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Emphasis on Early Tumors and Presursor Lesions

PRINCIPAL INVESTIGATOR: Helen Feiner, M.D.

CONTRACTING ORGANIZATION: New York University Medical Center
New York, New York 10010-2598

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13. ABSTRACT (Maximum 200) The Breast Cancer Resource for Research and Banking has accrued the number and types of samples anticipated during the second year of operation. The existence of the Resource continues to be publicized both within and outside the Medical Center. Utilization of the Resource is improved compared with the first year of operation. Additional utilization will be stimulated in year #3 by the internal funding of six pilot projects in collaborative breast cancer research in 1997.				
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FOREWORD

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PI - Signature

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INTRODUCTION

Improved detection has resulted in a reduction in the average size of breast cancers in screened populations during the past 15 years (Reference 1, Table 1). Contemporaneously increased funding of breast cancer research has increased the need for fresh tumor tissue.

Proliferative lesions that confer increased risk for breast cancer, and precursors of invasive breast cancer, have been defined histopathologically and epidemiologically, and the attention of researchers is beginning to focus on these lesions both nationally (reviewed in Reference 2) and at NYU Medical Center (Reference 3). Since these lesions are virtually always microscopic, unavailability of fresh tissue for research purposes may be a major impediment.

METHODS

To address the needs of investigators for at risk lesions (ductal and lobular hyperplasia, with and without atypia), precursor lesions (ductal and lobular carcinoma in situ), and early (small) invasive breast cancers we have been banking at risk and precursor lesions as well as small cancers in the form of slide imprints/scrapes of such lesions. In the case of larger tumors conventional snap freezing of paired samples of tumor and non-tumoral breast tissue have been collected. During the second year of the grant axillary lymph nodes that drain tumor-containing tissue have also been acquired.

The availability of the Breast Cancer Resource has been made known to investigators both within the NYU research community, and outside investigators, in the following ways:

Investigators Within the NYU Research Community:

(a) The Resource was described and its inventory published in the Kaplan Cancer Center quarterly publication, "LAB NOTES", in January, 1996 (See Appendix 1, page 11).

(b) To foster the development of the NCI supported Breast Cancer Program within the Kaplan Cancer Center (Breast Cancer Program Development Grant, 5R21 CA66229-02). Dr. Vittorio Defendi, principal investigator, convened a meeting on November 20, 1996 that was attended by all breast cancer investigators at NYU Medical Center. At that meeting the inventory of the Breast Cancer Resource was described as was the mechanism for obtaining specimens.

(c) Proposals for pilot projects in breast cancer research were again solicited in the Breast Cancer Program in 1996. Thirteen proposals were received of which 6 will be funded for 1997.

Investigators Outside the NYU Research Community:

The NYU Breast Cancer Resource has been included in the Breast Cancer Specimen and Data Information System, a collaborative project sponsored by the National Action Plan for Breast Cancer Biologic Resources Banks Working Group and

the National Cancer Institute. According to the contractor for this effort, Westat, 1650 Research Boulevard, Rockville, Maryland 20850, the database will be on the Internet this month (December 1996).

RESULTS

The numbers of the various types of breast tissue samples that have been banked and entered into our database between December 1, 1995 and December 1, 1996, the period covered by this report, as well as the cumulative numbers of samples for the entire collection period are shown in Tables 1 and 2.

In Table 1 the breakdown is by type of lesion as defined histopathologically. In Table 2 the breakdown is by type(s) of samples available. Total number of samples in Table 2 exceeds total number of cases in Table 1 because some cases (patients) generated more than one sample type.

TABLE 1

BANKED CASES OF BREAST CANCER AND PRECURSOR LESIONS
AT NYU MEDICAL CENTER BY HISTOPATHOLOGIC DIAGNOSIS

	<u>As of 12/95</u>	<u>12/95 to 12/96</u>	<u>Total Banked</u>
Invasive ductal carcinoma	118	112	230
Invasive lobular carcinoma	16	20	36
Invasive carcinoma, special types	17	19	36
Ductal carcinoma in situ	55	50	105
Lobular carcinoma in situ	11	25	36
Secondary carcinoma, lymph node	25	23	48
Lymph node without tumor	0	30	30
Fibrocystic disease, proliferative	86	92	178
Fibrocystic disease, non proliferative	71	107	178
Other	<u>48</u>	<u>54</u>	<u>102</u>
TOTAL	447	532	979

TABLE 2

BANKED SAMPLES OF BREAST CANCER AND PRECURSOR LESIONS
AT NYU MEDICAL CENTER BY SAMPLE TYPE

	<u>As of 12/95</u>	<u>12/95 to 12/96</u>	<u>Total Banked</u>
Imprints/touch preps	367	415	782
Aspirated cells	149	219	368
Snap frozen tissue fragments	<u>233</u>	<u>199</u>	<u>432</u>
TOTAL	749	833	1,582

Materials Distributed:

During the period covered by this report five requests for specimens were received and satisfied, 4 from within NYU Medical Center and 1 from outside (Stanford University, California). Types of specimens disbursed were:

Frozen sections of invasive cancers and normal tissue (2 investigators), frozen pieces of invasive cancer tissue (2 investigators), slide imprints of at risk and precursor lesions (1 investigator)

Two of these requests were stimulated by the award of pilot project grants (See Appendix 1 - "LAB NOTES", page 15) in 1995/1996.

