Cancer Research

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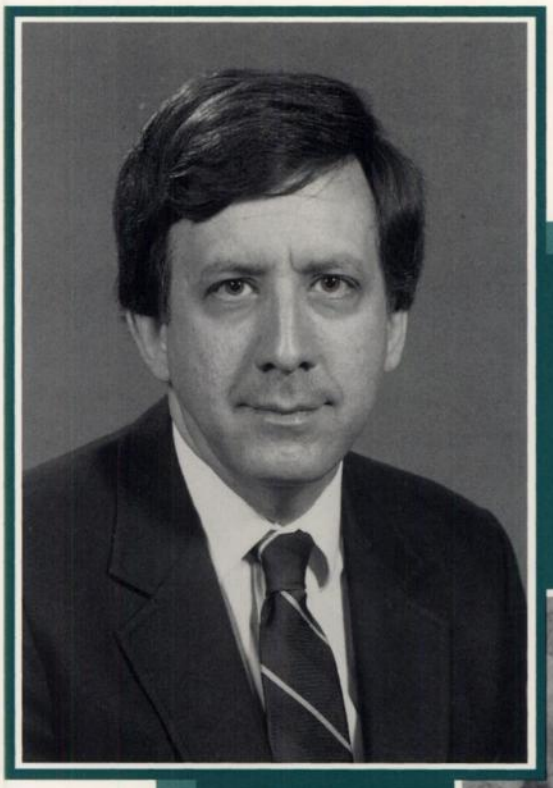

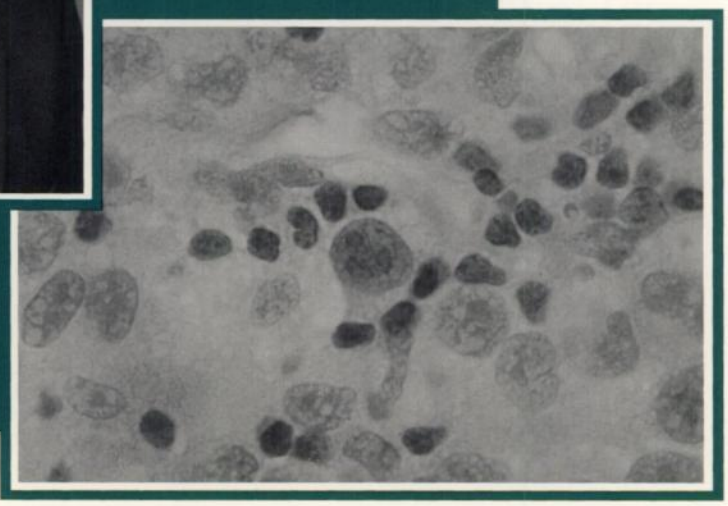

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New Telomerase PCR ELISA Offers Simplified, Nonradioactive TRAP Assay for Measuring Telomerase, A Potential Marker for Cancer Research

Boehringer Mannheim is now offering a Telomerase PCR ELISA for the highly sensitive, nonradioactive detection of telomerase activity in extracts from cell cultures and tissue samples.

Telomerase as an important parameter in cancer research

Telomeres, the specialized DNA/protein structures at the end of eukaryotic chromosomes, contain tandemly repeated DNA sequences that are believed to protect genomic DNA from degradation and deleterious recombination events. During normal somatic cell proliferation, telomeric ends are progressively shortened with each replication cycle, which may play a role in limiting the proliferative capacity of normal cells. Germline cells, many tumor cells, and "immortalized" cell lines are believed to circumvent this telomere shortening using telomerase, a ribonucleoprotein that adds new repeats to the ends of chromosomes. Telomerase activity has recently been identified in many cancers (e.g., prostate cancers [1], advanced-stage breast cancers [2], neuroblastomas [3], and primary lung cancer tissues [4]) that have been confirmed by other methods (*e.g.*, histochemical staining). Thus, telomerase reactivation may allow cells to escape from the proliferative limitations of cellular senescence and could be further investigated as a potential marker for the development of malignant tumor cells.

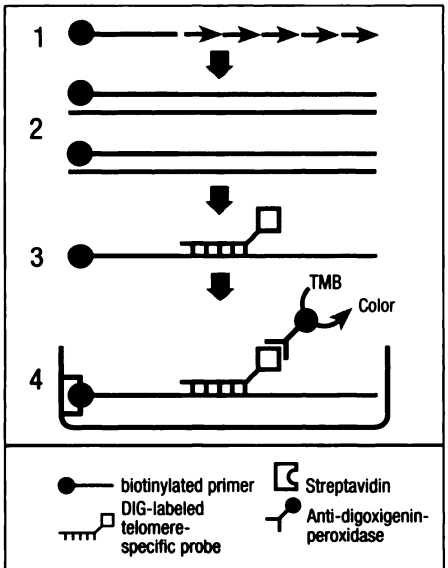

Figure 1. Detection of telomerase activity with the Telomerase PCR ELISA.

