in previously untreated patients (58% objective response) and as alternating non-cross-resistant therapy with CMFVP (Table 54.11) (175).

Several other clinical trials have tested doxorubicin in combination with a vinca alkaloid in previously treated patients. Tannir et al. (184) from the M.D. Anderson Cancer Center reported a 43% response (18 of 42 patients) to sequential *continuous infusion doxorubicin and vinblastine* in patients previously treated with CMFVP. Doxorubicin was given as 25 mg/m^2/day as a continuous intravenous infusion for 2 days, followed by vinblastine 1.4 mg/m^2/day for 4 days. The cycle was repeated every 3 weeks. The North Central Cancer Treatment Group randomized 173 previously treated patients to doxorubicin (D) versus *DVM* (vincristine, mitomycin C) (180). Doxorubicin dose was 60 mg/m^2; the DVM dosage schema are in Table 54.12. There was an objective response of 24% (20 of 83 patients) to D and 37% (29 of 78 patients) to DVM ($p = 0.036$). A similar DVM trial was performed at the University of Glasgow; a 39% response (15 of 38 patients) was reported for DVM (185).

The *VAM* combination of vinblastine, adriamycin, and mitomycin C has been tested in two clinical trials. Osties and Park from Columbia University evaluated 15 previously treated patients; 11 responded (3 complete responses, 8 partial responses) for a response rate of 73% (186). However, Luikart et al. (181) observed only a 33% response (9 of 27 patients, with 3

Table 54.12. Second-Line Chemotherapy Regimens for Advanced Breast Cancer[a]

1. *One-Day VATH* (Cancer and Leukemia Group B) (179)
   - Vinblastine 4.5 mg/m² day 1
   - Adriamycin 45 mg/m² day 1
   - Thiotepa 12 mg/m² day 1
   - Haloperidol 10 mg p.o. tid continuously
   - Repeat every 21 days.
2. *DVM* (North Central Cancer Treatment Group) (180)
   - Doxorubicin 50 mg/m² days 1 and 28
   - Vincristine 1 mg/m² days 1 and 28
   - Mitomycin C 10 mg/m² day 1
   - Repeat cycle every 8 weeks
3. *VAM* (Yale University) (181)
   - Vinblastine 6 mg/m² days 1 and 28
   - Adriamycin 30 mg/m² days 1 and 28
   - Mitomycin C 10 mg/m² day 1
   - Repeat cycle every 8 weeks
4. *Vinblastine + Mitomycin C* (University of Arizona) (182)
   - First two cycles:
     - Mitomycin C 10 mg/m² i.v. days 1 and 28
     - Vinblastine 5 mg/m² i.v. days 1, 14, 28, 42
   - Subsequent cycles:
     - Mitomycin C 10 mg/m² i.v. day 1
     - Vinblastine 5 mg/m² days 1 and 21
   - Repeat every 6 to 8 weeks

[a]From Kuminal CG. Chemotherapy. In: Donegan WL, Spratt JS, eds. Cancer of the breast. 2nd ed. Philadelphia: WB Saunders, 1989:405–447

complete responses and 6 partial responses) to VAM. The VAM dosage schema is in Table 54.12.

It appears that the addition of a vinca alkaloid enhances the response of doxorubicin in previously treated patients. The vinca alkaloid of choice is vinblastine because of its lack of neurotoxicity and equivalent efficacy to vincristine in advanced breast cancer. Mitomycin C and even thiotepa might enhance the response of the doxorubicin-vinblastine combination.

Vinca Alkaloids

In the management of previously treated patients with advanced breast cancer, *vinblastine* has assumed a prominent role which may not be totally justified. VATH and VAM have already been discussed (Table 54.12). *Vinblastine* as a single agent administered in continuous infusion has been reported to give a 40% objective response (12 of 30 patients, with 1 complete response and 11 partial responses) in refractory advanced breast cancer (187). Vinblastine was administered as a 5-day continuous infusion at 1.4 to 2.0 mg/m²/day. Myelosuppression was mild to moderate at doses between 1.4 and 1.8 mg/m² but became severe at 2 mg/m². Two subsequent studies utilizing 1.2 to 1.8 mg/m² of vinblastine by continuous i.v. infusion have been reported (188, 189). No responses were observed in either (0 of 17 patients in the first trial and 0 of 15 patients with measurable disease in the second trial). No response (0 of 18) were observed in a trial of continuous infusion *vincristine* (190). The efficacy of continuous-infusion vinca alkaloids as single agents in advanced, previously treated breast cancer is questionable.

Vinblastine plus mitomycin C has been evaluated in several clinical trials in advanced, previously treated breast cancer. A 40% objective response rate (12 of 30 cases) was reported in a trial evaluating mitomycin C, 20 mg/m² given on day 1, and vinblastine, 0.15 mg/kg given on days 1 and 21. The cycles were repeated every 6 to 8 weeks (191). Similarly, a 32% response (7 of 22 patients) was noted in a second trial of mitomycin C and vinblastine (dosage schedule in Table 54.12) (182). However, only a 14% response to mitomycin C and vinblastine (3 of 22) was noted in a third reported trial (192). Mitomycin C plus vinblastine appears to be an active combination in advanced, refractory breast cancer. Median response durations, however, are relatively short, between 127 and 164 days for the trials reported above.

Mitomycin C

Mitomycin C is an active drug in breast cancer, with a response rate of 38 to 41% in previously untreated patients and 20% in previously treated patients (193). The mitomycin C combinations of DVM, VAM, and mitomycin C plus vinblastine has already been discussed, and the dosage schema for these regimens are in Table 54.12. A variety of other mitomycin C combinations have been tested in phase II studies in previously treated patients with advanced breast cancer: mitomycin C plus mitolactol plus doxorubicin; mitomycin C plus mitolactol plus vinblastine; mitomycin C plus melphalan plus vincristine; mitomycin C plus melphalan plus methotrexate; mitomycin C plus teniposide; mitomycin C plus mitoxantrone; and mitomycin C plus a variety of hormonal agents including tamoxifen, fluoxymesterone, and megestrol acetate. The response rates in each of these trials were disappointing. However, mitomycin C does have a role in the treatment of advanced, pre-

viously treated breast cancer, particularly when it is used in combination with vinblastine, doxorubicin, or both (194).

Cisplatin and Etoposide

Cisplatin plus etoposide has been very active and even curative therapy in testicular and ovarian germ cell tumors. This combination has been tested extensively in previously treated patients with advanced breast cancer (195–197). The toxicity is substantial, and treatment-related deaths from leukopenia and sepsis as well as from renal failure have been reported. Cisplatin plus etoposide is active in advanced breast cancer but cannot be recommended with any degree of enthusiasm.

AUTOLOGOUS BONE MARROW TRANSPLANTATION

Dose intensity is a significant factor influencing response rate and duration in the chemotherapy of metastatic breast cancer (198). Based on the step dose-intensity curves for many chemotherapeutic agents, it is hypothesized that increasingly higher doses should be associated with higher response rates and even potential cure in advanced breast cancer (198, 199). A safe means of administration of potentially lethal doses of chemotherapy is needed to test this hypothesis. Autologous bone marrow transplant (ABMT) is currently being tested as a technique for safely administering these dose levels. It should be kept in mind that the transplant per se is not therapeutic but is a salvage technique for the administration of high-dose chemotherapy. Inherent in ABMT is the fact that the dose-limiting toxicity of the chemotherapeutic agent employed is myelosuppression. Toxicity to the gastrointestinal, cardiac, neurologic, and other systems is not influenced by this technique.

Antman and Gale (200) have reviewed 27 trials of ABMT involving 172 breast cancer patients. The overall response rate was 58%. Response rates were highest in trials involving multiple alkylating agents (76%) or in previously untreated patients (81%). These data must be interpreted cautiously since each individual trial was small (from 2 to 21 patients; mean 6.4 per trial) and the data were pooled for analysis. Response durations were only 3 to 8 months in previously untreated patients and 2 to 5 months in previously treated patients, which is similar to those of standard first- or second-line chemotherapy. Nonetheless, it has been demonstrated that single- or multiple-agent, high-dose chemotherapy, with or without total body radiation with ABMT, can induce remissions in patients with advanced breast cancer that is resistant to conventional chemotherapy.

It is by no means clear which chemotherapeutic agents should be used prior to ABMT or which doses or dose schedules are most appropriate. Other unknowns associated with ABMT are whether surgical debulking or total body or regional radiotherapy should accompany the procedure. ABMT in advanced breast cancer remains a highly experimental procedure and cannot be justified outside the setting of a well-designed, controlled clinical trial.

CHEMOHORMONAL THERAPY

Since breast cancers are heterogeneous, that is, composed of varying proportions of receptor-positive and receptor-negative cells, the use of combined hormonal and cytotoxic drug therapy should be more effective than either alone if the following two assumptions are valid: (*a*) there is a differential response between receptor-positive and receptor-negative cells at both chemotherapy and hormonal therapy; and (*b*) there is no antagonism of chemotherapy-induced cytotoxicity by hormonal agents or, conversely, no antagonism of the effects of hormonal therapy by chemotherapy. If these assumptions are true, the response rates for combined therapy should be at least additive.

With regard to the first assumption, estrogen receptor (ER) is strongly predictive of response to hormonal therapy; however, there is no differential response to cytotoxic chemotherapy between ER+ and ER-negative (ER−) tumors. This was confirmed in a CALGB trial of CAF (cyclophosphamide, Adriamycin, 5-fluorouracil) chemotherapy in which patients were stratified by ER status. The response rates of ER+ and ER− patients to CAF were identical (56%). However, the time to treatment failure, response duration, and survival were significantly longer in ER+ patients than in ER− patients (Table 54.13) (201). This lack of differential response of ER+ and ER− breast cancer to cytotoxic chemotherapy has been confirmed in a second prospective, randomized trial (202). Cytotoxic chemotherapy kills ER+ and ER− cells indiscriminately. At least with reference to ER+ cells, chemotherapy and hormonal therapy are competing for the same cell population.

Table 54.13. Results of CAF Treated Patients by ER Status[a]

	Median (mo)		
ER + PR/Total	TTF[b]	Response Duration	Survival
ER + 15 + 17/57 (50%)	15.3	19.1	23.7
ER − 9 + 33/75 (56%)	8.3	8.9	15.7
p value	0.0061	0.0015	0.0058

[a]Modified from Kardinal CG, Perry MC, Korzun AH, Wood W. Lack of differential response of estrogen receptor positive (ER +) vs ER negative (ER −) breast cancer to Cytoxan + Adriamycin + 5-fluorouracil (CAF) chemotherapy (abst). Proc Am Soc Clin Oncol 1986;5:74.
[b]Time to treatment failure

With regard to the second assumption, antagonism between the antiestrogenic drug, tamoxifen, and various chemotherapeutic agents has been demonstrated in human breast cancer cell lines in tissue culture (203). Since tamoxifen acts as a cell cycle inhibitor arresting cells in G_1 and G_0, and since chemotherapeutic agents are most active in actively proliferating cell systems, the antagonism between tamoxifen and chemotherapy is not unexpected.

Several clinical trials have been reported which evaluate combination chemotherapy with and without tamoxifen. The largest of these trials was conducted by the CALGB (153, 204). In the CALGB trial, patients were stratified by ER status, dominant site of metastatic disease, menopausal status, and prior adjuvant chemotherapy. They were then randomized to CAF chemotherapy (as in Table 54.11), with or without tamoxifen 10 mg orally twice daily. A total of 474 patients were entered; less than 5% were ineligible or inevaluable. Regardless of ER status or menopausal status, the addition of tamoxifen conferred no significant advantage or disadvantage in response rate, response duration, time to treatment failure, or survival over CAF alone. The North Central Cancer Treatment Group, in a similar trial, was unable to demonstrate a difference in time to disease progression or survival by the addition of tamoxifen to CFP (cyclophosphamide, 5-FU, prednisone) over CFP alone (205). These trials have confirmed that the mere addition of a hormonal agent, such as tamoxifen, to combination chemotherapy, such as CAF or CFP, adds nothing to the response rate or response duration regardless of receptor status. This confirms in vivo that chemotherapy kills breast cancer cells indiscriminately regardless of receptor status. It also confirms that chemotherapy and hormonal therapy compete for the same pool of ER + cells.

Despite negative therapeutic results, chemohormonal trials are important clinically since they verify that advanced receptor-positive breast cancer should be treated sequentially and not concurrently with hormonal therapy and chemotherapy. Advanced ER + breast cancer should be treated initially with hormonal manipulation; at treatment failure, combination chemotherapy should be initiated. Hormonally responsive breast cancer may respond to secondary, tertiary, or even quaternary forms of endocrine manipulation before cytotoxic drugs are necessary. The exception to the rule is the ER + patient with life-threatening metastatic disease in whom rapid onset of chemotherapeutic action is necessary.

SYSTEMIC ADJUVANT THERAPY

Perioperative Adjuvant Trials

The first prospective, randomized breast cancer adjuvant therapy trial was conducted by the National Surgical Adjuvant Breast Project (NSABP) between April 1958 and October 1961. This initial trial, NSABP B-01, was designed to test the hypothesis that chemotherapy would cure patients by destroying tumor cells disseminated in the blood at the time of surgery. A total of 826 patients were randomly assigned to *perioperative thiotepa* (TT) or placebo administered at the time of surgery and on each of the first 2 postoperative days. One subset of patients, premenopausal women with four or more positive nodes who received perioperative TT, had significantly greater 5- and 10-year survival (56.3% versus 24.3% 5-year and 24.8% versus 13.5% 10-year) than those who received placebo (206–208).

The Scandinavian Adjuvant Chemotherapy Group chaired by R. Nissen-Meyer randomized 1026 women (507 treated and 519 controls) to no treatment or to cyclophosphamide (5 mg/kg/day for 6 days, with the first injection immediately after wound closure). With 20-year follow-up, the relapse rate was 60.5% in the control group and 48% in the treatment group ($p < 0.001$) (209).

Adjuvant Therapy of Stage II Breast Cancer

THE NSABP L-PAM STUDIES

Between September 1972 and February 1975, the NSABP conducted a landmark clinical trial comparing L-phenylalanine mustard (L-PAM)

or melphalan to placebo in 370 women with stage II breast cancer (208, 210). These patients were stratified by menopausal status (≤49 versus ≥50 years of age) and by the number of positive axillary nodes (one to three versus four or more). L-PAM was administered in a dose of 0.15 mg/kg/day for 3 consecutive days every 6 weeks for 2 years. This study demonstrated improvement in disease-free and absolute survival in women 49 years of age or younger. In this age group there was a 37% reduction in mortality. Women with one to three as well as those with four or more positive nodes benefited, but the advantage was greater for women with fewer positive nodes (Fig. 54.4) (208). No advantage or disadvantages of L-PAM was observed in patients 50 years of age or older (Fig. 54.5) (208).

Based upon the data of the initial L-PAM trial, the NSABP conducted a series of clinical trials sequentially: P versus PF; PF versus PMF; and PF versus PAF (P = L-PAM, F = 5-FU, M = methotrexate, A = Adriamycin). The P versus PF trial was important since the addition of 5-FU to L-PAM increased disease-free survival in postmenopausal women with four or more positive nodes (Fig. 54.6) (208). This study established that adjuvant chemotherapy is also of value in postmenopausal women.

