PROGRESS in CLINICAL and BIOLOGICAL RESEARCH VOLUME 204

TUMOR MARKERS AND THEIR SIGNIFICANCE IN THE MANAGEMENT OF BREAST CANCER

EDITORS: Thomas Dao
Angela Brodie
Clement Ip

ALAN R. LISS, INC., NEW YORK

TUMOR MARKERS AND THEIR SIGNIFICANCE IN THE MANAGEMENT OF BREAST CANCER

Proceedings of a Workshop held in Bethesda, Maryland
March 6, 1985

Editors

Thomas Dao
Department of Breast Surgery
Roswell Park Memorial Institute
Buffalo, New York

Angela Brodie
Department of Pharmacology and
Experimental Therapeutics
University of Maryland School of Medicine
Baltimore, Maryland

Clement Ip
Organ Systems Coordinating Center
Roswell Park Memorial Institute
Buffalo, New York

ALAN R. LISS, INC. • NEW YORK

Address all Inquiries to the Publisher
Alan R. Liss, Inc., 41 East 11th Street, New York, NY 10003

Printed in the United States of America

Library of Congress Cataloging-in-Publication Data
Main entry under title:

Tumor markers and their significance in the management of breast cancer.

Sponsored by the Breast Cancer Working Group of the Organ Systems Program, National Cancer Institute.
Includes index.
1. Breast—Cancer—Diagnosis—Congresses. 2. Tumor markers—Congresses. 3. Tumor antigens—Congresses. I. Ip, Clement. II. Dao, Thomas L. (Thomas Ling-Yuan), 1922– . III. Brodie, Angela. IV. Organ Systems Program (National Cancer Institute). Breast Cancer Working Group. [DNLM: 1. Antigens, Neoplasms—analysis—congresses. 2. Breast Neoplasms—diagnosis—congresses. 3. Neoplasms Proteins—analysis—congresses. W1 PR668E v.204/WP 870 T925 1985]
RC280.B8T86 1986 616.99'449075 85-23902
ISBN 0-8451-5054-5

PROGRESS IN CLINICAL AND BIOLOGICAL RESEARCH

RECENT TITLES

Vol 163: **Prevention of Physical and Mental Congenital Defects**, Maurice Marois, *Editor.* Published in 3 volumes: Part A: *The Scope of the Problem.* Part B: *Epidemiology, Early Detection and Therapy, and Environmental Factors.* Part C: *Basic and Medical Science, Education, and Future Strategies*

Vol 164: **Information and Energy Transduction in Biological Membranes,** C. Liana Bolis, Ernst J.M. Helmreich, Hermann Passow, *Editors*

Vol 165: **The Red Cell: Sixth Ann Arbor Conference**, George J. Brewer, *Editor*

Vol 166: **Human Alkaline Phosphatases,** Torgny Stigbrand, William H. Fishman, *Editors*

Vol 167: **Ethopharmacological Aggression Research**, Klaus A. Miczek, Menno R. Kruk, Berend Olivier, *Editors*

Vol 168: **Epithelial Calcium and Phosphate Transport: Molecular and Cellular Aspects,** Felix Bronner, Meinrad Peterlik, *Editors*

Vol 169: **Biological Perspectives on Aggression,** Kevin J. Flannelly, Robert J. Blanchard, D. Caroline Blanchard, *Editors*

Vol 170: **Porphyrin Localization and Treatment of Tumors,** Daniel R. Doiron, Charles J. Gomer, *Editors*

Vol 171: **Developmental Mechanisms: Normal and Abnormal,** James W. Lash, Lauri Saxén, *Editors*

Vol 172: **Molecular Basis of Cancer,** Robert Rein, *Editor.* Published in 2 volumes: Part A: *Macromolecular Structure, Carcinogens, and Oncogenes.* Part B: *Macromolecular Recognition, Chemotherapy, and Immunology*

Vol 173: **Pepsinogens in Man: Clinical and Genetic Advances,** Johanna Kreuning, I. Michael Samloff, Jerome I. Rotter, Aldur Eriksson, *Editors*