The Breast Cancer Program pilot projects that will be funded for 1997 and utilize human tissue are:

- 1) Sandra Reynolds - "Peptide Epitopes Recognized by CD8+ T Cells in Patients with Breast Cancer"
- 2) Herbert Samuels - "Retinoid-Regulated Genes and Breast Cancer"
- 3) Jan Sap - "Receptor Protein Tyrosine Phosphatases and Breast Cancer"
- 4) Stephen Tomlinson - "Role of Complement Inhibitors in Tumorigenicity"
- 5) Stanislav Vukmanovic - "Effector Function of Vaccine Induced CD8+ Cells"

A questionnaire will soon be distributed to users of the Resource to determine how the tissue material was used and their level of satisfaction.

CONCLUSIONS

Our goals in terms of the amount and types of material acquired in the second year of the grant have been met. Utilization of the resource appears to have been stimulated by the measures outlined under "Methods".

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KAPLAN COMPREHENSIVE CANCER CENTER



New York University Medical Center

550 First Avenue
New York, NY 10016
(212) 263-5349

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activities at the Kaplan Comprehensive Cancer Center of NYU Medical Center.

CORE FACILITIES

Biostatistics
Roy Shore, Ph.D.
263-6500

Cell Sorting
Ross Basch, M.D.
263-5344

Coordinated Computing Facility
Ross Smith, M.D., Ph.D.
263-5356

Core Clinical Laboratory
Leonard Liebes, Ph.D.
263-5757

Histopathology & Electron
Microscopy
Maarten Bosland, D.V.Sc., Ph.D.
(885)-5216

Imaging & Microscopy
John Hill, Ph.D.
263-7689

Inhalation & Animal Care
Maarten Bosland, D.V.Sc., Ph.D.
(885)-5297

NCI APPROVES CENTER'S ENHANCED CLINICAL MANAGEMENT SYSTEMS

Two of the important clinical program management systems of the Cancer Center underwent successful interim review by the NCI as required by the terms of the current core grant. In September the Center submitted for NCI review an Interim Report on its 1993 Plan to Enhance Gender and Minority Accrual in Clinical Programs. The Report noted the progress being made towards increasing the gender and minority enrollment in clinical trials; in it was described the Center's Task Force on Gender/Minority Enrollment in Clinical Programs chaired by Dr. Harold Ballard, which has: a) raised the awareness of the Medical Center and the Cancer Center for clinical investigators to enter minority patients on clinical trials; b) initiated a number of activities intended to promote gender/minority consideration in protocol development; and c) proposed a number of actions to enhance protocol recruitment.

CORE FACILITIES

Library and Information Services
Max Costa, Ph.D.
(885)-5213

Macromolecular Sequencing &
Synthesis
Bernard Goldschmidt, Ph.D.
263-5743

Mass Spectrometry
Jerome Solomon, Ph.D.
(885)-5284

Molecular Epidemiology
Elizabeth Snow, Ph.D.
(885)-3172

Patient Research Resources
Ruth Oratz, M.D.
263-6485

Resource for Tumor Tissue
Rita Demopoulos, M.D.
263-6451

Transgenic Mouse Research
Elizabeth Newcomb, Ph.D.
263-8757

PROGRAMS

Cancer Epidemiology & Prevention
Paolo Toniolo, M.D.
263-6499

Environmental Carcinogenesis
Seymour Garte, Ph.D.
263-8903

Molecular & Tumor Immunology
G. Jeanette Thorbecke, M.D., Ph.D.
263-5345

Cell Interactions
Daniel Rifkin, Ph.D.
263-5109

Genetic & Molecular Toxicology
George Teebor, M.D.
263-5473

Molecular & Viral Oncology
Angel Pellicer, M.D., Ph.D.
263-5342

Clinical Oncology Research
Ronald Blum, M.D. (Acting Head)
263-6485

Growth Regulation
Claudio Basilio, M.D.
263-5341

NCI APPROVES CENTER'S ENHANCED CLINICAL MANAGEMENT SYSTEMS (CONTINUED):

Looking towards the future, the Report cited concern over the impact of proposed Medicare and Medicaid cuts and the increased penetration of managed care on clinical research.

The Center also in September submitted to the NCI an interim report in the form of a Request for Evaluation of the Clinical Protocol Scientific Review and Monitoring System. A sub-committee of the parent NCI Cancer Centers study section granted approval to the interim report in early December.

In the interval since the June 1993 site visit, the Center has reorganized the clinical protocol scientific review and monitoring system to strengthen the entire protocol management process. In October 1993 the Protocol Review Committee was dissolved because of considerable overlap between it and the Clinical Executive Management Committee (CEMC), chaired by Dr. Ronald Blum. The separation of functions of protocol approval and monitoring was cumbersome, thus protocol review and monitoring functions were consolidated into one committee, the CEMC. Protocol management has since been strengthened by expanding CEMC membership, integrating protocol development, approval, monitoring, and closure as a CEMC function.