The addition of methotrexate to PF (PF versus PMF) failed to produce an advantage over PF in any menopausal or nodal subset. However, the addition of doxorubicin (Adriamycin) to PF (PAF) in receptor-negative, stage II breast cancer resulted in significantly better disease-free and absolute survival (211). The sequential development and results of the NSABP clinical trials in stage II breast cancer are illustrated in Figure 54.7. (208).

THE MILAN CANCER INSTITUTE CMF TRIAL

In parallel with the NSABP studies, Bonadonna of the Instituto Nazionale Tumori of Milan initiated a series of clinical trials in stage II breast cancer evaluating the three-drug combination CMF (cyclophosphamide, methotrexate, 5-FU; see Table 54.9). The first of these trials was published in 1976, and 14-year follow-up data are now available (145, 146). Stratifications, that is, menopausal status (pre- versus post-) and axillary nodal status (1 to 3 versus >3) were the same as in the NSABP trial. A total of 386 patients were randomized, 207 to CMF and 179 as controls. All patients had a standard radical mastectomy. As in the NSABP L-PAM study, the greatest benefit was in premenopausal women (Fig. 54.8) (146). Close analysis of these data illustrates that the maximal recurrence rate for both CMF-treated and control patients is within the first 3 years. After 3 years, the difference between the control and CMF groups remained the same for the subsequent 10 years. The overall survival difference between CMF-treated and control patients continues to increase gradually. At 14 years, the median sur-

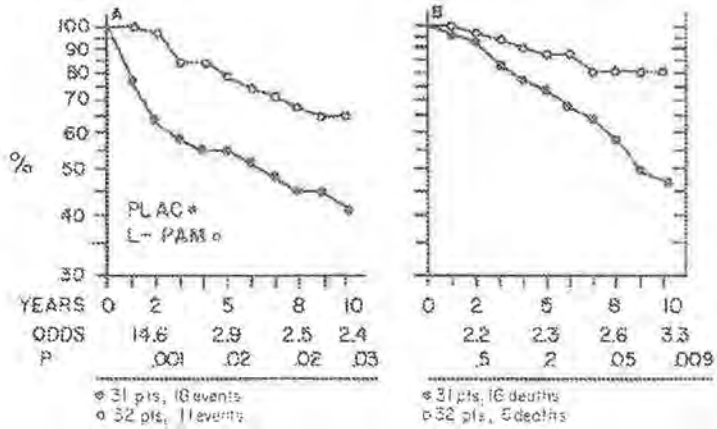

Figure 54.4. Disease-free survival (A) and survival (B) of patients ≤49 years with 1 to 3 positive nodes. Cumulative odds ratio and cumulative p values demonstrate the benefit in this nodal group. NSABP B-05: L-PAM versus Placebo (PLAC) in stage II breast cancer. (From Fisher B, Redmond C, Fisher ER, Wolmark N. Systemic adjuvant therapy in treatment of primary operable breast cancer: National Surgical Adjuvant Breast and Bowel Project experience. JNCI Monogr 1986;1:35–43.)

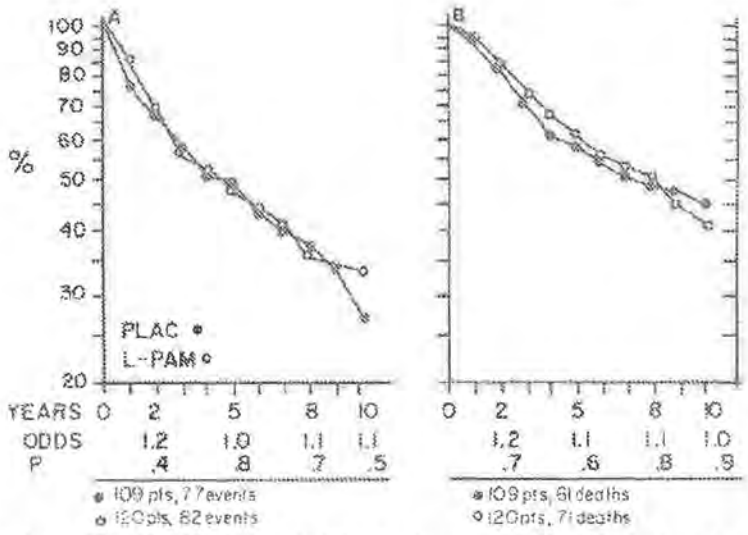

Figure 54.5. Disease-free survival (A) and survival (B) of patients ≥50 years; no benefit shown by L-PAM. NSABP B-05: L-PAM versus Placebo (PLAC) in stage II breast cancer. (From Fisher B, Redmond C, Fisher ER, Wolmark N. Systemic adjuvant therapy in treatment of primary operable breast cancer: National Surgical Adjuvant Breast and Bowel Project experience. NCI Monogr 1986;1:35–43.)

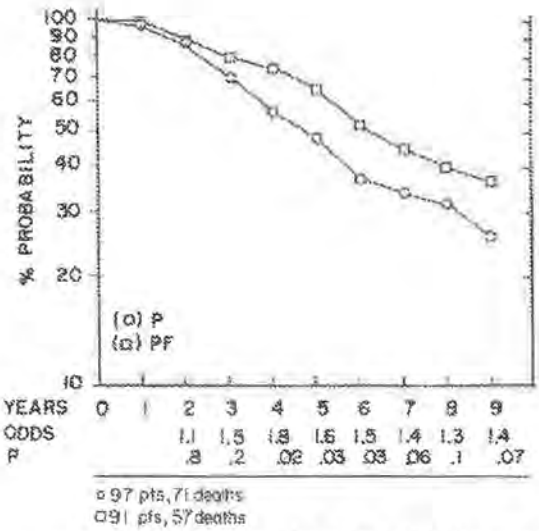

Figure 54.6. Survival of patients ≥30 years with ≥4 positive nodes. NSABP B-07: L-PAM (P) vs L-PAM + 5-FU (PF) in stage II breast cancer. (From Fisher B, Redmond C, Fisher ER, Wolmark N. Systemic adjuvant therapy in treatment of primary operable breast cancer: National Surgical Adjuvant Breast and Bowel Project experience. NCI Monogr 1986;1:35–43.)

vival for the entire premenopausal group has not yet been reached.

The second Milan Cancer Institute CMF trial tested 6 versus 12 treatment cycles. This study was designed to empirically test equivalency of response, with the hope that a shorter duration of treatment would be associated with a decreased frequency of acute toxic reactions. Six months of CMF is equivalent to 12 months and may even have a slight advantage. It appears

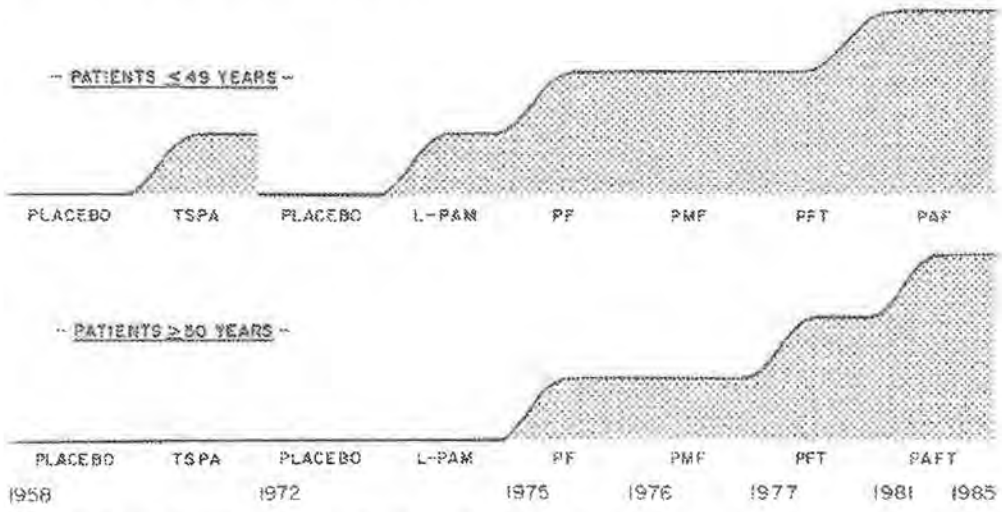

Figure 54.7. The NSABP experience with adjuvant therapy for primary breast cancer; incremental gains achieved in premenopausal and postmenopausal patients between 1958 and 1985. L-PAM = P. (From Fisher B, Redmond C, Fisher ER, Wolmark N. Systemic adjuvant therapy in treatment of primary operable breast cancer: National Surgical Adjuvant Breast and Bowel Project experience. NCI Monogr 1986;1:35–43.)

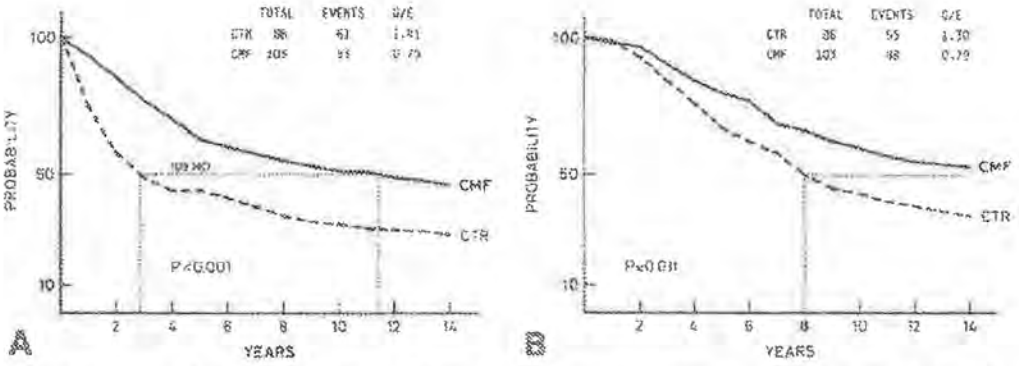

Figure 54.8. First CMF program. Comparative results at 14 years in premenopausal patients. (A) Relapse-free survival. (B) Total survival. CTR = Control. (From Bonadonna G. Conceptual and practical advances in the management of breast cancer. J Clin Oncol 1989;7(10):1368–1392.)

that there is no reason to prolong the same drug combination beyond six cycles. More prolonged therapy may facilitate the overgrowth of drug-resistant tumor cells (146). The NSABP has recently confirmed that four doses of Adriamycin plus cyclophosphamide (AC) given at 3-week intervals is equivalent to 6 months of CMF (150).

Since in both the NSABP L-PAM study and in the Milan CMF adjuvant trials the benefit was observed primarily in premenopausal women, and since a substantial proportion of premenopausal patients treated with adjuvant chemotherapy became amenorrheic, a *castration-mediated effect* was proposed. In the NSABP trial, 73% of women age 40 to 49 and 22% of women age 39 or younger became amenorrheic (212). A significant increase in LH and FSH and a decrease in estradiol were noted only in patients age 40 to 49. However, the greatest therapeutic benefit in terms of improved disease-free survival was observed in women 39 years of age or younger. In the Milan trial, women failing CMF responded to therapeutic castration with the same frequency regardless of previous CMF-induced amenorrhea (146). Therefore, it appears that chemotherapy induced amenorrhea

is not tantamount to a chemical castration and that the main therapeutic effect of adjuvant chemotherapy is cytotoxicity rather than induced ovarian failure.

CALGB TRIALS

The Cancer and Leukemia Group B (CALGB) has performed two major adjuvant chemotherapy trials in stage II breast cancer. The first was based on the clinical observations of Cooper and associates (213, 214), who empirically treated 100 women with primary breast cancer and four of more positive axillary nodes with 9 months of CMFVP (Table 54.9). Disease-free survival was 68% at 5 years. Since this was significantly greater than anticipated, the CALGB initiated a prospective, randomized trial comparing CMF to CMFVP (143). Women with four or more positive axillary nodes who were treated with CMFVP had significantly greater disease-free survival than those treated with CMF (144). In women with one to three positive nodes CMF was equivalent to CMFVP.

The second major CALGB adjuvant study analyzed 807 patients. This study also tested CMFVP but at two different dose intensities, each of which was administered for 8 months (215). There was then a second randomization to 6 additional months of CMFVP or VATH (Table 54.12). Disease-free survival was significantly superior in patients who were crossed over to VATH ($p = 0.01$). This was most marked in postmenopausal women with more than 4 positive nodes ($p = 0.02$) and in all patients with more than 10 positive nodes ($p = 0.006$). It was concluded that a second combination chemotherapy regimen, given after the tumor burden had been reduced by the initial chemotherapy, conveys a further cytocidal effect. This effect was most marked in postmenopausal patients and in all patients with high tumor burdens (216). Dose intensity is of particular importance in multinode positive breast cancer (217).

Tamoxifen as an Adjuvant in Stage II Breast Cancer

Tamoxifen has added a whole new dimension to the adjuvant therapy of breast cancer. Tamoxifen, both as a single agent and in combination with chemotherapy, has been evaluated extensively in operable breast cancer. Twenty-eight prospectively randomized controlled studies of tamoxifen involving a total of 16,513 women have been reported (13). In trials comparing tamoxifen with untreated controls, there is significant improvement in disease-free survival and reduction in mortality in treated patients ($p < 0.0001$). However, this improvement is restricted to women 50 years of age or older.

The largest controlled trials of tamoxifen as a single agent have been conducted in Europe. The *NATO trial* (Nolvadex Adjuvant Trial Organization), chaired by Michael Baum of Kings College Hospital Medical School in London, involved 1285 women 75 years of age or younger. The treatment group received 10 mg of tamoxifen twice daily for 2 years. In this study, there was a significant advantage in the treatment group independent of menopausal status, stage, grade, and ER status. The NATO group concurs that the failure of the ER content to predict response was "counterintuitive" (218–220).

The other major European trials have all noted a strong relationship between ER and response to tamoxifen. In the *Danish trial* of 1650 patients, only patients with ER ≥100 fmol/mg had a significantly improved recurrence-free survival (221). The *Scottish trial* of 1312 evaluable patients also noted the greatest benefit in disease-free survival in patients with ER ≥100 fmol/mg (222, 223). The *Swedish trial* of 1407 patients noted no benefit with tamoxifen in ER-negative patients but did note a strong correlation between ER content and benefit from tamoxifen (224).

The disease-free (event-free) survival in the NATO trial is illustrated in Figure 54.9 (220); the disease-free survival in the Scottish trial is illustrated in Figure 54.10 (222). Although a direct comparison between two trials is difficult, two observations should be made: (*a*) in neither trial does the disease-free survival plateau, that is, despite treatment with tamoxifen, patients continue to relapse, even beyond 7 years, and (*b*) although the median disease-free survival has not yet been reached in either trial, it appears that it will be longer in the Scottish trial. In the Scottish trial, patients received tamoxifen for 5 years as opposed to 2 years in the NATO trial.