Vol 174: **Enzymology of Carbonyl Metabolism 2: Aldehyde Dehydrogenase, Aldo-Keto Reductase, and Alcohol Dehydrogenase,** T. Geoffrey Flynn, Henry Weiner, *Editors*

Vol 175: **Advances in Neuroblastoma Research,** Audrey E. Evans, Giulio J. D'Angio, Robert C. Seeger, *Editors*

Vol 176: **Contemporary Sensory Neurobiology,** Manning J. Correia, Adrian A. Perachio, *Editors*

Vol 177: **Medical Genetics: Past, Present, Future,** Kåre Berg, *Editor*

Vol 178: **Bluetongue and Related Orbiviruses,** T. Lynwood Barber, Michael M. Jochim, *Editors*

Vol 179: **Taurine: Biological Actions and Clinical Perspectives,** Simo S. Oja, Liisa Ahtee, Pirjo Kontro, Matti K. Paasonen, *Editors*

Vol 180: **Intracellular Protein Catabolism,** Edward A. Khairallah, Judith S. Bond, John W.C. Bird, *Editors*

Vol 181: **Germfree Research: Microflora Control and Its Application to the Biomedical Sciences,** Bernard S. Wostmann, *Editor*, Julian R. Pleasants, Morris Pollard, Bernard A. Teah, Morris Wagner, *Co-Editors*

Vol 182: **Infection, Immunity, and Blood Transfusion,** Roger Y. Dodd and Lewellys F. Barker, *Editors*

Vol 183: **Aldehyde Adducts in Alcoholism,** Michael A. Collins, *Editor*

Vol 184: **Hematopoietic Stem Cell Physiology,** Eugene P. Cronkite, Nicholas Dainiak, Ronald P. McCaffrey, Jiri Palek, Peter J. Quesenberry, *Editors*

Vol 185: **EORTC Genitourinary Group Monograph 2,** Fritz H. Schroeder, Brian Richards, *Editors.* Published in two volumes: Part A: *Therapeutic Principles in Metastatic Prostatic Cancer.* Part B: *Superficial Bladder Tumors*

Vol 186: **Carcinoma of the Large Bowel and Its Precursors,** John R.F. Ingall, Anthony J. Mastromarino, *Editors*

Vol 187: **Normal and Abnormal Bone Growth: Basic and Clinical Research,** Andrew D. Dixon, Bernard G. Sarnat, *Editors*

Vol 188: **Detection and Treatment of Lipid and Lipoprotein Disorders of Childhood,** Kurt Widhalm, Herbert K. Naito, *Editors*

Vol 189: **Bacterial Endotoxins: Structure, Biomedical Significance, and Detection With the Limulus Amebocyte Lysate Test,** Jan W. ten Cate, Harry R. Büller, Augueste Sturk, Jack Levin, *Editors*

Vol 190: **The Interphotoreceptor Matrix in Health and Disease,** C. David Bridges, *Editor,* Alice J. Adler, *Associate Editor*

Vol 191: **Experimental Approaches for the Study of Hemoglobin Switching,** George Stamatoyannopoulos, Arthur W. Nienhuis, *Editors*

Vol 192: **Endocoids,** Harbans Lal, Frank LaBella, John Lane, *Editors*

Vol 193: **Fetal Liver Transplantation,** Robert Peter Gale, Jean-Louis Touraine, Guido Lucarelli, *Editors*

Vol 194: **Diseases of Complex Etiology in Small Populations: Ethnic Differences and Research Approaches,** Ranajit Chakraborty, Emöke J.E. Szathmary, *Editors*

Vol 195: **Cellular and Molecular Aspects of Aging: The Red Cell as a Model,** John W. Eaton, Diane K. Konzen, James G. White, *Editors*

Vol 196: **Advances in Microscopy,** Ronald R. Cowden, Fredrick W. Harrison, *Editors*

Vol 197: **Cooperative Approaches to Research and Development of Orphan Drugs,** Melvin Van Woert, Eunyong Chung, *Editors*

Vol 198: **Biochemistry and Biology of DNA Methylation,** Giulio L. Cantoni, Aharon Razin, *Editors*