The approval of these management/monitoring systems will provide for continued funding of the NCI core grant through 1997.

WEB HOME PAGE CANCER FUNDING OPPORTUNITIES

Cancer related grant opportunities are now on the Cancer Center's Web Home Page at <http://www.med.nyu.edu/KCCC/grant.html>. This section, a joint effort of the Center's Coordinated Computer Resource and Administration Unit, contains up to date agency deadlines for cancer related research support. The service, which is maintained by Mr. Jim Kannengieser, links to other sources of information including the NIH Guide to Grants and Contracts and sponsors with Web sites. The NIH Guide link includes only cancer/oncology opportunities.

Further development of this Web site will provide for articles of interest on cancer related funding and new sources of research support. For information or comment, please contact Mr. Ira Goodman at extension 6703.

EXPERIMENTAL ONCOLOGY LECTURE SERIES - 1996

The Experimental Oncology Lecture Series will continue for 1996 on **alternate Wednesdays beginning at 5:00 p.m. in the Skirball Institute's 3rd Floor Jacob Bleibtreu Seminar Room**. The series was begun by Drs. Defendi and Philipson as an experiment to enhance interprogrammatic dialogue, and it's success has relied entirely on interaction among the participants. Please mark your calendars with the following lectures for the next six months and make every effort to attend:

February 7, 1996	Miroslav Blumenberg, Ph.D. Depts. of Dermatology and Biochemistry	Molecular Biology of Human Keratin Genes
February 21, 1996	Jerome Solomon, Ph.D. Dept. of Env. Medicine	3-Hydroxyalkyl-Uracil: An Epoxide Induced DNA Lesion
March 6, 1996	Naoko Tanese, Ph.D. Dept. of Microbiology	Mechanism of Transcriptional Activation Mediated by the Human TAF Proteins
March 20, 1996	Joel Buxbaum, M.D. Dept. of Medicine	Diseases of Secondary Protein Structure
April 3, 1996	NO SEMINAR	

EXPERIMENTAL ONCOLOGY LECTURE SERIES - 1996 (CONTINUED)

April 17, 1996	Robert Boorstein, M.D, Ph.D. Dept. of Pathology	A Model System to Study Mammalian DNA Base Excision Repair
May 1, 1996	Kenichi Takeshita, M.D. Dept. of Medicine	Homeobox Gene Function During Hematopoiesis
May 15, 1996	Michael Rindler, Ph.D. Dept. of Cell Biology	Biogenesis of Secretory Granules Granules
May 29, 1996	Herbert Samuels, M.D. Dept. of Medicine	Control of Gene Expression by Thyroid Hormone and Retinoid Receptors
June 12, 1996	Peter D'Eustachio, Ph.D. Dept. of Biochemistry	The Ran Family of GTPases
June 26, 1996	Michael Ittmann, M.D., Ph.D. Dept. of Pathology	Molecular Genetics of Prostate Cancer

FACULTY HIGHLIGHTS

The following individuals have been appointed members of the Kaplan Comprehensive Cancer Center:

Mimi Kim, Sc.D., Assistant Professor in the Department of Environmental Medicine.

Dr. Kim's research focuses on developing statistical methods for evaluating the association between repeat determinations of serum levels of endogenous hormones and other biologic measurements with risk of breast cancer.

Nancy Mills, M.D., Instructor in the Department of Medicine. Dr. Mills' research focuses on the biology of retinoids and their receptors in breast cancer.

Arturo Zychlinsky, Ph.D., Assistant Professor in the Department of Microbiology and the Skirball Institute. Dr. Zychlinsky's work focuses on apoptosis induced by pathogens.

SPECIAL RECOGNITION/HONORS

Max Costa, Ph.D. has been selected to participate in the Wellcome Visiting Professorship in the Basic Medical Sciences for this academic year. The Wellcome Visiting Professorship is administered by the Federation of American Societies for Experimental Biology and sponsored by the Burroughs Wellcome Fund. The purpose of the whole program is to stimulate interest in the basic medical sciences, to recognize eminent scientists, and to encourage careers in this field.

Dr. Costa will be able to visit the University of South Alabama lecturing and interacting with faculty, students, and staff. During the visit he will deliver a Wellcome Lecture on a subject pertinent to the basic medical sciences.

RESEARCH RESOURCES

Biostatistics Unit

Effective January 1996, Dr. Roy Shore will assume responsibility as the Director of the Biostatistics Shared Resource. Dr. Shore is a leader in radiation epidemiology and brings to the shared resource an

international reputation in the field of epidemiology and biostatistics. Dr. Shore is assisted by a team of faculty who aid Cancer Center members in their biostatistics needs. Dr. Ann Zeleniuch-Jacquotte will still be available for biostatistical consulting, and in addition, the team also includes Drs. Ikuko Kato, Karen Koenig, Mimi Kim and Eric Lee who are well trained in biostatistics and are available for consulting.

Any Cancer Center member who has a need for biostatistics consulting should contact Dr. Roy Shore at 263-6498.