Tamoxifen Combinations in Stage II Breast Cancer

In 1977, concurrent with the initiation of the NATO trial evaluating tamoxifen as a single agent, the NSABP initiated a prospective, randomized trial in stage II breast cancer (225). In this study, 1891 patients were randomized to

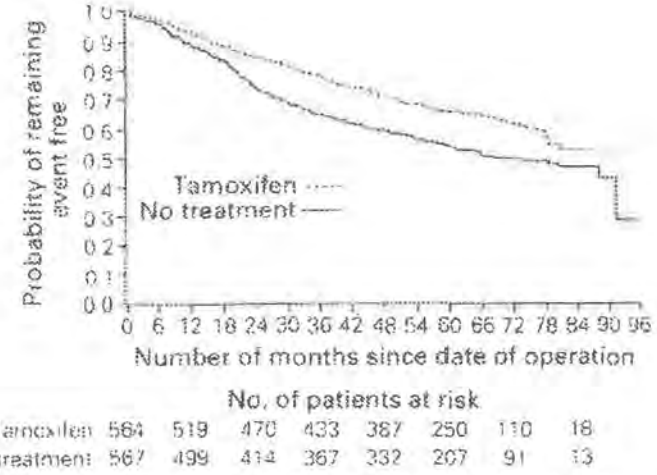

Figure 54.9. Event-free survival in stage II breast cancer. Tamoxifen versus no treatment. Nolvadex Adjuvant Treatment Organization (NATO) Trial. (From Controlled trial of tamoxifen as a single adjuvant agent in the management of early breast cancer. Analysis at eight years by Nolvadex Adjuvant Trial Organisation (Baum M, Chairman of Steering Committee). *Br J Cancer* 1988;57:608–611.)

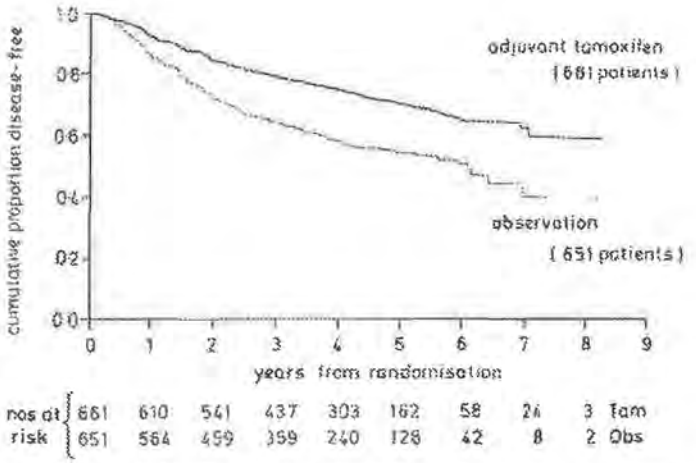

Figure 54.10. Disease-free survival in stage II breast cancer. Tamoxifen vs observation. The Scottish Trial. (From Adjuvant tamoxifen in the management of operable breast cancer: the Scottish trial. Report from the Breast Cancer Trials Committee. *Lancet* 1987;2:171–175.)

receive L-PAM (P) and 5-fluorouracil (PF) with or without tamoxifen (T). P (4 mg/m^2 p.o.) and F (300 mg/m^2 i.v.) were administered in 5-day cycles every 6 weeks for 2 years. Tamoxifen, 10 mg orally twice daily, was given for the entire 2 years of therapy. At 5 years median follow-up, the benefit from the addition of tamoxifen to PF (PFT) was restricted to women 50 years of age or older (226). The advantage of PFT over PF was strongly associated with both estrogen and progesterone receptor levels. Postmenopausal patients with ER levels <10 fmol/mg demonstrated no benefit from the addition of tamoxifen; however, disease-free survival in postmenopausal women treated with tamoxifen improved progressively with increasing ER levels ≥10 fmol/mg (Fig. 54.11) (225). In premenopausal women, the addition of tamoxifen to PF was of no benefit regardless of ER or PR level. As can be seen in Figure 54.11, there was

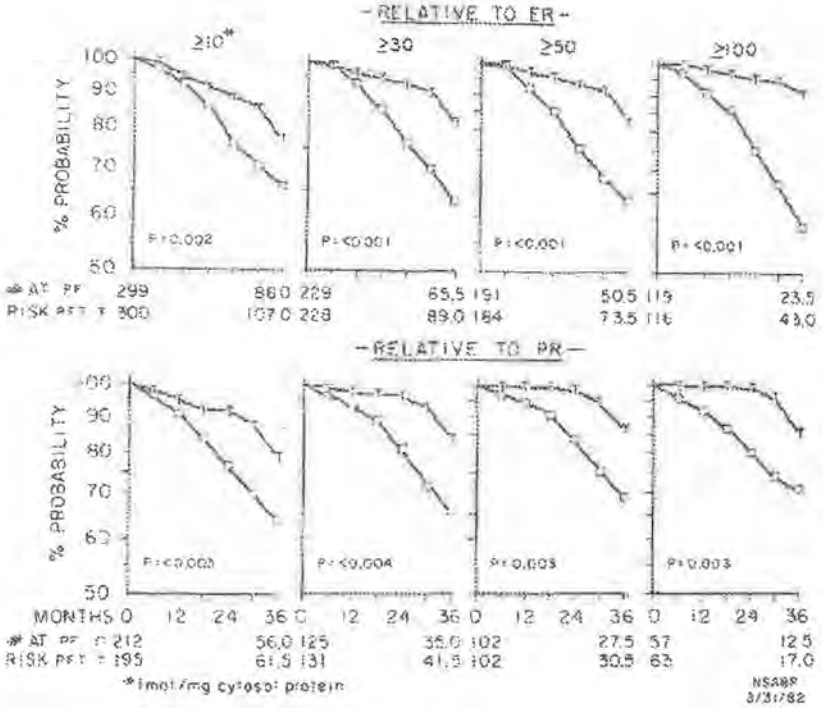

Figure 54.11. Disease-free survival of women ≥50 years of age with stage II breast cancer treated with PF versus PFT (t-PAM + 5-FU versus t-PAM + 5-FU + Tamoxifen). Analysis relative to ER (estrogen receptor) and PR (progesterone receptor). (From Fisher B, Redmond C, Brown A et al. Influence of tumor estrogen and progesterone receptor levels on the response to tamoxifen and chemotherapy in primary breast cancer. J Clin Oncol 1983;1(4):227–241.)

a fairly rapid increase in recurrence rate after the 24 months of tamoxifen was completed. This observation prompted the NSABP to offer a third year of tamoxifen to women who were disease-free at 2 years. Women receiving a third year of tamoxifen had further improvement in disease-free survival (Fig. 54.12) (53).

The *Eastern Cooperative Oncology Group* (ECOG) performed a similar trial in 265 node-positive postmenopausal women (227). ECOG compared CMFP administered with or without tamoxifen versus a no-treatment control. The tamoxifen was given for 1 year. There was no benefit in ER-positive patients with either CMFP or CMFPT, but ER-negative patients treated with CMFP or CMFPT had improved disease-free survival when compared with the no-treatment control group ($p = 0.0003$). However, a subsequent ECOG trial comparing CMFPT for 12 cycles to CMFPT followed by continuous tamoxifen demonstrated a significant improvement in disease-free survival in postmenopausal, receptor-positive patients treated with continuous tamoxifen (228).

These studies have raised three important questions: (*a*) Is the beneficial effect observed in axillary node-positive, receptor-positive, postmenopausal women due to tamoxifen alone or to the combination of tamoxifen plus cytotoxic drugs? (*b*) In tamoxifen-responsive stage II patients, is disease-free survival enhanced by the addition of cytotoxic drugs? (*c*) What is the optimal duration of tamoxifen therapy? Should tamoxifen be given indefinitely?

The issue of the optimal duration of adjuvant tamoxifen has not yet been resolved, but the NSABP is testing 5 versus 10 years of tamoxifen in stage I receptor-positive breast cancer. The current recommendation is that tamoxifen should be continued for a minimum of 5 years.

The major European trials (the NATO trial, the Scottish trial, the Danish trial, and the Swedish trial) have all confirmed the value of single-agent tamoxifen in stage II, receptor-pos-

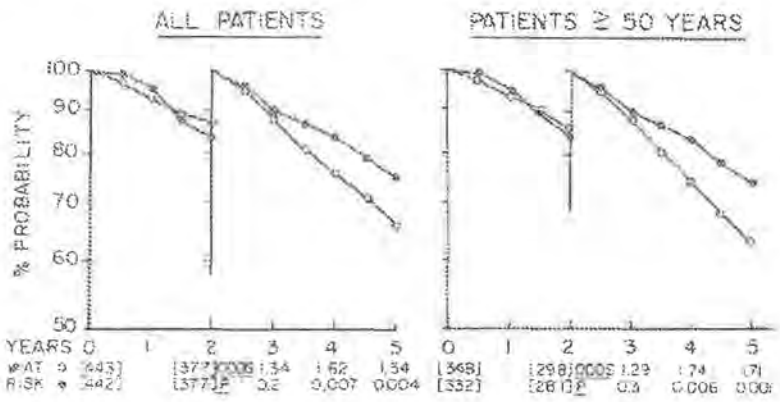

Figure 54.12. Effect of an additional year of tamoxifen on the disease-free survival of patients who were free of disease 2 years after surgery. Open circles indicate women who received melphalan, fluorouracil, and tamoxifen alone, and closed circles indicate women who received this treatment plus an additional year of tamoxifen. (From Fisher B, Brown A, Wolmark N et al. Prolonging tamoxifen therapy for primary breast cancer. Findings from the National Surgical Adjuvant Breast and Bowel Project clinical trial. Ann Intern Med 1987;106:649–654.)

tive breast cancer in postmenopausal women (218–224). The question is: Does chemotherapy used in conjunction with tamoxifen prolong disease-free survival and absolute survival in node-positive, tamoxifen-responsive breast cancer?

The NSABP has performed the definitive study (151). Receptor-positive, node-positive postmenopausal women were randomized to tamoxifen alone (for 5 years), four cycles of AC (Adriamycin, cyclophosphamide) plus tamoxifen (ACT), or PF (L-PAM plus 5-FU) plus tamoxifen (PFT). The PFT arm was subsequently modified to PAFT. There were 1226 eligible patients randomized. Follow-up data through 3 years are available on 1124 patients. Patients receiving ACT had significantly better disease-free survival (84%) than those who received tamoxifen alone (67%) ($p = 0.0002$). The groups receiving PFT and PAFT also did significantly better than the group receiving tamoxifen alone.

Two important conclusions can be drawn from this study: (*a*) Chemotherapy significantly improves disease-free survival in node-positive, receptor-positive, tamoxifen-responsive women. (*b*) Tamoxifen alone is not optimal treatment for node-positive, receptor-positive, postmenopausal women with breast cancer.

Adjuvant Therapy of Stage I Breast Cancer

The adjuvant therapy of stage I breast cancer is a controversy that should be resolved in the 1990s. Standard prognostic criteria, based on the status of axillary lymph nodes or hormonal receptors, are not sensitive and specific enough determinants in stage I breast cancer to identify the patient group requiring adjuvant therapy. Nevertheless, it is well-recognized that in 25 to 30% of stage I breast cancer patients the disease will recur and the patients will ultimately die. Because of the limitations of available prognostic determinants and the generally good prognosis of stage I patients, it was concluded at the National Institute of Health Consensus Development Conference in 1985 that adjuvant therapy is not generally recommended for patients with negative axillary nodes (229). Since then, several node-negative trials have been reported. These trials have altered the approach to stage I breast cancer.

Bonadonna evaluated six cycles of CMF versus an untreated control group in 98 node-negative, receptor-negative patients and reported a highly significant improvement in both disease-free and absolute survival with treatment (Fig. 54.13) (146). The NSABP analyzed 12 cycles of sequential methotrexate and 5-FU followed by leucovorin in 679 women with negative nodes and negative receptors (230, 231). Disease-free survival improved significantly in both premenopausal and postmenopausal women (Fig. 54.14) (231). The NSABP performed a concurrent study in node-negative, ER+ women, comparing tamoxifen (for 5 years) versus placebo in 2644 patients (232). Again, disease-free survival improved significantly in both pre-

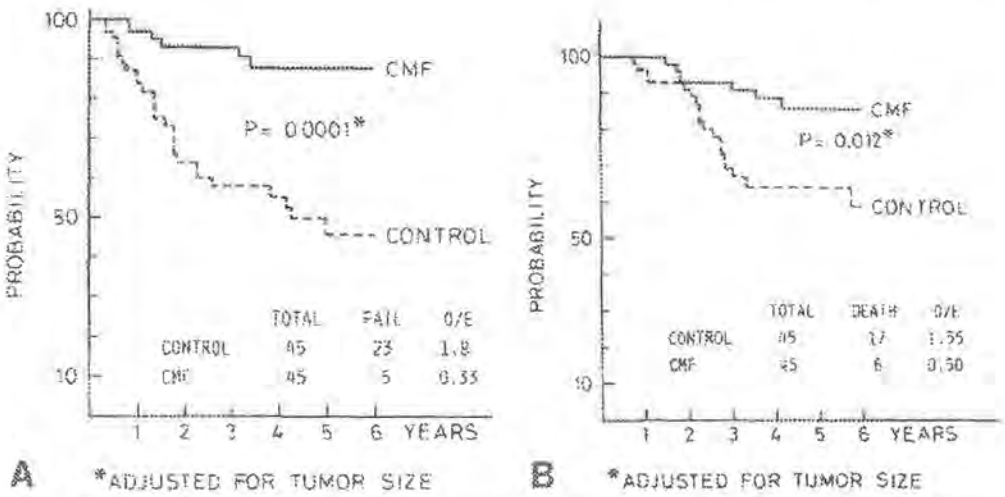

Figure 54.13. Node-negative, ER-negative breast cancer: comparative 6-year results. (A) Relapse-free survival. (B) Total survival. (From Bonadonna G. Conceptual and practical advances in the management of breast cancer. J Clin Oncol 1989;7(10):1380–1397.)

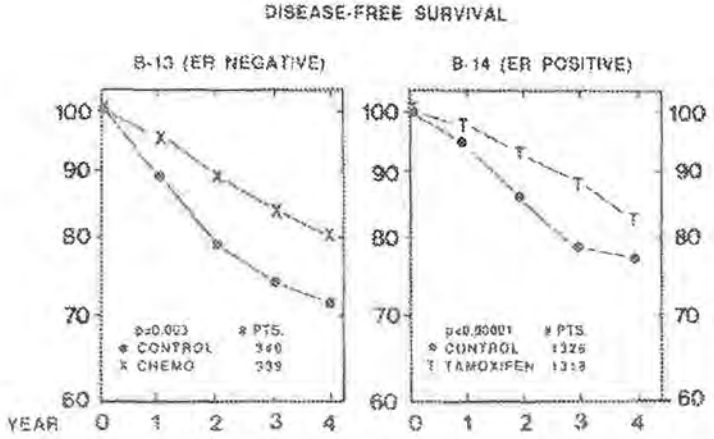

Figure 54.14. Disease-free survival, NSABP protocol 13 (patients with receptor-negative tumors) and NSABP protocol 14 (patients with receptor-positive tumors). (From Wolmark N. 1989: the year of adjuvant therapy in node-negative breast cancer. Principles & Practice of Oncology 1989;3(12):1–10.)

menopausal and postmenopausal patients (Fig. 54.14). Finally, the ECOG/SWOG/CALGB Intergroup Study tested CMFP versus no treatment in 536 patients with high-risk (ER− tumor of any size or an ER+ tumor ≥3 cm), node-negative breast cancer. Disease-free survival was 84% in the CMFP-treated group and 69% in the control group ($p = 0.0001$) (233).