Vol 199: **Leukotrienes in Cardiovascular and Pulmonary Function,** Allan M. Lefer, Marlys H. Gee, *Editors*

Vol 200: **Endocrine Genetics and Genetics of Growth,** Costas J. Papadatos, Christos S. Bartsocas, *Editors*

Vol 201: **Primary Chemotherapy in Cancer Medicine,** D.J. Theo Wagener, Geert H. Blijham, Jan B.E. Smeets, Jacques A. Wils, *Editors*

Vol 202: **The 2-5A System: Molecular and Clinical Aspects of the Interferon-Regulated Pathway,** Bryan R.G. Williams, Robert Silverman, *Editors*

Vol 203: **Testicular Cancer,** Saad Khoury, René Küss, Gerald P. Murphy, Christian Chatelain, James P. Karr, *Editors*

Vol 204: **Tumor Markers and Their Significance in the Management of Breast Cancer,** Thomas Dao, Angela Brodie, Clement Ip, *Editors*

Vol 205: **Comparative Endocrinology: Developments and Directions,** Charles L. Ralph, *Editor*

Vol 206: **Genetic Toxicology of the Diet,** Ib Knudsen, *Editor*

Vol 207: **Monitoring of Occupational Genotoxicants,** Marja Sorsa, Hannu Norppa, *Editors*

Please contact the publisher for information about previous titles in this series.

TUMOR MARKERS AND THEIR SIGNIFICANCE IN THE MANAGEMENT OF BREAST CANCER

Contributors

H. Leon Bradlow, The Rockefeller University, 1230 York Avenue, New York, NY 10021 **[91]**

Francoise Capony, Unité d'Endocrinologie Cellulaire et Moléculaire (U 148), INSERM, 60, rue de Navacelles - 34100 Montpellier, France **[125]**

Roberto L. Ceriani, John Muir Cancer and Aging Research Institute, 2055 North Broadway, Walnut Creek, CA 94561 **[3]**

Mark W. Chandler, John Muir Cancer and Aging Research Institute, 2055 North Broadway, Walnut Creek, CA 94561 **[3]**

Thomas L. Dao, Department of Breast Surgery, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, NY 14263 **[31]**

Parimal R. Desai, Department of Immunochemistry Research, Evanston Hospital, 2650 Ridge Avenue, Evanston, IL 60201 **[47]**

Lynn G. Dressler, Department of Medicine, University of Texas Health Sciences Center, 7703 Floyd Curl Drive, San Antonio, TX 78284 **[71]**

Dean P. Edwards, Department of Pathology, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Denver, CO 80262 **[71]**

Jack Fishman, The Rockefeller University, 1230 York Avenue, New York, NY 10021 **[91]**

Marcel Garcia, Unité d'Endocrinologie Cellulaire at Moléculaire (U 148), INSERM, 60, rue de Navacelles - 34100 Montpellier, France **[125]**

Fred J. Hendler, Department of Internal Medicine, The University of Texas Health Science Center, 5323 Harry Hines Blvd., Dallas, TX 75235 **[105]**

Richard J. Hershcopf, The Rockefeller University, 1230 York Avenue, New York, NY 10021 **[91]**

Diane House, Cancer Center, The University of Texas Health Science Center, 5323 Harry Hines Blvd., Dallas, TX 75235 **[105]**

Clement Ip, Department of Breast Surgery, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, NY 14263 **[31]**

David Kessel, Departments of Medicine and Pharmacology, Wayne State University School of Medicine, 540 E. Canfield Street, Detroit, MI 48201 **[21]**

G.S. Kishore, Department of Breast Surgery, Roswell Park Memorial Institute, Buffalo, NY 14263 **[31]**

William L. McGuire, Department of Medicine, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284 **[71]**

Muriel Morisset, Unité d'Endocrinologie Cellulaire et Moléculaire (U 148), INSERM, 60, rue de Navacelles- 34100 Montpellier, France **[125]**

The number in brackets is the opening page number of the contributor's article.