Breast Tissue Resource for Research and Banking

The Breast Tissue Resource for Research and Banking has increased its store of small invasive breast cancers and precursor lesions, which are available for research use. The current inventory of tissue samples by histopathologic diagnosis includes: invasive ductal carcinoma; invasive lobular carcinoma; in-situ ductal carcinoma; the precursor lesion of proliferative fibrocystic disease; fibroadenomas; and normal breast tissue. Beginning January 1996 the Resource is also collecting samples of normal (non-tumoral) lymph nodes. All members of the Cancer Center who require this type of tissue are urged to utilize this Resource.

The Breast Tissue Resource for Research and Banking was established in January, 1995 to provide investigators at NYU with neoplastic, preneoplastic and normal breast tissue. The Resource is funded as an infrastructure grant by the Department of the Army. The emphasis of this effort is on the acquisition of precancerous breast lesions and early cancers.

Inventory by diagnosis is:

Invasive ductal carcinoma	118
Invasive lobular carcinoma	16
In-situ ductal carcinoma	55
In-situ lobular carcinoma	11
Secondary carcinoma	25
Fibroadenoma	48
Proliferative fibrocystic disease	86
Normal/non proliferative, fibrocystic, other	<u>71</u>
TOTAL	456

On larger tumors snap frozen tissue is available. For small tumors and precancerous lesions the material available is slide imprints and frozen cells.

Inventory by specimen type is:

Imprints/touch preps	367
Aspirated cells	149
Snap frozen tissue fragments	<u>233</u>
TOTAL	749

For consultation or access to the tissue samples, please call Dr. Helen Feiner at extension 8826.

Resource for Tumor Tissue

The Resource for Tumor Tissue, under the direction of Rita Demopoulos, M.D., encourages sharing of expertise among basic research and clinical investigators who require human tissues, either fresh from the operating room or archival (paraffin blocks) for their studies. The Unit provides the following services:

- ◇ delivery of fresh human tumor and control tissues;
- ◇ provision of clinical data which is required for quality control as well as for correlation of research findings with clinical outcome;
- ◇ rapid identification of newly diagnosed hospital patients with malignant disease for the administration of epidemiologic questionnaires and for recruitment into clinical trials;
- ◇ Selection of appropriate tissue blocks by review of previously prepared histologic slides for research studies requiring archival material. Slide review is carried out by Dr. Demopoulos.

In addition, this Unit provides ongoing daily/weekly identification of newly diagnosed cancer patients to investigators for epidemiologic studies or participation into randomized clinical trials.

Preference for service is given to investigators with peer reviewed funded grants; service will be provided to developmental studies if available following assistance to funded investigators. For further information or service please call 263-6845.

NEW CLINICAL TRIALS

A Phase II Trial of Intravenous Navelbine (Vinorelbine Tartrate) in Combination with Ifosfamide as First- or Second-Line Treatment of Patients with Advanced Non-Small Cell Lung Cancer. Investigator: Howard Hochster, M.D., beginning November 1, 1995.

Phase II Study of TLC D-99 (Liposomal Doxorubicin) in the Treatment of AIDS-Related Kaposi's Sarcoma. Investigator: James Wernz, M.D., beginning November 1, 1995.

A Phase II, Double-Blind, Placebo-Controlled Study of Lisofylline in Patients with de novo Acute Myeloid Leukemia Undergoing Induction Chemotherapy. Investigator: Alec Goldenberg, M.D., beginning December 26, 1995.

Phase II Pilot Evaluation of the Efficacy of Interferon-alpha-2a for the Treatment of Progressive Craniopharyngiomas. Investigator: Jeffrey Allen, M.D., beginning January 10, 1996.

Neo-Adjuvant High-Dose Carboplatin in Newly Diagnosed High Grade (WHO Grade II and III) Astrocytoma. Investigator: Michael Gruber, M.D., beginning January 15, 1996.

Phase III Double-Masked, Randomized Study of Recombinant Human Interleukin Eleven (NEUMEGATM rhIL-11 Growth Factor) at a Dose of 50 ug/kg Subcutaneously Once Daily for 14 Days vs. Placebo in Adult Cancer Patients with Severe Thrombocytopenia Due to Chemotherapy. Investigator: Michael Gruber, M.D., beginning January 15, 1996.

Phase I Trial of Adriamycin, Zinecard, and Escalating Doses of Taxol Plus G-CSF in Advanced Breast Cancer. Investigators: Howard Hochster, M.D. and James Speyer, M.D., beginning January 15, 1996.

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NEW SPONSORED RESEARCH AND TRAINING AWARDS

Pamela COWIN, Ph.D., Department of Cell Biology, received a 1 year pilot project (1/1/96-12/31/96) from the Cancer Center's NCI Breast Cancer Program Grant, entitled "The Role of Plakoglobin in Breast Cancer", \$30,000.

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Mary Ann PERLE, Ph.D., Department of Pathology, received a 1 year pilot project (1/1/96-12/31/96) from the Cancer Center's NCI Breast Cancer Program Grant, entitled "Chromosomes 7, 18, 20 and X in Mammogram Detected Atypical Ductal Hyperplasia and Ductal Carcinoma In Situ", \$8,950.

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For information on LAB NOTES or to include items in future issues, please contact Ms. Gwynne Nemcek at extension 5349.