There is now no doubt that the natural history of node-negative breast cancer can be affected by systemic therapy but questions linger. Do all patients with stage I breast cancer require adjuvant therapy? The answer is emphatically no. Which patients are most likely to benefit from treatment? Can the group not requiring treatment be clearly defined? These questions are much more difficult to answer. However, there is a growing body of data that women with lesions smaller than 1 cm in diameter have an exceedingly good prognosis without adjuvant treatment (234–236). This is at least one group which adjuvant therapy is probably not indicated.

Systemic Failure After Adjuvant Therapy

Systemic relapse after adjuvant therapy is an increasing problem and a major therapeutic dilemma. If the patient is hormonally responsive (receptor positive) and has metastatic disease that is not immediately life-threatening, a primary or secondary type of hormonal manipulation is the treatment of choice (see pp 951–956). If the patient is receptor negative or has extensive liver or lymphagitic pulmonary metastases, regardless of receptor status, cytotoxic chemotherapy is indicated. However, if the patient has relapsed following a standard course of combination chemotherapy such as CMF, CMFVP, CFP, or AC, what are the treatment options? Have these patients become refractory to standard chemotherapeutic regimens? Will patients respond to the same or similar regimens in the future?

The efficacy of salvage treatment in 240 patients who failed adjuvant CMF has been reviewed by the Milan group (237). Results were compared with outcomes of 100 women who relapsed after mastectomy alone. The response rate and duration of response to subsequent CMF are equivalent: 37% response for 17 months in patients with prior adjuvant CMF; 43% response for 16 months in surgery-only controls. In patients failing adjuvant CMF who had a disease-free interval longer than 12 months from the completion of adjuvant therapy, the response to CMF was 52%.

The CALGB evaluated CAF, with or without tamoxifen, in 46 patients with advanced breast cancer who had received prior adjuvant chemotherapy completed 6 months or more prior to protocol entry (238). This was compared to the outcomes of 379 patients treated identically who had not received prior adjuvant chemotherapy. The response rates and response durations were equivalent: a 50% response lasting 10.7 months for patients receiving prior adjuvant chemotherapy, and a 59% response lasting 12 months for patients with no prior chemotherapy. Median survival was also equivalent: 17.5 months for patients who received prior adjuvant chemotherapy and 19.6 for patients who did not receive prior chemotherapy.

Two important conclusions are apparent: (*a*) Women with stage II breast cancer who relapse more than 6 months after completion of adjuvant chemotherapy do not have inherently drug-resistant tumors; that is, chemosensitive clones of cells may have been suppressed but not eradicated. (*b*) Patients may respond to subsequent chemotherapeutic regimens containing two or more of the same agents used as adjuvants and may have the same response rate, response duration, and survival as patients with advanced breast cancer who have not received prior adjuvant chemotherapy.

Neoadjuvant Chemotherapy of Local-Regional Advanced Breast Cancer

The treatment of local-regional, advanced (LRA), nonmetastatic breast cancer has undergone a major change in recent years, based upon the recognition that this condition is a systemic disease at the time of diagnosis. With this recognition, the emphasis of therapy has changed from local surgery and radiation therapy to primary systemic chemotherapy. With systemic chemotherapy as the initial therapeutic modality, inoperable tumors can frequently be converted to operable tumors, and many large, technically operable tumors can be reduced in size sufficiently that breast preservation may be possible.

LRA breast cancer is not a well-defined, solitary entity but rather one that represents a spectrum of disorders with divergent biologic behaviors. These range from indolent, slow-growing tumors that have been hidden by the patient for years prior to discovery to aggressive inflammatory cancers. This divergent biologic behavior requires individualization of therapy, but these tumors do have features in common. Basically, there are three subsets of LRA breast cancer: (*a*) *stage* III_A: large primary lesions ≥5 cm (T_3) or a tumor of any size with fixed ipsilateral axillary adenopathy (N_2); (*b*) *stage* III_B: tumors with direct extension to the chest wall or skin, satellite skin nodules, or breast edema (peau d'orange) (T_4), with or without fixed matted nodes (N_2), or any tumor associated with internal mammary adenopathy (N_3); (*c*) *inflammatory carcinoma* (T_{4d}).

The recommended multimodality approach to the management of LRA breast cancer is shown in Table 56.14. This approach to the management of LRA breast cancer has been derived from the data of Perloff et al. (239), Lippman et al. (240), Swain et al. (241), Olson et al. (242), Loprinzi et al. (243), Hortobagyi et al. (244), and Bonadonna (146). With this approach to LRA breast cancer, an objective response with a 50% or greater reduction in the size of the primary

Table 54.14. Recommended Multimodality Approach to Management of Local-Regional Advanced Breast Cancer

1. Confirm diagnosis of breast cancer by fine-needle aspiration cytology of the breast. A second fine-needle aspiration is performed for estrogen receptors by immunocytochemical (ERICA) assay.
2. Rule out systemic metastases with a chest x-ray, bone scan, liver imaging procedure, basic blood chemistries, and blood count.
3. Initiate systemic Adriamycin-based chemotherapy with CAF, FAC, or AC (Table 54.10), unless there is a medical contradiction.
4. Treat to maximal tumor response (three to five cycles).
5. Evaluate operability.
   (a) If the tumor has converted to operable, a modified radical mastectomy is performed. If there is minimal or no residual tumor in the surgical specimen, postoperative radiation is not recommended. If there is gross residual tumor, postoperative radiation is performed.
   (b) If the tumor has not converted to operable, local radiation is initiated.
6. Completion of systemic chemotherapy: three to five additional cycles of postoperative or postradiation chemotherapy (to a total of eight cycles) are administered.
7. If the patient is ERICA positive, treatment with long-term tamoxifen is continued after completion of the final cycle of chemotherapy.

tumor will occur in 70 to 90% of cases following the initial induction chemotherapy. From 16 to 40% of these responses will be clinically complete responses. Fewer than 10% of patients will have no response to induction chemotherapy, and in essentially none will progression occur during induction chemotherapy. Up to 80% of previously inoperable patients will be converted to operable. In the M.D. Anderson series, only 6 to 174 (3%) were not disease-free after completion of induction chemotherapy and local treatment (245). Five-year survival rates are in excess of 80% for stage III_A and up to 40% for stage III_B. With multimodality therapy, local-regional recurrences have developed in only 15% of patients.

Inflammatory cancer of the breast is currently managed in the same manner as are other LRA cancers of the breast. Inflammatory carcinoma is a relatively rare, distinct clinicopathologic entity (1% of breast cancers). Clinically, it is associated with a seemingly inflamed, warm, tender, erythematous breast, usually without a distinct mass. Pathologically, tumor cells infiltrate dermal lymphatics. With local therapy alone, the 5-year survival rate is less than 5%. However, with the use of initial induction chemotherapy prior to local treatment, the 5-year survival rate has now increased to 30% and may be as high as 80% in patients with no residual disease after induction chemotherapy (245–247).

MALE BREAST CANCER

Male breast cancer (MBC) is an uncommon disease accounting for less than 1% of all breast cancers (248–250). The median age of patients with MBC is approximately one decade older (64 to 71 years) than for female breast cancer patients. When MBC develops, its clinical course and metastatic pattern are similar to those of female breast cancer, with a few notable exceptions: (*a*) overall, MBC is more hormonally responsive than female breast cancer; nearly 90% are ER+ and 75% are PR+; and (*b*) at presentation, MBC is locally advanced more frequently than female breast cancer, with more than 30% of cases having nipple ulceration, bloody nipple discharge, and/or nipple retraction at diagnosis (251, 252).

Since MBC tends to present with locally advanced disease, and since there is a paucity of tissue between the tumor and the chest wall, patients are at significant risk of chest wall recurrence following modified radical or radical mastectomy. It is therefore recommended that patients with MBC receive routine postoperative radiation therapy.

Hormone Therapy of MBC

MBC is responsive to both additive and ablative hormonal therapy, and, as in female breast cancer, response is dependent on hormone receptor status. ER – MBC is unresponsive to hormonal manipulation, while 80% or more of ER+ MBC is hormonally responsive. *Orchiectomy* has been the most frequent ablative procedure in MBC: 177 of 319 (55%) unselected patients with advanced MBC and 25 of 32 (78%) ER+ patients achieved a major response to orchiectomy (251, 253, 254).

In addition, MBC is responsive to a variety of additive hormonal agents. *Tamoxifen* is an active agent in MBC, with a 49% (42 of 86) response in unselected and an 82% (18 of 22) response in ER+ patients. Tamoxifen is equally active before or after an orchiectomy has been

performed. The recommended dose of tamoxifen in MBC is the standard dose of 10 mg twice daily. *Estrogens* (diethylstilbestrol, 1 mg three times daily) are active in advanced MBC, with a 32% (51 of 161) response rate. However, as in males treated with DES for advanced prostatic cancer, there is an increased risk of thromboembolic complications (251, 253–257).

Other hormonal agents such as *progestins* (medroxyprogesterone acetate, 500 to 1500 mg i.m. daily), aminoglutethimide, and adrogens (fluoxymesterone, 10 mg three times daily) have been reported to have activity in MBC in small groups of patients (258). LHRH agonists, with and without the antiandrogen flutamide, are now being tested in MBC with promising results (259).

Chemotherapy in MBC

MBC responds to *cytotoxic chemotherapy* with the same frequency as female breast cancer. Also, MBC responds to the same basic regimens of CMF, CMFVP, CAF, and FAC (Tables 54.9 and 54.10) as female breast cancer (260). Chemotherapy in advanced MBC should be restricted to patients resistant to hormonal therapy.

Adjuvant Therapy in MBC

Degree of axillary nodal involvement, tumor size, and hormone receptor status have the same prognostic implications in MBC as in breast cancer in women. Since MBC is a rare disease, no large adjuvant trials have been possible. Bagley et al. (261) from the National Cancer Institute treated 24 stage II patients with adjuvant CMF. With a median follow-up of 46 months, four patients had developed recurrences and two of them had died. Patel et al. (262) from the M.D. Anderson Cancer Center treated 11 patients with stage II and III MBC with adjuvant FAC. With a median follow-up of 52 months, four had relapsed and seven remained disease-free. Ribeiro (263) treated 23 patients with stage II and III MBC with adjuvant tamoxifen for 1 year. At 5 years, 12 of 23 were disease-free.

The data on the adjuvant therapy of MBC are sketchy, and it is difficult to make firm recommendations. However, it seems prudent to assume that since MBC is responsive to cytotoxic chemotherapy and even more responsive to hormonal therapy than female breast cancer, adjuvant therapy for stage II MBC may be beneficial.

For stage II MBC, the following seems reasonable:

1. For patients with 1 to 3 positive axillary nodes: CMF × 6 cycles (6 months)
2. For patients with ≥4 positive axillary nodes: CAF × 6 cycles (6 months)
3. ER+ patients, in addition to adjuvant chemotherapy, should also receive tamoxifen, 10 mg twice daily for 5 years
4. All patients should receive postoperative radiation therapy after completion of the 6 cycles of chemotherapy

Stage III MBC should be approached in the same manner as stage III breast cancer in women utilizing neoadjuvant chemotherapy. The treatment of stage III breast cancer is summarized in Table 54.14.

MBC remains a complex clinical problem and because of its rarity may remain an enigma.

FUTURE DIRECTIONS

The standard prognostic factors of tumor size, axillary nodal involvement, histologic grade, and hormone receptor status are inadequate predictors of outcome, especially in stage I breast cancer. Currently 75% of axillary node-negative and axillary node-positive patients are cured by local therapy alone, but exactly who will relapse and who will remain disease-free cannot be reliably predicted.

A large number of new tumor markers are now being evaluated clinically. These include a whole series of growth factors, growth factor receptors, DNA flow cytometry, growth fraction (S-phase), oncogenes, and metabolic breakdown products. Ideally, the presence of one or a series of these markers will be absolutely predictive of relapse or absolutely predictive of disease-free survival. Selected patients could then be spared the rigors of adjuvant therapy, and high-risk patients could be treated aggressively.

Growth Factors

Several *growth factors* such as epidermal growth factor, insulin-like growth factor I or somatomedin-c, somatostatin, prolactin, and leuteinizing-hormone-releasing hormone (LHRH) appear to have a role in the regulation of human breast cancer (36, 264–267). There is an inverse correlation between epidermal growth factor receptor content and ER status, with ER− tumors tending to have a higher level of epi-

dermal growth factor receptor than ER+ tumors. Within the ER- group, tumors with higher levels of epidermal growth factor receptor tend to have a poorer prognosis. Inhibiting growth factors or blocking growth factor receptors may have therapeutic implications in the future.

DNA Flow Cytometry

DNA flow cytometric measurements of ploidy and the fraction of cells in the synthesis phase of the cell cycle (S-phase fraction) appear to have prognostic value in women with node-negative breast cancer (268, 269). Approximately one-third of breast cancers have normal or *diploid* chromosomes while two-thirds have excess or abnormal DNA (*aneuploid*). In retrospective studies, stage I patients with diploid tumors appear to have a better prognosis than those with aneuploid tumors. Diploid tumors with a high S phase (≥6.7%) have a poorer prognosis than those with a low S phase; S phase was not a significant determinant in aneuploid tumors (Fig. 54.4). However, Muss et al. (270), in a prospective study of 101 women with stage I breast cancer, and Keyhani-Rofagha et al. (271), in a retrospective study of 165 stage I patients, were unable to confirm a correlation between DNA index (ploidy) and any clinical variable, including time to recurrence and survival. Therapeutic decisions based upon ploidy and S phase cannot be justified outside the context of a controlled clinical trial.

HER-2/neu Oncogene

Amplification of the *HER-2/neu (erb B-2) oncogene* has been reported to be of prognostic importance in stage I and stage II breast cancer (273–275). In these studies, patients with higher levels of HER-2/neu protein had shorter disease-free intervals and overall survival than patients with lower levels. HER-2/neu (erb B-2) gene amplification may prove to be an important independent prognostic variable.

Other prognostic variables such as haptoglobin-related protein epitopes, nm23 RNA, and cathepsin D are being investigated (206, 207, 276, 277). Of these, *cathepsin D*, an estrogen-induced lysosomal protease, has stimulated the greatest interest. Conflicting results have been reported. Using a quantitative technique, Tandon et al. (278) have reported that in stage I breast cancer, high levels of cathepsin D are associated with a reduced disease-free survival. However, using an immunohistochemical technique, Henry et al. (279) noted that patients with tumors that are cathepsin D positive (CD+) have a significantly prolonged disease-free survival and that tumors that were both CD+ and ER+ have an especially good prognosis. Tandon and Henry must not be measuring the same thing.

It is hoped that we are entering an era in which breast cancer therapy will be individualized and based upon a series of prognostic factors determined by analysis of the primary tumor (280, 281).