Beverly Myers, Department of Pathology, Mt. Zion Hospital, 1600 Divisadero, San Francisco, CA 94115 **[3]**

J.K. Patel, Department of Breast Surgery, Roswell Park Memorial Institute, Buffalo, NY 14263 **[31]**

Kelly Patrick, Department of Internal Medicine, The University of Texas Health Science Center, 5323 Harry Hines Blvd., Dallas, TX 75235 **[105]**

Michael K. Robinson, Department of Immunochemistry Research, Evanston Hospital, 2650 Ridge Avenue, Evanston, IL 60201 **[47]**

Henri Rochefort, Unité d'Endocrinologie Cellulaire et Moléculaire (U 148), INSERM, 60, rue de Navacelles - 34100 Montpellier, France **[125]**

Ernest H. Rosenbaum, Department of Hematology-Oncology, Mt. Zion Hospital, 1600 Divisadero, San Francisco, CA 94115 **[3]**

Matthew Sakada, Department of Pathology, French Hospital, 4131 Geary Blvd., San Francisco, CA **[3]**

Edward F. Scanlon, Department of Surgery, Evanston Hospital, 2650 Ridge Avenue, Evanston, IL 60201 **[47]**

Georg F. Springer, Department of Immunochemistry Research, Evanston Hospital, 2650 Ridge Avenue, Evanston, IL 60201 **[47]**

Herta Tegtmeyer, Department of Immunochemistry Research, Evanston Hospital, 2650 Ridge Avenue, Evanston, IL 60201 **[47]**

Isabelle Touïtou, Unité d'Endocrinologie Cellulaire et Moléculaire (U 148), INSERM, 60, rue de Navacelles - 34100 Montpellier, France **[125]**

Tracy T. Trujillo, John Muir Cancer and Aging Research Institute, 2055 North Broadway, Walnut Creek, CA 94561 **[3]**

F. Vignon, Unité d' Endocrinologie Cellulaire et Moléculaire (U 148), INSERM, 60, rue de Navacelles - 34100 Montpellier, France **[125]**

David T. Zava, John Muir Aging and Cancer Institute, 2055 North Broadway, Walnut Creek, CA 94596 **[71]**

Preface

The Breast Cancer Working Group of the Organ Systems Program, National Cancer Institute, sponsored a Workshop through the Organ Systems Coordinating Center, entitled "Tumor Markers and Their Significance in the Management of Breast Cancer." The Workshop was held at the National Institutes of Health, Bethesda, Maryland. The Program Committee consisted of Angela Brodie and Thomas Dao (Co-Chairpersons), and Roberto Ceriani, Irma Russo, and Albert Segaloff. The Workshop program is reproduced as follows:

Session I Circulating tumor markers

Role of circulating human mammary epithelial antigens as serum markers for breast cancer
Roberto Ceriani, M.D., Ph.D., John Muir Cancer & Aging Institute

Monoclonal antibodies, tests and breast cancer
William Feller, M.D., Ph.D., Georgetown University Hospital

Glycosyltransferases as tumor markers
David Kessel, Ph.D., Wayne State University

Sialyltransferase in serum and tumor tissue in women with breast cancer
Thomas Dao, M.D., Roswell Park Memorial Institute

Session II Breast cancer antigens

Monoclonal antibodies, oncogenes, and human carcinomas
Jeffrey Schlom, Ph.D., National Cancer Institute

Monoclonal antibodies to surface antigens of the T47D human breast carcinoma cell line
Ricardo Mesa-Tejada, M.D., Columbia-Presbyterian Medical Center

Studies with a monoclonal antibody which defines a tumor-associated antigen in human breast cancer
Dean Edwards, Ph.D., University of Colorado

Serological analysis of human breast cancer with human and mouse monoclonal antibodies
Richard Cote, M.D., Memorial Sloan-Kettering Cancer Center

Session III Estrogen metabolites and estrogen-induced proteins

Biochemical epidemiology of breast cancer
Jack Fishman, Ph.D., Rockfeller University

Estrogen regulation of H59 in breast cancer *in vivo* and *in vitro*
Fred Hendler, M.D., Ph.D., University of Texas, Dallas

Estrogen-regulated proteins secreted by breast cancer cells
Henri Rochefort, M.D., Ph.D., Montpellier, France

This volume contains a partial collection of manuscripts submitted by the participants. Four of them chose not to publish. A manuscript is included from Dr. Georg Springer, who was invited to give a talk at the Breast Cancer Working Group meeting on March 7th.