Morphological and Biological Characteristics of Mammogram-Detected Invasive Breast Cancer

M. MOEZZI, MD, J. MELAMED, MD, E. VAMVAKAS, MD,
G. INGHIRAMI, MD, J. MITNICK, MD, A. QUISH, MD,
S. BOSE, MD, G. ZELMAN, BA, D. ROSES, MD,
M. HARRIS, MD, AND H. FEINER, MD

Thirty-nine mammographically detected, (M-detected) small invasive carcinomas of the breast (≤ 5 mm) were compared with 78 consecutive clinical cancers (≥ 10 mm) for a variety of morphological and biological markers of prognostic importance. There were more tubular carcinomas in the M-detected group (12.8% *v* 3.8%), but this did not reach statistical significance. Incidences of other histological types were similar. The types of associated in situ component were similar in the two groups. M-detected cancers were of lower overall grade ($P < .001$), lower architectural and nuclear grades ($P = .0164$ and $P < .0001$ respectively), and had fewer mitotic cells ($P < .0001$). None showed positive lymph nodes ($P < .0001$). Estrogen and progesterone receptor expression was similar in both groups. M-detected cancers expressed p53 nuclear protein less frequently than clinical cancers ($P = .0398$), had lower levels of microvessel density ($P = .0001$), and were more often diploid ($P = .0131$). S-phase of diploid

At the University Hospital of New York University Medical Center, the average size of invasive breast cancer has dropped significantly during the past decade (Table 1). During the same period, cases of subclinical cancer, that is, nonpalpable breast cancers detected by mammography, have accounted for a progressively increasing percentage of breast cancers.

Definitions of minimal invasive breast cancer have varied. Most often this term has referred to tumors that measured 1 cm or less.¹ Using that definition, 30% of the invasive breast cancers seen at this institution in 1994 qualified as minimal (Table 1). These minimal cancers differ from those seen in the premammographic era not only in their overall incidence but in the occurrence of tiny (≤ 5 mm) tumors, pT1a, according to the pTNM pathological classification of breast carcinoma.² The morphological and biological features of such tiny invasive breast cancers have not been described in detail.

Herein we report on the pathological features of 39 invasive carcinomas that measured 5 mm or less at gross examination, usually of segmental resections for mammographically detected densities or clustered microcalcification. A variety of morphological and biological features of known prognostic importance were

tumors in the two groups was similar, but S-phase of aneuploid tumors was lower in the M-detected group ($P = .0057$). Ki67 expression was lower in M-detected cancers ($P < .0001$). In conclusion, M-detected small breast cancers, although invasive, represent an evolutionary phase of breast cancer that generally lacks morphological and biologic markers of aggressive behavior. The presence or absence of these markers, collectively, may explain the influence of tumor size on survival in patients with breast cancer. HUM PATHOL 27:944-948. Copyright © 1996 by W.B. Saunders Company

Key words: breast carcinoma, early breast cancer, histological grade, hormone receptors, p53, microvessel density, DNA ploidy, Ki67.

Abbreviations: M-detected, mammographically detected; hpf, high power field; DCIS, ductal carcinoma in situ; SE, standard error; MVD, microvessel density.

evaluated³ and compared with similar data from 78 consecutive breast cancers that presented as mass lesions measuring 1 cm or more. In preliminary studies we have shown significant differences between the two groups.^{4,5} These differences indicate that in many breast cancers, prognostically important phenotypic characteristics develop only after the invasive state has been achieved.

METHODS

From among more than 800 invasive breast cancers accessioned in the Pathology Department at Tisch Hospital between July 1991 and December 1994, all solitary lesions that measured 3 to 5 mm on gross examination were selected. These tumors were usually embedded in a single paraffin block such that the tumor could easily and accurately be re-measured histologically.

Only those cases where the microscopic measurement was 7 mm or less were retained in the study. This microscopic measurement ensured that we excluded cases in which gross measurements were inaccurate. Tumors less than 3 mm were often only tentatively recognized at gross examination. When an invasive component of less than 3 mm was confirmed microscopically, such a tumor was classified as "microinvasive"⁶ and excluded from this study. The pathological features of 39 invasive breast cancers with gross measurements of 3 to 5 mm and a microscopic measurement of 7 mm or less are described. We will refer to these as mammographically detected (M-detected) cancers because none was clinically palpable. All were discovered on screening mammography. Six of the patients had had previous contralateral breast cancer. M-detected cancers were compared with 78 consecutive clinical cancers all equal to or exceeding 10 mm based on the gross pathological measurement.

From the Departments of Pathology, Radiology, and Surgery, and Kaplan Cancer Center, New York University Medical Center, New York, NY; and the Department of Pathology, Massachusetts General Hospital, Boston, MA. Accepted for publication February 22, 1996.

Address correspondence and reprint requests to Helen Feiner, MD, Department of Pathology, Tisch Hospital, New York University Medical Center, 560 First Ave, New York, NY 10016.