SUMMARY

There have been dramatic advances in the systemic treatment of breast cancer. Many of these advances have been "conceptual" rather than therapeutic "breakthroughs." The adjuvant therapy of stage I and stage II breast cancer is a classic example. Standard chemotherapeutic or hormonal regimens are now being utilized postoperatively to reduce the risk of recurrence, based upon the conceptual change that operable breast cancer is frequently a systemic disease at diagnosis. Neoadjuvant chemotherapy of local-regional advanced breast cancer is also a conceptual change. There is every reason to be optimistic about the future of breast cancer therapy. There will be greater selectivity of adjuvant treatments based upon improved prognostic parameters. Biological response modifiers, which have been very useful in other malignancies, have hardly been tested in breast cancer. LHRH antagonists have been synthesized which may prove more potent than the currently available LHRH agonists. Chemoprevention techniques are entering clinical trials, and breast cancer in high-risk groups may become a preventable disease (282). Breast cancer remains the most feared disease among women, but important advances are being made and there is now even greater reason for optimism (281).

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EXHIBIT 9

REDACTED IN ITS ENTIRETY

EXHIBIT 10

Trials@uspto.gov
571-272-7822

Paper No. 83
Entered: October 3, 2018

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

HOSPIRA, INC., and
SAMSUNG BIOEPIS CO., LTD.
Petitioners,

v.

GENENTECH, INC.,
Patent Owner.

Case IPR2017-00804[1]
Patent 6,627,196 B1

Before ZHENYU YANG, CHRISTOPHER G. PAULRAJ, and ROBERT A. POLLOCK, *Administrative Patent Judges.*

PAULRAJ, *Administrative Patent Judge.*

FINAL WRITTEN DECISION
35 U.S.C. § 318(a) and 37 C.F.R. § 42.73

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[1] Case IPR2017-01958 has been joined with IPR2017-00804

I. INTRODUCTION

Hospira, Inc. ("Hospira") filed a Petition (Paper 1, "Pet."), requesting institution of an *inter partes* review of claims 1–3, 5, 7, 9–11, and 17–33 of U.S. Patent No. 6,627,196 B1 (Ex. 1001, "the '196 patent"). Genentech, Inc. timely filed a Patent Owner Preliminary Response (Paper 6, "Prelim. Resp."). We determined, based on the information presented in the Petition and Preliminary Response, that there was a reasonable likelihood that Hospira would prevail in challenging claims 1–3, 5, 7, 9–11, and 17–33 as unpatentable under 35 U.S.C. § 103(a). Pursuant to 35 U.S.C. § 314, the Board instituted trial on July 27, 2017, as to those claims of the '196 patent. Paper 13 ("Institution Decision" or "Inst. Dec."). Following our institution based on Hospira's Petition, Samsung Bioepis Co., Ltd. ("Samsung") filed a substantially identical Petition challenging the same claims of the '196 patent and requested joinder in this proceeding, which we granted. Paper 40. Thus, Hospira and Samsung together are the "Petitioners" in this proceeding.

Patent Owner filed its Response to the Petition (Paper 41, "PO Resp.") and Petitioners filed a Reply to Patent Owner's Response (Paper 55, "Reply"). Patent Owner filed a Motion to Exclude certain evidence (Paper 68), to which Petitioners filed an Opposition (Paper 69) and Patent Owner filed a Reply in support thereof (Paper 73). Patent Owner also filed a Motion for Observations on Cross-Examination of Petitioners' Reply Declarants (Drs. Allan Lipton and William Jusko) (Paper 64) to which Petitioners filed a Response (Paper 70). Additionally, pursuant to our authorization, Patent Owner filed an Identification of Improper New Reply Materials (Paper 67), to which Petitioners filed a Response (Paper 72) and Patent Owner filed a Reply (Paper 74). An oral hearing was held on May 8,

2018. The transcript of the hearing has been entered into the record. Paper 80 ("Tr.").

We have jurisdiction under 35 U.S.C. § 6. This Final Written Decision is issued pursuant to 35 U.S.C. § 318(a) and 37 C.F.R. § 42.73. Based on the record before us, we conclude that Petitioners have *not* demonstrated by a preponderance of the evidence that claims 1–3, 5, 7, 9–11, and 17–33 of the '196 patent are unpatentable.

A. Related Proceedings

As a related matter, Petitioners and Patent Owner identify a concurrently-filed petition for *inter partes* review (IPR2017-00805) for a related patent, U.S. Patent 7,371,379 ("the '379 patent"). *See* Pet. 2. We issue our Final Written Decision in IPR2017-00805 concurrently with this decision. Additionally, also concurrently with this Decision, we issue Final Written Decisions in two other *inter partes* review proceedings concerning the '196 and '379 patents brought by another petitioner. IPR2017-01139; IPR2017-001140.

The parties also identify litigation matters pending in the U.S. District Courts for the Northern District of California and the District of Delaware and on appeal before the Federal Circuit Court of Appeals concerning the '379 and '196 patents, as well as foreign proceedings concerning counterparts to these patents, as related matters. Paper 81; Paper 82.

B. The '196 Patent (Ex. 1001)

The '196 patent issued on September 30, 2003, with Sharon A. Baughman and Steven Shak as the listed co-inventors. Ex. 1001, (45), (75). The '196 patent issued from an application filed August 25, 2000, and claims priority to provisional applications filed June 23, 2000, and August

27, 1999. *Id.* at (22), (60). The parties have not disputed the claimed priority date for the '196 patent.

The '196 patent relates generally to dosages for the treatment of disorders characterized by the overexpression of ErbB2 (also known as HER2), which encodes a 185-kd transmembrane glycoprotein receptor ($p185^{HER2}$) related to the epidermal growth factor receptor (EGFR). *Id.* at 1:13–25, 42–48. The overexpression of ErbB2 has been associated with breast cancer. *Id.* As noted in the '196 patent, a recombinant humanized anti-ErbB2 monoclonal antibody (alternatively referred to as "rhuMab HER2," "trastuzumab," or by its tradename "Herceptin")[2] had been clinically tested and approved for patients with ErbB2-overexpressing metastatic breast cancers who received prior anti-cancer therapy. *Id.* at 3:54–60. The recommended initial "loading dose" for trastuzumab was 4 mg/kg administered as a 90-minute infusion, and the recommended weekly "maintenance dose" was 2 mg/kg, which could be administered as a 30-minute infusion if the initial loading dose was well-tolerated. *Id.* at 3:61–65.

The invention described in the '196 patent "concerns the discovery that an early attainment of an efficacious target trough serum concentration by providing an initial dose or doses of anti-ErbB2 antibodies, followed by subsequent doses of equal or smaller amounts of antibody (greater front loading) is more efficacious than conventional treatments." *Id.* at 4:21–27. The method of treatment, according to the invention described in the patent, "involves administration of an initial dose of anti-ErbB2 antibody of more

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[2] For consistency's sake, we will refer to the antibody at issue in this proceeding as trastuzumab unless we are directly quoting one of its alternative names from another document.

than approximately 4 mg/kg, preferably more than approximately 5 mg/kg," with the maximum dose not to exceed 50 mg/kg. *Id.* at 4:47–51. "[T]he initial dose or doses is/are followed by subsequent doses of equal or smaller amounts of antibody at intervals sufficiently close to maintain the trough serum concentration of antibody at or above an efficacious target level." *Id.* at 4:61–65. Preferably, "the amount of drug administered is sufficient to maintain the target trough serum concentration such that the interval between administration cycles is at least one week," and "the trough serum concentration does not exceed 2500 μg/ml and does not fall below 0.01 μg/ml during treatment." *Id.* at 4:67–5:5. The patent explains that "[t]he front loading drug treatment method of the invention has the advantage of increased efficacy by reaching a target serum drug concentration early in treatment." *Id.* at 5:5–8. As a result, "[t]he efficacious target trough serum concentration is reached in 4 weeks or less . . . and most preferably 1 week or less, including 1 day or less." *Id.* at 4:26–29. Additionally, the patent states that the method of therapy may involve "infrequent dosing" of the anti-ErbB2 antibody, wherein the first and second dose are separated by at least two weeks, and optionally at least about three weeks. *Id.* at 6:20–31.

The '196 patent describes embodiments in which the initial dose of trastuzumab is 6 mg/kg, 8 mg/kg, or 12 mg/kg, followed by subsequent maintenance doses of 6 mg/kg or 8 mg/kg administered once every 2 or 3 weeks, in a manner such that the trough serum concentration is maintained at approximately 10–20 μg/ml during the treatment period. *Id.* at 5:16–55, 45:23–28. The treatment regimen according to the invention may further comprise administration of chemotherapy along with trastuzumab. *Id.* at 6:4–8, 7:22–25, 45:64–65. Of particular relevance, the '196 patent includes

a prophetic example describing the administration of trastuzumab intravenously every three weeks in combination with the chemotherapeutic agent paclitaxel. *Id.* at 46:5–48:4. According to this example, "[s]imulation of the proposed treatment regimen suggests that the trough serum concentrations will be 17 [μ]g/ml, in the range (10–20 [μ]g/ml) of the targeted trough serum concentrations from previous HERCEPTIN® IV clinical trials." *Id.* at 46:12–16. The example sets forth inclusion criteria for a study in which patients will be administered trastuzumab every three weeks. *Id.* at 47:9–48:12. The '196 patent concludes that "[i]t is believed that the above treatment regimen will be effective in treating metastatic breast cancer, despite the infrequency with which HERCEPTIN® is administered to the patient." *Id.* at 48:1–4.

C. Illustrative Claim

Petitioners challenge claims 1–3, 5, 7, 9–11, and 17–33 of the '196 Patent. Independent claim 1 is illustrative, and is reproduced below:

> 1. A method for the treatment of a human patient diagnosed with cancer characterized by overexpression of ErbB2 receptor, comprising administering an effective amount of an anti-ErbB2 antibody to the human patient, the method comprising:
> administering to the patient an initial dose of at least approximately 5 mg/kg of the anti-ErbB2 antibody; and
> administering to the patient a plurality of subsequent doses of the antibody in an amount that is approximately the same or less than the initial dose, wherein the subsequent doses are separated in time from each other by at least two weeks.

Ex. 1001, 55:63–57:2.

D. The Asserted Ground of Unpatentability

Petitioners challenge the patentability of the claims of the '196 Patent based on the following ground:

References	**Basis**	**Claims challenged**
Herceptin label,[3] Baselga '96,[4] Pegram '98,[5] and the knowledge of a person of ordinary skill in the art	§ 103(a)	1–3, 5, 7, 9–11, and 17–33

Petitioners further rely upon the declarations of Allan Lipton, M.D. (Ex. 1002; Ex. 1056) and William Jusko, Ph.D. (Ex. 1003; Ex. 1057). Patent Owner relies upon the declarations of George Grass, Ph.D. (Ex. 2039) and Karen Gelmon, M.D. (Ex. 2040).

II. ANALYSIS

A. Claim Construction

We interpret claims using the "broadest reasonable construction in light of the specification of the patent in which [they] appear[]." 37 C.F.R. § 42.100(b); *see also Cuozzo Speed Techs., LLC v. Lee*, 136 S. Ct. 2131, 2144–46 (2016). Under the broadest reasonable construction standard, claim

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[3] Genentech, Inc, Herceptin® Trastuzumab, Sept. 1998 (hereinafter "Herceptin Label" (Ex. 1008).

[4] Jose Baselga, *Phase II Study of Weekly Intravenous Recombinant Humanized Anti-p185^{HER2} Monoclonal Antibody in Patients With HER2/neu-Overexpressing Metastatic Breast Cancer*, 14 JOURNAL OF CLINICAL ONCOLOGY 737–744 (1996) (hereinafter "Baselga '96") (Ex. 1013).

[5] Mark D. Pegram, *Phase II Study of Receptor-Enhanced Chemosensitivity Using Recombinant Humanized Anti-p185$^{HER2/neu}$ Monoclonal Antibody Plus Cisplatin in Patients With HER2/neu-Overexpressing Metastatic Breast Cancer Refractory to Chemotherapy Treatment*, 16 JOURNAL OF CLINICAL ONCOLOGY 2659–71 (1998) (hereinafter "Pegram '98") (Ex. 1014).

terms are generally given their ordinary and customary meaning, as would be understood by one of ordinary skill in the art at the time of the invention. *In re Translogic Tech., Inc.*, 504 F.3d 1249, 1257 (Fed. Cir. 2007). "Absent claim language carrying a narrow meaning, the PTO should only limit the claim based on the specification . . . when [it] expressly disclaim[s] the broader definition." *In re Bigio*, 381 F.3d 1320, 1325 (Fed. Cir. 2004). "Although an inventor is indeed free to define the specific terms used to describe his or her invention, this must be done with reasonable clarity, deliberateness, and precision." *In re Paulsen*, 30 F.3d 1475, 1480 (Fed. Cir. 1994).

Petitioners propose a construction for "ErbB2 receptor." *See* Pet. 24. Patent Owner does not propose any terms to be construed in its post-institution Response. We find that no explicit construction of any claim term is necessary to decide the issues presented in this case. *See Wellman, Inc. v. Eastman Chem. Co.*, 642 F.3d 1355, 1361 (Fed. Cir. 2011) ("[C]laim terms need only be construed 'to the extent necessary to resolve the controversy.'" (quoting *Vivid Techs., Inc. v. Am. Sci. & Eng'g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999))).

B. Level of Skill in the Art

Petitioners contend that a person of ordinary skill in the art for the '196 patent would be a "team" that includes both (1) a clinical or medical oncologist specializing in breast cancer with several years of experience in breast cancer research or clinical trials, and (2) a person with a Ph.D. in pharmaceutical sciences or a closely related field with an emphasis in pharmacokinetics with three years of relevant experience in protein based

drug kinetics. Pet. 23–24 (citing Exs. 1002 ¶ 14; 1003 ¶ 15; 1006 ¶ 32). Patent Owner does not address the requisite level of skill in its Response.

Because it is otherwise undisputed and consistent with the evidence of record, we adopt Petitioners' proposed definition of a person of ordinary skill in the art ("POSITA" or "skilled artisan") for purposes of our analysis. We further note that the prior art itself demonstrates the level of skill in the art at the time of the invention. *See Okajima v. Bourdeau*, 261 F.3d 1350, 1355 (Fed. Cir. 2001) (explaining that specific findings regarding ordinary skill level are not required "where the prior art itself reflects an appropriate level and a need for testimony is not shown") (quoting *Litton Indus. Prods., Inc. v. Solid State Sys. Corp.*, 755 F.2d 158, 163 (Fed. Cir. 1985)).

C. Patentability Analysis

1. Content of the Prior Art

Petitioners rely upon, *inter alia*, the following prior art teachings to support their challenge.

a. Herceptin Label (Ex. 1008)

As recognized in the '196 patent, trastuzumab was already FDA-approved and commercially sold in the U.S. by 1998 under the tradename Herceptin. Ex. 1001, 3:54–60. The Herceptin label teaches:

> The pharmacokinetics of Trastuzumab were studied in breast cancer patients with metastatic disease. Short duration intravenous infusions of 10 to 500 mg once weekly demonstrated dose-dependent pharmacokinetics. Mean half-life increased and clearance decreased with increasing dose level. The half-life averaged 1.7 and 12 days at the 10 and 500 mg dose levels, respectively. Trastuzumab's volume of distribution was approximately that of serum volume (44 mL/kg). At the highest weekly

> dose studied (500 mg), mean peak serum concentrations were 377 microgram/mL.

Ex. 1008, 1.