This book is dedicated to Dr. Albert Segaloff, who passed away on February 27, 1985, at the age of 68, at his home in New Orleans, Louisiana. Dr. Segaloff played a prominent role in the development of the National Cancer Institute's breast cancer programs, serving as chairman of the Cooperative Breast Cancer Group from 1956 to 1968, as a member of the Breast Cancer Task Force from 1966–1970, and as a member of the Breast Cancer Working Group until his death.

Clement Ip, Ph.D.
Scientific Administrator
Breast Cancer Program
Organ Systems Coordinating Center

Contents

I. Circulating Tumor Markers

Tumor Markers and Their Significance in the Management of Breast Cancer, pages 3–19

ROLE OF CIRCULATING HUMAN MAMMARY EPITHELIAL ANTIGENS (HME-Ags) AS SERUM MARKERS FOR BREAST CANCER*,**

Roberto L. Ceriani, M.D., Ph.D. ***, Ernest H. Rosenbaum, M.D.[2], Mark Chandler, B.A.[1] and Tracy T. Trujillo, B.S.[1], Beverly Myers, M.D.[2], Matthew Sakada, M.D.[3]
John Muir Cancer and Aging Research Inst., 2055 N. Broadway, Walnut Creek, CA 94561[1], Mt. Zion Hosp. San Francisco, CA[2], French Hosp., San Francisco, CA[3]

INTRODUCTION:

The idea of screening and following patients with breast cancer by a serum test is appealing from several points of view including its easiness, its economic advantages, its possibility of repeated sampling and its non-invasiveness. So strong and pervasive are these and other reasons that this approach has been continuously proposed, and perhaps with limited success, newer tests have been made available intermittently. As expected the aim to be achieved by every novel test, thus guaranteeing advantage over previous ones, has been greater specificity and higher sensitivity. In spite of the two latter proclaimed goals, by force or necessity, most tests available had to rely on less than ideal specificity (they are usually pan-carcinoma-detecting tests). In addition their sensitivity usually was restricted to a certain

ABBREVIATIONS: anti-HME = anti-human mammary epithelial antigen; CEA = carcinoembryonic antigen; HME-Ags = human mammary epithelial antigen; HMFG = human milk fat globule; LDH = lactic dehydrogenase; ng/ml = nanograms/ml; NPGP = non-penetrating glycoprotein.

** This work was supported by grants from the Dept. of Human Health Services, NIH, NCI, CA-39932 and 39933.

*** From whom reprints should be requested.

percentage of positivity in known breast cancer patients, even among those with important tumor load, thus yielding many false negatives.

In search for specificity the notion that breast tissue had differentiation antigens of its own (Ceriani et al., 1977) created newer expectations. These differentiation antigens, that we discovered and called human mammary epithelial antigens (HME-Ags), identified cell surface components of human breast epithelial cells that were found in every human breast cell line (Peterson et al., 1978) and human breast tumors (Sebesteny, et al., 1979; Peterson et al., 1981) tested to date. These components have been identified by rabbit antisera (anti-HME) created by injections of human milk fat globule membrane (HMFG) which are subsequently absorbed with human epithelial cells (Ceriani et al., 1977). In every instance rabbit antisera with surprisingly similar specificity were prepared that recognized 3 components of the breast epithelial cell membrane (Ceriani et al., 1977; Sasaki et al., 1981; Sasaki, Wara et al., 1981). These components were glycoproteins of apparent molecular weight of 150,000, 70,000 and 40,000 daltons (Ceriani et al., 1977; Sasaki et al., 1981). Anti-HME was used immediately to quantitate the presence of their corresponding antigens in a first generation radioimmunoassay (RIA) to measure HME-Ags on human breast epithelial cells (Sasaki et al., 1981). This RIA used a precipitation with protein-A-laden bacteria of immune complexes formed between anti-HME and its antigens. With this same assay it was possible to find high values of circulating HME-Ags in nude mice implanted with human breast carcinomas (Sasaki, Wara et al., 1981). Mice grafted with human non-breast tumors did not have HME-Ags in their sera (Sasaki, Wara et al., 1981). The fact that promoted HME-Ags as a serum-marker for breast cancer was that surgical removal of these human breast tumors in the nude mice eliminated the high titers of HME-Ags (Sasaki, Wara et al., 1981).