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Histology and Tumor Grading

The standard histological classification of "ordinary" and "special" types of breast cancer was applied⁷ (Table 2). Tumors were classified as tubular adenocarcinoma only when the entire tumor had a tubular pattern. Tumors in which the infiltrative component had both ductal and lobular patterns were classified as infiltrative ductal carcinoma. Criteria for architectural and nuclear grading were as previously described.⁸ Mitotic figures were counted in 10 high-power fields (hpfs), or in all hpfs, with extrapolation to 10, if a cross-section of the entire tumor yielded less than 10 hpfs. Using an Olympus microscope (Olympus Corp, Lake Success, NY) and a $\times 40$ objective, the field diameter was 0.4 mm, and the field area 0.126 mm². Mitotic counts were defined as low ($\leq 5/10$ hpfs), intermediate (6 to 10/10 hpfs), or high ($>10/10$ hpfs). An overall tumor grade (I to III) was computed from the sum of architectural grade, nuclear grade, and mitotic cell count.⁸ For each tumor, the in situ component was classified as comedo ductal carcinoma in situ (DCIS), noncomedo DCIS (including lobular carcinoma in situ), or no in situ component. Comedo DCIS was defined as centrally necrotic DCIS with intermediate or high-grade cytology.⁹

Biological Characteristics

Immunohistochemical analyses used formalin-fixed, paraffin-embedded tissue, and antibodies to estrogen receptor (clone ER1D5; AMAC; Westbrook, ME); progesterone receptor (clone PR4-12, CAS); p53 protein (clone DO-1; Santa Cruz Biotechnology, Santa Cruz, CA); factor 8-related antigen (clone F8-86, DAKO; Carpinteria, CA) and the Ki67 protein (clone MIB1, AMAC). A standard avidin-biotin-peroxidase technique was used, with ethyl green or hematoxylin counterstain. The use of paraffin immunohistochemistry for determining these prognostic features of breast cancer has been previously validated.¹⁰⁻¹⁴ A tumor was classified as positive for estrogen and progesterone receptor when $>10\%$ of tumor cell nuclei expressed these antigens. p53 positivity was defined by expression of this antigen in at least 5% of tumor cell nuclei. Microvessel density was calculated as maximum microvessel count among 3 $20 \times$ fields, representing areas of maximal staining on factor 8 antibody-stained sections. Ki67 expression was evaluated in 10 $40 \times$ fields in a CAS Image Analyzer (CAS) and expressed as percentage positive nuclear area. If there were fewer than 10 $40 \times$ fields in a tumor, an entire cross-section was evaluated.

DNA ploidy and S-phase were assayed by flow cytometry on fresh cells obtained by bench aspiration, as previously described.¹⁵ The authors similarly evaluated the previously mentioned morphological and biological features of 78 consecutive invasive breast cancers ≥ 1 cm in diameter, obtained in 1994.

TABLE 1. Size of Invasive Carcinoma of the Breast at Tisch Hospital, New York University Medical Center, by Year

	1983	1987	1991	1994
No. of cases*	78	96	91	101
Mean size (cm)	2.12	1.97	1.63	1.58
No. (%) ≤ 1 cm	5 (6.4)	15 (15.6)	23 (25.3)	31 (30.7)
No. (%) ≤ 0.05 cm	2 (2.5)	2 (2.1)	10 (11.0)	14 (13.9)

* Data spans the 6-month period January to June for each year.

TABLE 2. Comparison of Histological Types of Mammogram Detected 3- to 5-mm Invasive Breast Carcinomas With Clinical Carcinomas

	Ordinary Ductal	Lobular	Tubular	Other
M-detected ($n = 39$) (%)	26 (66.7)	6 (15.4)	5 (12.8)	2* (5.1)
Clinical ($n = 78$) (%)	55 (70.5)	13 (16.7)	3 (3.8)	7† (9.0)

* One mucinous and one low grade adenosquamous.

† Two mucinous, two medullary (variant), two apocrine, and one metaplastic.

Statistical Analysis

The association of each individual morphological or biological characteristic (except for S-phase, microvessel density, and Ki67 growth fraction) with the occurrence of M-detected versus clinical invasive carcinoma was examined by two-tailed Fisher's exact test (for variables with two categories) or by Pearson's chi-squared test (for variables with three categories). Comparisons of S-phase, microvessel density, and Ki67 expression between M-detected and clinical carcinomas were made by the Mann-Whitney rank-sum test. Multivariate logistic regression analysis was used to evaluate the probability of a breast cancer's being M detected versus clinical, based on its biological characteristics and overall tumor grade.