The Herceptin label also teaches that "[i]n studies using a loading dose of 4 mg/kg followed by a weekly maintenance dose of 2 mg/kg, a mean half-life of 5.8 days . . . was observed," and "[b]etween week 16 and 32, Trastuzumab serum concentration reached a steady state with a mean trough and peak concentrations of approximately 79 [mg]/mL and 123 [mg]/mL, respectively. *Id.* The label further describes clinical studies in which metastatic breast cancer patients with certain levels of HER2 overexpression were administered chemotherapy either alone or in combination with trastuzumab given intravenously as a 4 mg/kg loading dose followed by weekly doses at 2 mg/kg. *Id.* The chemotherapy in these clinical studies (e.g., paclitaxel) was administered every 3 weeks (21 days). *Id.*

b. Baselga '96 (Ex. 1013)

Baselga '96 reports the results of a phase II clinical trial in which patients with ErbB2-overexpressing metastatic breast cancer were treated with trastuzumab. Ex. 1013, 737. The pharmacokinetic goal of the trial "was to achieve rhuMAb HER2 trough serum concentrations greater than 10 μg/mL, a level associated with optimal inhibition of cell grown in the preclinical model." *Id.* at 738. Further, the "[s]erum levels of rhuMAb HER2 as a function of time were analyzed for each patient using a one-compartment model." *Id.*

According to the results reported in Baselga '96, "[m]ore than 90% of the examined population (41 patients) had rhuMAb HER2 trough levels above the targeted 10 μg/mL level. *Id.* at 739. Moreover, the treatment "was remarkably well tolerated." *Id.* "Toxicity [from rhuMAb HER2] was

minimal," and no immune response against the antibody was detected. *Id.* at 737. Out of the 768 times trastuzumab was administered, "only 11 events occurred that were considered to be related to the use of the antibody." *Id.* at 739. Baselga '96 also teaches that in preclinical studies (both *in vitro* and in xenografts), trastuzumab "markedly potentiated the antitumor effects of several chemotherapeutic agents, including cisplatin, doxorubicin, and paclitaxel, without increasing their toxicity." *Id.* at 743.

c. Pegram '98 (Ex. 1014)

Pegram '98 reports the results of a phase II clinical trial using a combination of trastuzumab plus cisplatin. Ex. 1014, 2659. Pegram '98 states that "[t]hese studies showed that the pharmacokinetics of rhuMAb HER2 were predictable, and that the doses delivered achieved a target trough serum concentration of 10 to 20 μg/mL, which is associated with antitumor activity in preclinical models." *Id.* at 2660. Pegram '98 also reports a toxicity profile of the combination that paralleled the toxicity of cisplatin alone, thereby leading to the conclusion that trastuzumab did not increase toxicity. *Id.* at 2668.

2. Obviousness Based on the Herceptin Label, Baselga '96, Pegram '98, and the Knowledge of a Person of Ordinary Skill in the Art of the Prior Art

Petitioners have provided a claim-by-claim explanation for the basis of their contention that claims 1–3, 5, 7, 9–11, and 17–33 are obvious over the Herceptin label in view of Baselga '96, Pegram '98, and the Knowledge of a Person of Ordinary Skill in the Art. Pet. 29–54.

In general terms, the challenged claims are directed to a dosing regimen for the treatment of cancer in which trastuzumab is administered at an initial dose, followed by administration of the antibody at subsequent

doses that are the same or less than the initial dose and separated in time by at least about two weeks. Independent claim 1 specifies an initial dose of approximately 5 mg/kg, while certain dependent claims specify higher initial doses of 6 mg/kg, 8 mg/kg, or 12 mg/kg (e.g., cls. 2, 3, 9), whereas other dependent claims specify that the subsequent doses are separated in time by at least three weeks (e.g., cls. 5, 10). Our obviousness analysis assumes a treatment method in which trastuzumab is administered once every three weeks, as that dosing interval is encompassed by all the challenged claims and is the focus of the parties' arguments and evidence in this proceeding.

Petitioners rely upon the teaching in the Herceptin label that trastuzumab doses of up to 500 mg had been successfully administered to patients. Pet. 31 (citing Ex. 1008, 1). Based on a patient weight range of 55–85 kg, Petitioners calculate that the weight-based dose for the 500 mg absolute dose taught by the Herceptin label ranges from 5.88–9.09 mg/kg. *Id.* at 31–32 (citing Ex. 1002 ¶¶ 55–57; Ex. 1003 ¶ 45; Ex. 1026, 3; Ex. 1027, 334 (Table 7-2)). Petitioners further rely upon the Herceptin label's teaching that trastuzumab doses should be "front-loaded" with a higher initial dose of 4 mg/kg followed by a lower weekly maintenance dose of 2 mg/kg. *Id.* at 33. Additionally, Petitioners rely upon the teaching in the Herceptin label describing the administration of trastuzumab in combination with chemotherapeutic agents, and that these chemotherapeutic agents are administered once every three weeks to patients. *Id.* at 35–36, 43–44. Petitioners further rely upon Baselga '96 and Pegram '98 insofar as they confirm that the weekly dosing regimen encompassed by the Herceptin label was successfully administered to patients in phase II clinical trials, and that

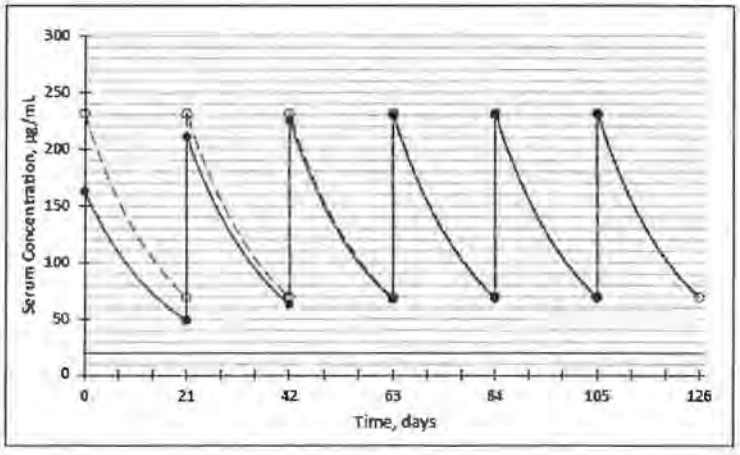
the skilled artisan would have been aware of a target trough serum concentration of 10–20 μg/mL for trastuzumab. Pet. 33, 37.

Petitioners acknowledge that the Herceptin label, along with Baselga '96 and Pegram '98, teach only a *weekly* dosing regimen, but assert that the skilled artisan would nonetheless have been motivated to decrease the frequency of trastuzumab administration to once every three weeks for several reasons. *Id.* at 34–42. First, Petitioners contend that "a skilled artisan would decrease the frequency of injections to improve efficiency, to provide a more convenient dosing regimen—particularly for terminally ill patients—, and to improve patient compliance and quality of life." *Id.* at 34. Second, Petitioners contend that the skilled artisan would have been motivated to apply a tri-weekly (i.e., once every three weeks) regimen for the antibody in order to align with the dosing schedules of the chemotherapy so that a patient would only have to make one trip to the clinic to receive both doses. *Id.* at 36. In support, Petitioners rely upon their oncology expert, Dr. Lipton, who attests that each trip to the clinic to receive even a single infusion of antibody treatment often takes between a half and a full day, which can result in additional time and costs for the patient. Ex. 1002 ¶¶ 42–43.

Petitioners further contend that the skilled artisan would confidently decrease the frequency of injections and use a tri-weekly dosing regimen in view of trastuzumab's known pharmacokinetic properties. *Id.* at 36. Petitioners contend that arriving at the tri-weekly dosing schedule was merely a matter of "routine calculation and optimization" of the therapy outlined in the Herceptin label. *Id.* at 37. In this regard, Petitioners rely upon data from the Herceptin label and Dr. Jusko's opinions to assert that it

would have been a matter of routine calculation for a skilled artisan to determine that a tri-weekly 500 mg trastuzumab dosing regimen would have resulted in a serum concentration well above the target minimum trough concentration of 10–20 μg/mL reported in the prior art. *Id.* at 37–39 (citing Ex. 1003 ¶¶ 46–47, 49–51, 56–58, 62).

Specifically, Dr. Jusko, assuming a "one-compartment" model to approximate drug concentration over time, calculated the initial minimum drug concentration three weeks after first administering a 500 mg antibody dose to a 70 kg patient to be 48.3 μg/mL and the steady-state trough concentration after multiple doses to be 68.7 μg/mL. Ex. 1003 ¶¶ 46–58. Additionally, assuming linear (first-order) kinetics, Dr. Jusko calculated that a 712 mg loading dose followed by 500 mg tri-weekly maintenance doses could be administered to patients while keeping serum drug concentrations within acceptable levels. *Id.* ¶¶ 59–66. Dr. Jusko provides the following graph depicting expected trastuzumab concentrations over time for a 70 kg patient administered 500 mg of trastuzumab every three weeks, with or without an initial 712 mg loading dose (broken and solid lines, respectively):



Ex. 1003 ¶ 62 (Fig. 2). As shown in the figure above, when administering either calculated dosing regimen, Dr. Jusko concludes that the trastuzumab serum concentration would have been expected to stay well above the target minimum trough concentration of 10–20 μg/ml (with 20 μg/ml shown in red). *Id.* ¶ 63.

As noted by Petitioners, Dr. Jusko made three assumptions in performing his calculations: (1) that trastuzumab exhibits non-exponential kinetics; (2) that the initial concentration (C_0) can be estimated by multiplying the dose by the volume of distribution and average mass of a patient; and (3) that the kinetics of trastuzumab remain constant with multiple-dosing. Pet. 42 (citing Ex. 1003 ¶¶ 69–71; Ex. 1028, 91; Ex. 1029, 77).

The two main issues argued in this proceeding are: (a) whether there would have been a motivation to extend the weekly dosing interval taught in the prior art to a tri-weekly dosing interval based on concerns about patient convenience and quality of life, and (b) whether there would have been a reasonable expectation of success in implementing such a dosing regimen based on Dr. Jusko's pharmacokinetic analysis. It is Petitioners' burden to demonstrate both "that a skilled artisan would have been motivated to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success in doing so." *Intelligent Bio-Sys., Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359, 1367 (Fed. Cir. 2016) (internal citations omitted). As they are distinct legal requirements for obviousness, we address motivation and reasonable expectation of success separately in our analysis. For the reasons explained below, while skilled artisans may have

been motivated to extend the dosing interval, we find that they would not have had a reasonable expectation of success in doing so based on the prior art. Thus, we determine that Petitioners have not shown that the challenged claims are unpatentable for obviousness.

a. Motivation

As discussed above, Petitioners' primary arguments on motivation for extending the dosing interval of trastuzumab from the weekly administration taught in the prior art to tri-weekly is based on a desire to improve patient "convenience," "compliance," "efficiency," and "quality of life." Pet. 34. In its Response, Patent Owner contends these "patient-related" factors would not have served as a reason to extend the dosing interval because the primary focus for skilled artisans in developing a treatment regimen for HER2-positive breast cancer would have been on efficacy. PO Resp. 28–36. Moreover, instead of extending trastuzumab's dosing interval to a tri-weekly schedule, Patent Owner asserts that skilled artisans were actually increasing the frequency of the chemotherapy (paclitaxel) administration in numerous clinical trials so that both drugs could be administered on a weekly schedule. *Id.* at 31–32. Patent Owner also argues that this is not simply a case of selecting an optimal doses from known range of doses in the prior art since the only dosing interval disclosed was weekly. *Id.* at 26. Patent Owner notes that "at the time of the invention, developing an antibody dosing regimen for clinical use was described as a "complicated task" and such drugs "defy easy quantitative description and prediction." *Id.* at 26 (citing Ex. 2004, 11; Ex. 1022, 3:109).

We find that the skilled artisan would have been motivated to extend the dosing interval for the simple (yet compelling) reasons that doing so

would have been more cost-effective and less burdensome for the patient undergoing such treatment, which required in-person visits to the clinic for each antibody infusion. As previously recognized by the Federal Circuit, "[a] relatively infrequent dosing schedule has long been viewed as a potential solution to the problem of patient compliance." *Hoffman-La Roche Inc. v. Apotex Inc.*, 748 F.3d 1326, 1329 (Fed. Cir. 2014). Patent Owner seeks to limit this statement in *Hoffman-La Roche* to the specific issue addressed in that case, which was whether once-monthly administration of bisphosphonate ibandronate to treat osteoporosis would have been obvious. PO Resp. 38–39. Patent Owner contends that, unlike the facts of *Hoffman-La Roche*, the claimed treatment regimen at issue in this proceeding involves a "first-in-class" therapeutic (i.e., trastuzumab was the only antibody approved at the time for the treatment of "solid" tumors), a fatal disease condition (breast cancer), and a completely different set of prior art. *Id.* at 39. Patent Owner argues that "[c]onvenience considerations that may be applicable in the context of treatments to prevent osteoporosis have little relevance in the context of treating HER2-positive breast cancer." *Id.* at 39. We do not read *Hoffman-La Roche* to stand for a *per se* rule that it would always have been obvious to extend the dosing interval in order to address patient compliance concerns regardless of the particular medical condition or drug at issue. Nonetheless, based on the specific facts of this case, we find that skilled artisans would have been similarly motivated to administer trastuzumab less frequently to treat breast cancer patients.

In support of this finding, we take into account the real-world experiences of the parties' oncology experts, Dr. Lipton (Petitioner's expert) and Dr. Gelmon (Patent Owner's expert), who are both physicians with

extensive experience treating breast cancer patients in clinical settings. Ex. 1002 ¶¶ 4–10; Ex. 2040 ¶¶ 2–5. Dr. Lipton attests that each trip to his clinic to receive even a relatively short infusion of antibody treatment often takes between a half and a full day, which can result in additional time and costs for the patient. Ex. 1002 ¶¶ 42–43. Indeed, some of his patients have had to travel up to one hundred miles each direction to receive treatment at the clinic. *Id.* ¶ 39. As such, we are not persuaded by Dr. Gelmon's contention that efficacy would have taken precedence over convenience as the focus of cancer treatment in the 1990s. Ex. 2040 ¶¶ 30–34. Of course, maintaining efficacy and safety would have been a paramount concern for the skilled artisan seeking to improve upon the weekly dosing regimen that was previously FDA-approved, but that does not mean improving convenience and quality of life for the patient would not have also been motivating concerns. By 1999, efficacy and safety had already been demonstrated for weekly trastuzumab administration as set forth in the Herceptin label. Ex. 1008. Notably, Dr. Gelmon admitted during her deposition that "before 1999 it was known that providing a drug less frequently might provide benefits to certain patients in terms of convenience, cost and quality of life as long as efficacy and safety were shown." Ex. 1058, 328:24-329:7. Indeed, these same concerns factored into Dr. Gelmon's own clinical study involving tri-weekly trastuzumab administration, which took place within months of the '196 patent priority date. *Id.* at 73:19–75:16.[6]

[6] While the publication of Dr. Gelmon's tri-weekly study does not qualify as prior art, we find the fact that she initiated the study so close to the priority date undermines the credibility of her testimony that skilled artisans

Contrary to Patent Owner's arguments, the prior art need not have expressly articulated or suggested patient convenience or quality of life concerns as the motivation to extend the dosing interval. *See KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 418 (2007) ("[T]he [obviousness] analysis need not seek out precise teachings directed to the specific subject matter of the challenged claim, for a court can take account of the inferences and creative steps that a person of ordinary skill in the art would employ."). Nonetheless, the motivation set forth by Dr. Lipton is supported by his citation to prior art articles indicating that quality of life issues for cancer patients have long been a concern to physicians. Ex. 1002 ¶ 44 (citing Coates, et al., *Quality of Life in Oncology Practice: Prognostic Value of EORTC QLQ-C30 Scores in Patients with Advanced Malignancy*, 33(7) EUROPEAN JOURNAL OF CANCER 1025–30 (1997) (Ex. 1019); Aaronson, et al., *The European Organization for Research and Treatment of Cancer QLQ-C30: A Quality-of-Life Instrument for Use in International Clinical Trials in Oncology*, 85(5) J. NAT'L CANCER INSTITUTE 365–76 (1993) (Ex. 1020); Ferrell, *Quality of Life in Breast Cancer*, 4(6) CANCER PRACTICE 331–40 (1996) (Ex. 1021)).