The potential value of these differentiation antigens of the breast prompted the search for a more sensitive RIA to measure the much lower serum levels to be expected in breast cancer patients. For this purpose a solid phase RIA was developed that used anti-HME

covalently bound to Sepharose-beads which bind HME-Ags present in the breast cancer patient's serum (Ceriani et al., 1982). These HME-Ags bound to the solid phase were then measured by the radioiodine labelled polyclonal anti-HME. A further refinement was the use of biotinilated anti-HME that was in turn recognized by radioiodinated avidin. With this assay it was possible to detect HME-Ags levels in 75% of stage IV patients and in 25% of stages I and II. Sera from non-breast cancer patients as well as patients with benign breast lesions and normal women were negative. In an effort to confirm the results obtained by the RIA a very sensitive analytical technique was developed to identify the breast HME-Ags that could be present in the human serum (Ceriani et al., 1982). This approach consisted of scavenging from the sera HME-Ags with anti-HME bound to Sepharose-beads. The antigens on the beads were then labelled in situ with radioiodine and ultimately released with low pH. Thus very small amounts of HME-Ags could be extracted from the serum, labelled and then analyzed for molecular characteristics. By this methodology, the 3 HME-Ags identified by anti-HME were consistently recovered from breast cancer patient serum, thus proving that these antigens are undoubtedly in circulation. Applying a similar technique it was also possible to recover from sera of breast cancer patients the corresponding antigen of an anti-breast monoclonal antibody that we recently prepared (Ceriani et al., 1983). This monoclonal antibody was prepared by hybridization of spleen cells of mice immunized with HMFG and has an apparent molecular weight of 45,000 daltons. Thus it was envisaged that possibly most, if not all, components of the cell surface of breast epithelial cells are voided into the circulation. In fact, a large molecular weight component of the HMFG that were originally described (Ceriani et al., 1983) has also been found in the circulation of breast cancer patients (unpublished results). This component (40,000 daltons, approximately molecular weight) was called by us non-penetrating glycoprotein (NPGP) and was found in the sera of breast cancer patients in fragments of reduced molecular weight (unpublished results).

Confirmatory evidence for our findings has come from other laboratories (Papsidero et al., 1984; Hilkens, et al., 1985) which using monoclonal antibodies have also detected breast epithelial cell components in the circulation of breast cancer patients. These findings of cell components in circulation are not isolated since less specific components of the breast epithelial cell have been detected in sera of breeast cancer patients. Among them sialytransferase (Ip and Dao, 1978) is one that has shown clinical application.

In this paper we describe comparative assays for HME-Ags, CEA and NPGP in longitudinally sampled breast and non-breast cancer patients. Specificity, sensitivity and predictive values for these assays as well as for LDH and alkaline phosphatase in serum are determined and guidelines for their future clinical use are presented.

MATERIALS, METHODS AND RESULTS

An important feature of a polyclonal antibody RIA of the type used is that it gathers its strength from specificity of the antibody for breast epithelial cells. This specificity was obtained in these antibodies by the repeated absorptions of the 40% ammonium precipitate of the rabbit antiserum, with several cellular materials. As previously mentioned (Sasaki et al., 1981), after each absorption the antiserum was again back tested to the absorbing cells for demonstration of its lack of cross-reactivity and to mammary epithelial cells to demonstrate its remaining titer.

The antiserum has been demonstrated to bind three components of the milk fat globule membrane (Ceriani et al., 1977; Sasaki et al., 1981), by affinity chromatography and double immunoprecipitations. These three components are different from another heavy molecular weight (NPGP) antigen of the human milk fat globule already described by us (Ceriani et al., 1983) and others. This antigen is present not only in breast