RESULTS

The distribution of morphological attributes of M-detected versus clinical carcinomas is shown in Tables 2 and 3. Although incidences of ordinary ductal and

TABLE 3. M-Detected Versus Clinical Cancers: Morphological Characteristics

Characteristic	M-Detected No. (%)	Clinical No. (%)	P Value
In situ component			
Comedo	11 (37.9)	31 (49.2)	.3714*
Noncomedo	18 (62.1)	32 (50.8)	
Infiltrative pattern			
Tubular	5 (12.8)	3 (3.8)	.1148*
Nontubular	34 (87.2)	75 (96.2)	
Architectural grade			
Low	9 (23.7)	8 (10.5)	
Intermediate	15 (39.5)	19 (25.0)	.0164†
High	14 (36.8)	49 (64.5)	
Nuclear grade			
Low	19 (50.0)	12 (16.0)	
Intermediate	17 (44.7)	36 (48.0)	<.0001†
High	2 (5.3)	27 (36.0)	
Mitotic rate			
Low	37 (94.9)	45 (57.7)	.0001†
Intermediate	2 (5.1)	16 (20.5)	
High	0 (0.0)	17 (21.8)	
Overall grade			
Low	28 (73.7)	23 (29.5)	
Intermediate	10 (26.3)	35 (44.9)	.0001†
High	0 (0.0)	20 (25.6)	
Lymph node involvement			
Absent	28 (100.0)	36 (59.0)	<.0001*
Present	0 (0.0)	25 (41.0)	

* Two-tailed Fisher's exact test.

† Pearson's chi-squared test.

TABLE 4. M-Detected Versus Clinical Cancers: Biological Characteristics

Characteristic	M-detected No. (%)	Clinical No. (%)	<i>P</i> Value
Estrogen receptor			
Positive	30 (88.2)	57 (73.1)	.0887*
Negative	4 (11.8)	21 (26.9)	
Progesterone receptor			
Positive	22 (62.9)	41 (53.9)	.4156*
Negative	13 (37.1)	35 (46.1)	
p53 expression			
Positive	3 (8.8)	19 (27.5)	.0398*
Negative	31 (91.2)	50 (72.5)	
DNA ploidy			
Diploid	28 (71.8)	34 (45.3)	.0131†
Tetraploid	4 (10.3)	7 (9.3)	
Aneuploid	7 (17.9)	34 (45.3)	

* Two-tailed Fisher's exact test.

† Pearson's chi-squared test.

lobular carcinoma in the two groups is similar, there were more tubular carcinomas among the M detected than among the clinical group (12.8% *v* 3.8%); however, this difference did not reach statistical significance ($P = .1148$). There were too few tumors of other special types to compare the incidences among the two groups. There was no statistically significant difference in distribution of types of in situ lesion between the two groups ($P = .3714$). M-detected carcinomas were of significantly lower mean architectural and nuclear grades than clinical cancers ($P = .0164$ and $P < .0001$, respectively). M-detected carcinomas had significantly lower mitotic cell counts than clinical cancers ($P = .0001$). In overall grade, computed from the previously mentioned three elements, M-detected cancers were significantly lower than clinical cancers ($P = .0001$). M-detected cancers were also significantly less likely to have regional lymph node metastases at presentation ($P < .0001$). In fact, there were no instances of metastatic carcinoma in regional lymph nodes in this group.

The biologic characteristics of the M-detected versus clinical cancers are shown in Table 4. Estrogen receptor was more often expressed in M-detected carcinoma, but the difference did not reach statistical significance ($P = .0887$). The frequency of progesterone receptor expression did not differ between the two categories ($P = .4670$). The expression of p53 nuclear protein was significantly lower among M-detected than among clinical cancers ($P = .0043$). M-detected carcinomas had significantly lower levels of microvessel density than clinical cancers. The microvessel density mean (\pm standard error [SE]) was 50.48 (± 4.66) for the former versus 80.50 (± 4.64) for the latter ($P = .0001$). Ki67 antigen expression was significantly lower in M-detected than in clinical cancers ($P < .0001$). The mean (\pm SE) level of Ki67 antigen expression was 7.50 (± 1.93) in M-detected compared with 19.18 (± 1.77) in clinical cancers.

The analysis of DNA ploidy, done by flow cytometry, is shown in Table 4. M-detected cancers were significantly more frequently diploid than clinical cancers ($P = .0131$). In S-phase comparisons, diploid tumors

were analyzed separately from tetraploid and aneuploid tumors. M-detected diploid cancers did not differ from clinical diploid cancers in S-phase size ($P = .6237$). The mean S-phase (\pm SE) was 3.51 (± 0.55) in M-detected carcinomas and 4.41 (± 0.73) in clinical carcinomas. However, M-detected nondiploid cancers had smaller S-phases than clinical nondiploid cancers ($P = .0131$). The mean S-phase (\pm SE) was 5.39 (± 0.98) in M-detected carcinomas and 10.19 (± 0.99) in clinical carcinomas.

Higher overall tumor grade was associated with nondiploid cancers ($P = .0064$), positive p53 antigen expression ($P = .0171$), Ki67 antigen expression ($P < .0001$), and larger S-phase size in the case of nondiploid cancers ($P = .0011$). However, the association of higher overall tumor grade with these adverse biological markers did not entirely explain the association of tumor size with adverse biological markers. In fact, in a multivariate analysis that evaluated each tumor's biological characteristics and overall grade as potential predictors of the tumor's size (M-detected vs clinical), Ki67 antigen expression, rather than overall grade was the only factor to predict a breast cancer's size ($P < .0001$).

DISCUSSION

Among women screened by mammography, the mean size of breast cancers has dropped compared with the premammographic and early mammographic era (Table 1). Tumors identified in this way are referred to as M- or screen-detected cancers. As one might expect, tiny invasive cancers are almost invariably discovered in screened populations.