Additionally, we find that the skilled artisan would have been motivated to match trastuzumab and chemotherapy dosing. As indicated in

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would not have considered extending the dosing interval at the time. In their Reply, however, Petitioners identify additional post-filing evidence supporting their contention that skilled artisans were motivated by "patient-related factors" to investigate tri-weekly dosing of trastuzumab. Reply 14–15. Insofar as these additional references do not qualify as prior art themselves, nor do they purport to recount what was publicly known in the prior art, we decline to give them any weight in our analysis.

the Herceptin label, patients were often prescribed chemotherapy, such as paclitaxel or anthracycline, in combination with trastuzumab. Ex. 1008, 1. The Herceptin label indicates that both paclitaxel and anthracycline were administered once every three weeks (21 days). *Id.* In addition to convenience for the patient, Dr. Lipton notes that "it is also beneficial for the clinic to administer the combined therapies on the same schedule because they only have to prep the patient once." Ex. 1002 ¶ 66. Patent Owner acknowledges that researchers at the time had explored the possibility of administering paclitaxel to match weekly trastuzumab administration. PO Resp. 9; Ex. 2040 ¶¶ 38, 57; *see, e.g.*, M Fornier, *Weekly (W) Herceptin (H) + 1 Hour Taxol (T): Phase II Study in HER2 Overexpressing (H2+) and Non-Overexpressing (H2-) Metastatic Breast Cancer (MBC)*, 18 PROC. AM. SOC'Y CLINICAL ONCOLOGY 126a (Abstract 482) (1999) (Ex. 2029). But, at the time, paclitaxel was FDA-approved for only tri-weekly treatment. Ex. 1058, 180:22–181:1. Regardless, the fact that skilled artisans were considering matching the antibody and chemotherapy treatments on a weekly basis does not mean that they would also not have considered matching the treatments on a tri-weekly basis. Obviousness does not require the claimed regimen to be the only or best choice, nor may a patentee defeat obviousness simply by identifying another alternative. *In re Fulton*, 391 F.3d 1195, 1200 (Fed. Cir. 2004) ("[O]ur case law does not require that a particular combination must be the preferred, or the most desirable, combination described in the prior art in order to provide motivation for the current invention.").

Patent Owner also contends that skilled artisans would not have had a reason to select a 500 mg maintenance dose or 712 mg loading dose, as

calculated by Dr. Jusko. PO Resp. 24–27. We are unpersuaded by these arguments because the Herceptin label expressly teaches that a 500 mg dose was considered safe and tolerable, at least when administered on a weekly basis. Dr. Jusko explained that the 500 mg dose level, and associated 12-day half-life, would have been the obvious starting point "because that was the highest reported tolerable weekly dose level with the longest half-life that would give the POSITA the best chance of achieving the minimum serum trough concentrations to establish efficacy at three weeks." Ex. 1057 ¶ 34. Dr. Jusko further notes that "[i]t would have made no sense to choose a lower dose level, as the result of any such simulation would not have been indicative of the feasibility of three-week dosing—a negative result would merely necessitate simulating at the higher dose level, i.e., 500 mg." *Id.* Furthermore, while the 712 mg loading dose is not expressly disclosed in the prior art (Ex. 1003 ¶¶ 59–63), Patent Owner's experts Dr. Grass and Dr. Gelmon do not dispute Dr. Jusko's calculation of this amount, which is based on equations set forth in a basic pharmacokinetics textbook. Ex. 1002 ¶ 72; *see* Rowland, *et al.*, CLINICAL PHARMACOKINETICS: CONCEPTS AND APPLICATIONS (3rd ed. 1995) (vol. 1), at 88 (Ex. 1022) ("Rowland").[7]

[7] Patent Owner also argues that the pharmacokinetic data in the prior art would not have motivated a skilled artisan to extend the dosing interval of trastuzumab. PO Resp. 40–43. We find that the skilled artisan would have been motivated to extend the dosing interval regardless of the pharmacokinetic data set forth in the prior art. But, as discussed below, we find that trastuzumab's non-linear kinetics would not have provided the skilled artisan with a reasonable expectation of success with such an extended dosing interval.

Accordingly, we find that skilled artisans would have been motivated to extend the dosing interval of trastuzumab to once every three weeks, with a 712 mg loading dose followed by 500 mg maintenance doses.

b. Reasonable Expectation of Success

Having found the requisite motivation to arrive at the claimed dosing regimen, we next turn to whether there would have had a reasonable expectation of success with such a treatment regimen. Based on our consideration of the record evidence, we find that Petitioners have not met their burden of establishing a reasonable expectation of success.

In evaluating reasonable expectation of success, we must "consider the appropriate scope of the patent's claimed invention." *Allergan, Inc. v. Apotex Inc.*, 754 F.3d 952, 965–66 (Fed. Cir. 2014). Here, the claims of the '196 patent are directed to a "method for the treatment of a human patient diagnosed with cancer characterized by overexpression of ErbB2 receptor, comprising administering an *effective* amount of an anti-ErbB2 antibody to the human patient." Ex. 1001, 55:63–66 (emphasis added). Petitioners and Patent Owner both focus their arguments and evidence on whether the skilled artisan would have reasonably expected that trastuzumab plasma concentrations would be maintained above 10–20 μg/mL, which the prior art identifies as the minimum serum trough concentration required for efficacy. In view of the claim scope, we agree that this is an appropriate definition of "success" for purposes of our analysis.

Petitioners contend that the skilled artisan would have extended the dosing interval based on Dr. Jusko's pharmacokinetic analysis as set forth above. Patent Owner disagrees that this type of mathematical analysis would have provided the requisite reasonable expectation of success for the

claimed dosing regimen. In particular, Patent Owner criticizes Dr. Jusko's application of linear pharmacokinetics to predict serum trough concentration insofar as the prior art taught that trastuzumab had demonstrated non-linear (dose-dependent) kinetics. PO Resp. 45–48. As noted by Patent Owner, "[f]or drugs with non-linear kinetics, pharmacokinetic parameters such as half-life do not remain constant but change as a function of the concentration of the drug in the plasma." *Id.* at 46 (citing Ex. 1022, 3:109; Ex. 2008, 123; Ex. 2038 ¶¶ 22–25, 27, 34–36). According to Patent Owner, there is insufficient data in the prior art to accurately predict whether a three-week dosing regimen would be clinically effective, and thus a clinical oncologist would not have confidently used three-week dosing based on Dr. Jusko's pharmacokinetic analysis. *Id.* at 55–57.

As part of our evaluation, we take into account the relative novelty of using antibodies for the treatment of cancer as of the August 27, 1999 priority date. Herceptin had been approved by the FDA for weekly administration in September 1998, less than a year before, was the first antibody approved to target "solid tumors," and the first approved to treat any form of breast cancer. Ex. 1008; Ex. 2003, 388; Ex. 2038, 33:8–17; Ex. 2040 ¶ 23.[8] Petitioners have not pointed to any prior art reference discussing the feasibility or viability of a tri-weekly antibody dosing regimen.

[8] Prior to August 1999, the FDA had approved only one other antibody for treating cancer—Patent Owner's rituximab product, which was approved for non-Hodgkin's lymphoma treatment in 1997. Ex. 2003, 388. We find no evidence of record indicating that rituximab had been approved or successfully tested for anything longer than weekly dosing.

While Dr. Jusko's calculations are based on "textbook" equations that were known in the prior art, the actual pharmacokinetic analysis set forth in his declaration for determining the serum trough concentration associated with a tri-weekly dosing regimen of trastuzumab was not found in any prior art reference. Thus, we find Dr. Jusko's analysis to be largely based on impermissible hindsight. *KSR*, 550 U.S. at 421 ("A factfinder should be aware . . . of the distortion caused by hindsight bias and must be cautious of arguments reliant upon ex post reasoning.").

Petitioners contend that Dr. Jusko applied the same model that Patent Owner and its collaborators did in the prior art. Reply 17. In particular, Petitioners rely upon Baselga '96's statement that "[s]erum levels of rhuMAb HER2 as a function of time were analyzed for each patient using a one-compartment model." Ex. 1013, 738. However, Baselga '96 did not mention a tri-weekly schedule, and instead determined that a regimen in which patients received an initial dose of 250 mg trastuzumab followed by 100 mg weekly doses was the "optimal dose and schedule." *Id.* Petitioners also speculate that the Herceptin label's reporting of only a single half-life for each dosage level "suggest[s] use of a one-compartment model." Reply 17; Ex. 1003 ¶ 34. But the Herceptin label does not explicitly indicate that a one-compartment model was used to model the weekly dosing regimen discussed therein. In any event, the pharmacokinetics discussed in the Herceptin label were based on actual clinical trials rather than just mathematical predictions. Ex. 1008, 1 ("The pharmacokinetics of Trastuzumab were studied in breast cancer patients with metastatic disease."). Baselga '96 and the Herceptin label both specifically recognize that trastuzumab has "dose dependent pharmacokinetics." Ex. 1008, 1;

Ex. 1013, 738. The very pharmacokinetics textbook relied upon by Dr. Jusko notes that "dose-dependent and time-dependent kinetic behaviors defy easy quantitative description and prediction." Ex. 1022, vol. 3, 395.

We recognize that Pegram'98 states that Phase I clinical "studies showed that the pharmacokinetics of rhuMAb HER2 were predictable." Ex. 1014, 2660. But as explained by Patent Owner's pharmacokinetic expert Dr. Grass, "[a] skilled artisan would understand 'predictable' in this context to mean that administration of the same dose with the same dosing schedule would likely yield the same serum concentrations if given to a similar patient population." Ex. 2039 ¶ 54. It does not suggest predictability across different dosing intervals. Insofar as the pharmacokinetics discussed in the prior art were only based on studies of weekly administration of lower trastuzumab doses, we do not find that the references support Petitioners' conclusion that the same "one-compartment" model could also be used to reasonably predict the expected serum concentrations for tri-weekly administration using higher doses of the antibody.

The evidence shows that the prior art did not contain sufficient data from which the skilled artisan could reliably predict the plasma concentration for trastuzumab over a three-week dosing interval using a one-compartment model. In this regard, we credit the testimony of Dr. Grass. Dr. Grass explains that one potential source of non-linear kinetics for trastuzumab was the presence of "shed antigens" in the patient's serum, which are extra-cellular domain HER2 receptors (ECD^{HER2}) "shed" from the tumor source that circulate in the patient's blood stream. Ex. 2039 ¶¶ 56, 71, 72. We are unpersuaded by Dr. Jusko's opinion that the effect of shed antigens on half-life and serum trough levels would not have been of

concern to the skilled artisan because it was "only shown to be significant in the small percentage of patients for which shed antigen reached 'high levels,' *i.e.*, greater than about 0.5 μg/mL." Ex. 1057 ¶ 46 (citing Ex. 1013 and Ex. 1014).

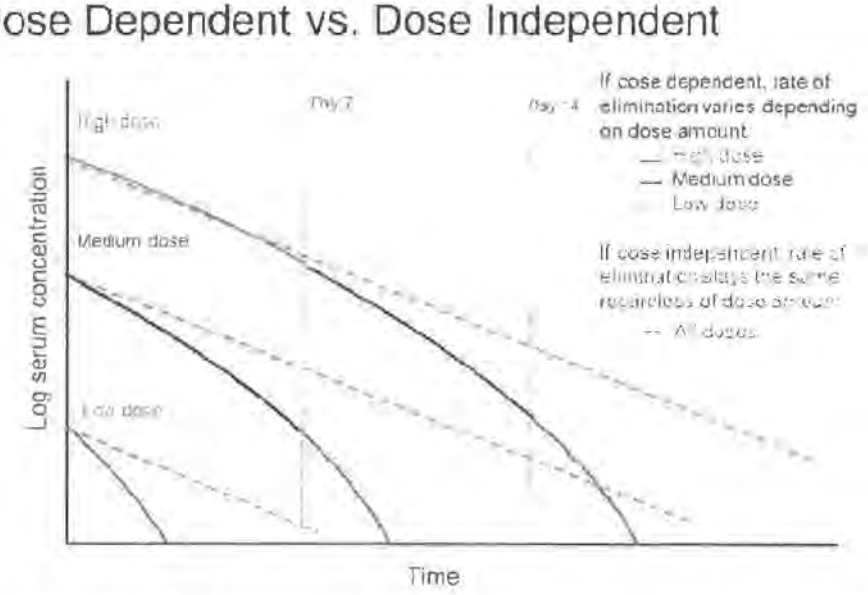
Petitioners' own prior art references highlight the uncertainty caused by the presence of shed antigens on the pharmacokinetics of trastuzumab. For instance, the Herceptin label notes that "64% of patients (287/447) had detectable shed antigen, which ranged as high as 1880 ng/mL (median = 11 ng/mL)," and that "[p]atients with higher baseline shed antigen levels were more likely to have lower serum trough concentrations." Ex. 1008, 1. Baselga '96 likewise teaches that "[t]he rhuMAb HER2 serum $t_{1/2}$ was found to be dependent on the presence of circulating ECD^{HER2} released from the tumor into the serum." Ex. 1013, 739. In fact, for those patients with high levels of shed antigen, Baselga '96 teaches that serum levels of the antibody were "suboptimal," and that "the trough levels of rhuMAb HER2 were consistently below detectable levels throughout the treatment course and until disease progression." *Id.* at 739–740 (Fig. 1B). Pegram '98 notes "there was an inverse relationship between rhuMAb HER2 serum half-life and serum shed HER2 ECD of 0.5 μg/mL or greater." Ex. 1014, 2665. Pegram '98 further indicates that "patients with any measurable shed [antigen] serum level, compared with patients without measurable circulating ECD, had lower mean trough rhuMAb HER2 concentrations (18.7 v. 43.6 μg/mL; $P = .0001$) across all time points (n = 443 observations; Fig. 1)." Notably, this prior art data appears to show that patients with *any* detectable shed antigen levels (i.e., 64% of patients as set forth in the Herceptin label) had a mean antibody trough level that was close to the 10–

20 μg/mL threshold for efficacy.[9] As such, we find that skilled artisan would have been concerned that the effect of shed antigens— not taken into account by Dr. Jusko's analysis—could indeed significantly affect serum trough concentrations for tri-weekly administration of trastuzumab.