Mammographic screening may preferentially detect indolent tumors, whereas a significant proportion of tumors that are aggressive ab initio are likely to present as clinical cancers or as so-called interval cancers in screened populations.¹⁷ This may explain the striking difference in incidence of axillary metastases in the study by Bedwani et al¹ from the premammographic/early mammographic era in which a subset of 157 invasive cancers that measured 5 mm or less included 23% with axillary metastases, and this series in which none of the patients had axillary metastases. Additionally, the accuracy of gross tumor measurements in Bedwani's multicenter study can be questioned. The method of measurement is not specified, but gross measurement is inferred. In selecting cases for this series, we rejected 25% of cases with gross measurements of 5 mm or less because the microscopic measurement of the tumors exceeded 7 mm. The question of how measurements of breast tumors are made is often not specified in the literature. Hutter¹⁶ defined minimal breast cancer as a lesion smaller than 5 mm. Issues of in situ versus invasive, and gross versus microscopic measurements were discussed but did not modify the definition. The 1989 TNM staging of breast cancer² subdivides primary invasive breast cancers (T1 lesions) into T1a, up to 0.5 cm in greatest dimension; T1b, 0.5 to 1.0 cm; and T1c, more than 1.0 but not more than 2 cm. This staging system as presented in Rosen and Oberman² does not

specify whether tiny invasive cancers should be defined based on gross or histological measurements. In this series, we used gross measurement as the defining determinant and modified it by microscopic measurement as a means of excluding those tumors whose size may have been incorrectly estimated at gross examination. In so doing, we rejected from this study 13 tumors that grossly measured 5 mm or less, but exceeded 7 mm on microscopic measurement. Outcome data should be excellent for these patients because in the study by Arnesson et al,¹⁸ node-negative tumors less than 1 cm in diameter were associated with survival without recurrence in 98.7% after a mean follow-up of 7 years regardless of detection method, and Rosen's study yielded a 95% survival rate for node-negative women with tumors 1 cm or less.¹⁹

Tumor size is the most important determinant of outcome in node-negative breast cancer. What is the nature of this influence? Tumor size reflects number of tumor cells, modified by cell size and amount of stroma. It may be that small tumors "shed" fewer cells that are destroyed by immune mechanisms, whereas larger tumors shed more cells with which the immune system cannot "cope." A more attractive hypothesis for the influence of tumor size on outcome, based on this study, is that small breast cancers, although already invasive, have not yet undergone the genetic changes that determine those phenotypic characteristics that represent poor prognostic indicators. Stated differently, small invasive cancers appear not to have undergone sufficient cycles of cell division to induce the genetic changes that determine the lethality of breast cancer. In this hypothesis, size can be viewed as a surrogate for the sum of most of the tumor characteristics evaluated in this study.

Differences in the biological characteristics between M-detected and clinical cancers did not merely reflect differences in the overall grade of these tumors. This was evident from the results of the multivariate analysis. A biological characteristic, Ki67 antigen expression, rather than a tumor's overall grade, was the factor to best predict the probability of each cancer's being M detected versus clinical.

Several additional conclusions can be drawn based on specific morphological and biological parameters measured in this study. First and unexpectedly, there was a similar incidence of comedo DCIS in the two groups, despite the fact that all but two of the M-detected cancers were of low and intermediate nuclear grades. Thus, comedo DCIS was usually associated with lower grades of invasive carcinoma. Second, the higher incidence of tubular carcinomas among M-detected than among clinical cancers, although not statistically significant, suggests that tubular carcinoma may be an evolutionary phase of invasive ductal carcinoma rather than a separate entity. However, because there was no statistically significant difference in occurrence of tubular carcinoma between the two groups, differences cannot be ascribed to differences in the frequency of tubular carcinoma. More generally, however, lower architectural and nuclear grades, fewer mitoses, and

lower overall grade predominated significantly among small tumors.

Among biological parameters measured, expression of estrogen and progesterone receptor was similar in M-detected and clinical tumors, suggesting that these parameters do not evolve in primary tumors. Conversely, p53 protein expression was significantly less common in small tumors, indicating that the p53 mutation that results in p53 protein overexpression, when it occurs, is a late phenomenon in many breast cancers. Microvessel density (MVD) was significantly lower in M-detected cancers. However, the significance of this can be questioned because MVD measurements are made in vascular "hot spots," and larger tumors provided a larger surface area from which to select such hot spots.

The more frequent diploid state of M-detected cancers follows a trend in the relationship between tumor size and diploid status.²⁰ When S-phase was analyzed for tumors in the two groups, S-phase was significantly lower in the aneuploid M-detected tumors compared with aneuploid clinical cancers. The percentage of Ki67-positive cells, as an index of proliferation, was also significantly lower among M-detected tumors.

The overall impression gained from this study is that a significant proportion of M-detected cancers, as described here, although invasive, may not have achieved metastatic potential as judged by their favorable morphological and biological features and the lack of lymph node metastases. This is supported by two large outcome studies of small breast cancers.^{18,19} The effect of tumor size on clinical outcome of breast cancer likely represents the cumulative effect of a variety of prognostically significant mutations that accumulate as the tumor grows.

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