Contrary to Dr. Jusko's assumptions, Dr. Grass attests that "applying a constant value for half-life over a three-week period, based on the one-week data reported in the prior art, to a dose-dependent drug like trastuzumab could overestimate trough serum concentration levels" because it "fail[s] to account for the nonlinear increase in elimination and corresponding decrease in the half-life that would be expected to occur as serum concentration declines." Ex. 2039 ¶ 25. Dr. Grass also contends that the actual rates of elimination for such a drug would be unpredictable without collecting sufficient data, such as by conducting a "washout study" where serum concentration is collected over several half-lives following a single administration of the drug, but notes that there is no prior art reference for trastuzumab that describes such data. *Id.* ¶ 24.

To illustrate this point, Dr. Grass provides the following graph showing differences that can potentially exist between dose-independent drugs (which exhibit linear kinetics) and dose-dependent drugs (which exhibit non-linear kinetics):

[9] Although Dr. Gelmon testified that later (post-filing) studies showed that shed antigens were not in fact a concern for efficacy of Herceptin, and that dosage is not adjusted based on shed antigen levels today, our analysis is based on what was known in the prior art. Ex. 1058, 62:20–65:6.



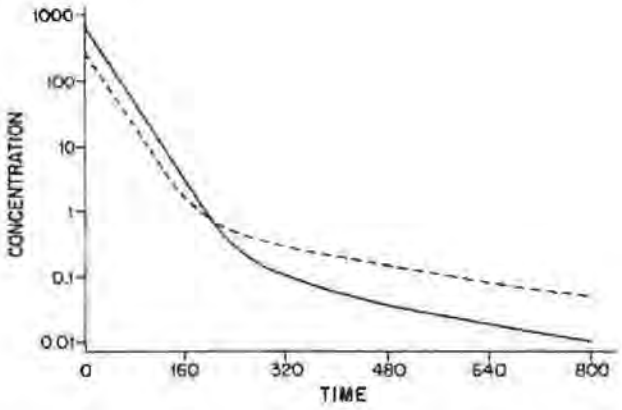
Id. ¶ 23. As shown by the solid lines in the graph above, which correspond to different dosage amounts of a dose-dependent drug, elimination increases (i.e., half-life decreases) as the drug concentration changes over time. Petitioners criticize this graph as being "made up" by Dr. Grass, as it was not derived from any particular data set forth in the prior art. Reply 20 (citing Ex. 1059, 116:16–21). Patent Owner, however, points to post-filing data concerning the anti-cancer agent indisulam as a "real-world example" of a dose-dependent drug that can behave this way, showing how assuming a constant half-life could greatly overestimate the predicted serum concentration over a longer interval. PO Resp. 49–50; Ex. 2039 ¶ 26; Anthe S. Zandvliet et al., *Saturable Binding of Indisulam to Plasma Proteins and Distribution to Human Erythrocytes*, 34 DRUG METABOLISM & DISPOSITION 1041 (2006) (Ex. 2052) ("Zandvliet"). While we recognize that Zandvliet does not qualify as prior art, and concerns a "small molecule" rather than an antibody, we find that it demonstrates at least one example in which assuming linear kinetics could result in an overestimation of trough serum

concentrations for a dose-dependent drug. From the perspective of a skilled artisan as of the August 27, 1999 priority date, we find nothing in the record to suggest that a similar overestimation would not have been a concern for tri-weekly trastuzumab administration.

With its Reply, Petitioners present additional evidence and arguments as to why Dr. Jusko's initial assumptions and analysis were reasonable. In particular, Petitioners contend that Dr. Jusko's analysis would, at worst, have underestimated, not overestimated, serum trough concentrations. Reply 18–23. In support of this contention, Petitioners cite King, APPLICATIONS AND ENGINEERING OF MONOCLONAL ANTIBODIES (1998) (Ex. 1029) ("King '98") as teaching that antibodies follow a common profile associated with "receptor-mediated" (or "target-mediated") drug disposition, with a quick initial clearance and short half-life ($t_{1/2}\alpha$), followed by slower clearance and a longer half-life ($t_{1/2}\beta$). While King '98 includes a table that identifies several antibodies known at the time to have a shorter $t_{1/2}\alpha$ followed by a longer $t_{1/2}\beta$, it *only* reports a $t_{1/2}\beta$ of 199 ± 120 hours for trastuzumab (citing Baselga '96), and Petitioners do not point to any other evidence suggesting a $t_{1/2}\alpha$ for trastuzumab. *See* Ex. 1029, 70 (Table 2.7). Furthermore, King '98 recognizes that the presence of circulating shed antigens could reduce antibody half-life in some cases, and that "[t]he pharmacokinetics of human IgG are unusual in that the half-life varies with concentration." *Id.* at 68, 70. As such, we find that King '98 does not show that Dr. Jusko's linear assumptions would have underestimated serum trough concentrations for trastuzumab.

In further support, Petitioners point to the following graph from Levy, *Pharmacologic target-mediated drug disposition*, 56(3) Clinical

Pharmacology & Therapeutics 248–52 (1994) ("Levy") as demonstrating this type of profile:

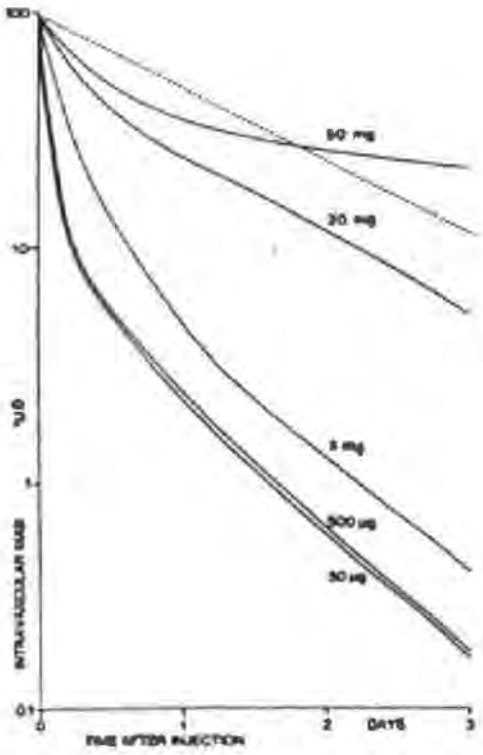


Ex. 1052, 249 (Fig. 1). The figure above shows "[t]ypical concentration-time profile in plasma *(continuous line)* and tissues *(broken line)* for a drug that is subject to high-affinity low-capacity binding in tissues." *Id.*

We do not find that the expected profile for receptor-mediated drug disposition, as shown in Levy, supports the reasonableness of Dr. Jusko's pharmacokinetic analysis for trastuzumab. Levy does not describe the kinetics of antibodies at all, but instead only identifies certain small molecules that might exhibit this "hypothetical behavior." Ex. 2084, 22:10–16, 59:8–16. Specifically, with reference to Figure 1 shown above, Levy notes that "the effect on pharmacokinetics can be quite striking in that the plasma concentration profile exhibits a terminal decay phase with a very long half-life ($t_{1/2}$), as is the case for certain angiotensin-converting enzyme (ACE) and aldose reductase inhibitors." Ex. 1052, 248. In criticizing Dr. Grass's reliance on the indisulam data discussed above, Dr. Jusko notes that skilled artisans would not "rely[] on pharmacokinetic behavior of ***small molecules***, which was known to be fundamentally different to that of antibodies." Ex. 1057 ¶ 5; *see also id.* ¶ 20 n.1 (noting "in addition to the

[differences in] molecular weight, the different mechanisms of disposition of small molecules and antibodies impacts their pharmacokinetic profiles"). Accordingly, we are not persuaded by Dr. Jusko's inconsistent opinion relying upon Levy's teachings with respect to target-mediated disposition of small molecules. Ex. 1057 ¶ 15. Moreover, even with respect to the ACE inhibitors discussed therein, Levy does not make any definitive conclusions as to their pharmacokinetic behavior, noting instead that "[m]ore definitive information can be obtained only in animal studies that permit opening of the 'black box' to explore what goes on in individual tissues." Ex. 1052, 248–49.

Petitioners also point to the following graph from Koizumi, *et al.*, *Multicompartmental Analysis of the Kinetics of Radioiodinated Monoclonal Antibody in Patients with Cancer*, 27(8) J. NUCLEAR MED. 1243–54 (1986) (Ex. 1054) ("Koizumi"):



Reply, 22; Ex. 1054, 1252 (Fig. 8) (annotation in red added by Petitioners). The annotated figure above shows "[m]odel simulated curves" for intravascular monoclonal antibodies (MAb) reflecting the "effect of different amount of injected MAb on blood clearance." *Id.* According to Petitioners, "for a given antibody dose (here 50mg), a linear model (shown in red) would underestimate the actual serum concentration (shown in black) soon after dosing." Reply 21.

We do not find that Koizumi supports the reasonableness of Dr. Jusko's application of a linear model. Indeed, Petitioners' own annotation in the figure above shows that a linear model could overestimate actual serum concentrations for certain doses (e.g., 20 mg) or at certain times after injection (e.g., less than 2 days). For tri-weekly trastuzumab administration, it was unknown whether the actual serum concentration would fall above or

below the linearity assumed in Dr. Jusko's model. Moreover, unlike Dr. Jusko's "one-compartment" analysis in this proceeding, Koizumi specifically describes a "multicompartmental" analysis conducted using a computer simulation. Ex. 1054, 1247. In this regard, Koizumi notes that "[i]nitial model solutions assumed that the model was linear," but "[u]sing this information it was not possible to fit the data observed for the patients with the model simulations." *Id.* at 1245–46. Furthermore, according to Koizumi:

> [C]ompartmental analysis also raises several problems. If the compartmental model is based upon unlikely assumptions, or inadequately validated, then misleading information follows. While this is self-evident, the complexity of a model addressing the pharmacokinetics of a MAb requires simplifications based upon assumptions in order to permit realistic mathematical handling. These simplifications and assumptions are particularly vulnerable to error in a system such as MAb, wherein many processes remain to be clarified.

Id. at 1252. As such, Koizumi underscores the inherent uncertainty associated with using mathematical models to predict the pharmacokinetic behavior of antibodies.

In sum, for the foregoing reasons, we determine Petitioners have not established the reasonable expectation of success required for obviousness. In reaching this conclusion, we are cognizant that "[c]onclusive proof of efficacy is not required to show obviousness." *Hoffman-La Roche*, 748 F.3d at 1331. Nonetheless, the Federal Circuit has also indicated that reasonable expectation cannot come from a mere "hypothesis" that might form the basis for further testing. *Sanofi v. Watson Labs. Inc.*, 875 F.3d 636, 647–49 (Fed. Cir. 2017) (finding prior art reference that stated the "expected" benefit of a

clinical trial did not establish a reasonable expectation of success); *see also In re Cyclobenzaprine Hydrochloride Extended-Release Capsule Patent Litig.*, 676 F.3d 1063, 1070 (Fed. Cir. 2012) ("While it may have been obvious to experiment with the use of the same PK profile when contemplating an extended-release formulation, there is nothing to indicate that a skilled artisan would have had a reasonable expectation that such an experiment would succeed in being therapeutically effective.").

III. ALLEGED IMPROPER REPLY MATERIALS/PATENT OWNER'S MOTION TO EXCLUDE

Pursuant to our authorization, Patent Owner filed a paper identifying allegedly improper arguments and evidence included with Petitioners' Reply. Paper 67. Specifically, Patent Owner identifies the following materials as improper: Exhibits 1043–1048, 1050, 1052, 1054, and 1055, and portions of Dr. Lipton's reply declaration (Ex. 1056) and Dr. Jusko's reply declaration (Ex. 1057) referencing those exhibits. *Id.* Patent Owner also separately filed a motion to exclude the same evidence it identifies as improper reply materials. Paper 68.

As a preliminary matter, a motion to exclude is not a proper vehicle for addressing "arguments or evidence that a party believes exceeds the proper scope of reply." Trial Practice Guide Update (August 13, 2018),[10] 16. Instead, "[i]f a party believes that a brief filed by the opposing party raises new issues, is accompanied by belatedly presented evidence, or otherwise exceeds the proper scope of reply . . . it may request authorization

[10] Available at https://www.uspto.gov/sites/default/files/documents/2018_Revised_Trial_Practice_Guide.pdf.

to file a motion to strike." *Id.* at 17. "In most cases, the Board is capable of identifying new issues or belatedly presented evidence when weighing the evidence at the close of trial, and disregarding any new issues or belatedly presented evidence that exceeds the proper scope of reply or sur-reply." *Id.*

Nevertheless, to the extent necessary, we treat Patent Owner's Motion to Exclude and Identification of Improper New Reply Materials as a motion to strike. We have not relied upon Exhibits 1043–1048, 1050, and 1055 in rendering this decision. We have not given any weight to this evidence to support Petitioners' obviousness arguments because they have publication dates after August 27, 1999, and thus do not qualify as prior art to the '196 patent. *See* Paper 68, 7–10 (explaining why post-priority date references relied upon by Petitioners are irrelevant to obviousness determination in this proceeding). Furthermore, Exhibit 1055 has not been cited or relied upon by Petitioners in their Reply, and we decline to incorporate by reference the opinion in Dr. Jusko's reply declaration concerning that exhibit. *See* 37 C.F.R. § 42.6(a)(3) ("Arguments must not be incorporated by reference from one document into another document."). Accordingly, we dismiss as moot Patent Owner's motion to strike this evidence.

We have taken into consideration Exhibits 1052 and 1054 in our analysis, as discussed above. We determine that these exhibits and Petitioners' arguments in relation to these exhibits are proper reply evidence as they seek to respond to Patent Owner's arguments concerning the reasonableness of Dr. Jusko's pharmacokinetic analysis. Specifically, in relying upon Exhibits 1052 and 1054, and the portions of Dr. Jusko's reply declaration citing those exhibits, Petitioners seek to respond to Patent Owner's criticism that Dr. Jusko's assumptions would have overestimated

serum concentration for dose-dependent drugs such as trastuzumab. With such evidence, Petitioners seek to further support, not modify, their basis for reasonable expectation of success set forth in the Petition. We do not find that Petitioners have presented an "entirely new rationale" worthy of being excluded in their Reply. *Ericsson Inc. v. Intellectual Ventures I LLC*, No. 2017-1521, 2018 WL 4055815, *6 (Fed. Cir. Aug. 27, 2018). Although we find the new exhibits unpersuasive, that does not render them improper reply evidence. We, therefore, deny Patent Owner's motion to strike this evidence.

IV. CONCLUSION

After reviewing the entire record and weighing evidence offered by both parties, we determine that although Petitioners have shown that a skilled artisan would have been motivated to extend the dosing frequency of trastuzumab from weekly to tri-weekly, Petitioners have not met their burden to show a reasonable expectation of success with respect to such a dosing regimen. As a result, Petitioners have not shown, by a preponderance of the evidence, that claims 1–3, 5, 7, 9–11, and 17–33 of the '196 patent would have been obvious over the combination of the Herceptin Label, Baselga '96, Pegram '98, and the knowledge of the skilled artisan.

V. ORDER

Accordingly, it is:

ORDERED that claims 1–3, 5, 7, 9–11, and 17–33 of the '196 patent have not been shown to be unpatentable;

FURTHER ORDERED that Patent Owner's Motion to Exclude is denied-in-part and dismissed-in-part; and

FURTHER ORDERED that, because this is a final written decision, parties to this proceeding seeking judicial review of our Decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.

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