Step 1. Telomerase, if present, adds multiple 6-nucleotide telomeric repeats to a biotinylated synthetic primer.

Step 2. The telomerase reaction product is amplified by PCR, using a biotinylated primer.

Step 3. After denaturation, the PCR product hybridizes to a digoxigenin-labeled probe specific for the telomeric repeat.

Step 4. The DNA hybrid binds to a streptavidin-coated microtiter plate, and anti-digoxigenin-peroxidase and TMB substrate generate a colored product measurable with a microplate reader.

Note: If desired, the TRAP reaction product from Step 2 can also be detected by the traditional gel electrophoresis method.

Telomerase PCR ELISA improves upon previous TRAP assays

Telomerase activity is most frequently detected by the Telomeric Repeat Amplification Protocol (TRAP) of Kim *et al.* (5), in which the telomerase-reaction product is amplified by PCR. However, the conventional TRAP assay achieves full sensitivity only when performed with a hazardous radioactive label, and visualization of results requires time-consuming gel electrophoresis and autoradiography. The new Telomerase PCR ELISA*,† combines a one-step/one-tube TRAP assay with nonradioactive detection in a highly sensitive photometric ELISA (Figure 1).

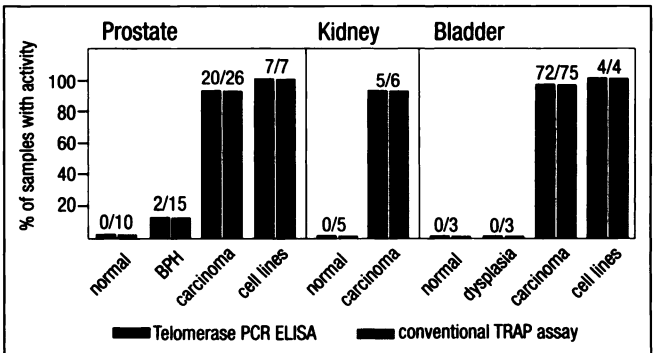

Figure 2. Correlation of results obtained with the Telomerase PCR ELISA and conventional, radioisotopic TRAP assays. Samples from known carcinomas, normal specimens (negative control), benign prostatic hyperplasia (BPH) specimens, and immortalized cell lines were tested with the Telomerase PCR ELISA and conventional, radioisotopic TRAP assays. In all sample types, the methods were able to identify the same number of samples featuring telomerase activity. Data provided by M. Müller and R. Heicappell (6) and by H. J. Sommerfeld.

Easy-to-use ELISA delivers results in less time

The Telomerase PCR ELISA delivers results within 6 hours, eliminating the need for laborious, time-consuming gel electrophoresis and autoradiography techniques. Its ready-to-use TRAP reaction mix (telomerase substrate, amplification primers, nucleotides, *Taq* DNA polymerase, reaction buffer) eliminates the need to prepare multiple solutions and minimizes the risk of assay failure caused by contamination. Up to 96 TRAP reactions can be simultaneously analyzed with an ELISA plate reader.

Sensitive results correspond closely with those of radioactive TRAP assays

Besides avoiding the use of hazardous radioisotopes, the Telomerase PCR ELISA produces sensitive results comparable to those of the radioisotopic TRAP assay (Figure 2). The kit's optimized detection probe and hybridization conditions maximize both specificity and sensitivity. Additionally, optimized primer sequences eliminate the need for "hot start" PCR while avoiding amplification artifacts (*e.g.*, primer dimers).

The Telomerase PCR ELISA is currently available

The Telomerase PCR ELISA (96 tests; Cat. No. 1 854 666) is now available from Boehringer Mannheim Biochemicals representatives. Additional information can also be found at http://biochem.boehringer-mannheim.com.

References:

1. Sommerfeld, H. J. *et al.* (1996) *Cancer Research* **56**:218-222.
2. Hiyama, E. *et al.* (1996) *J. National Cancer Institute* **88**:116-122.
3. Hiyama, E. *et al.* (1995) *Nature Medicine* **1**:249-255.
4. Hiyama, K. *et al.* (1995) *J. National Cancer Institute* **87**:895-902.
5. Kim, N. W. *et al.* (1994) *Science* **266**:2011-2015.
6. Müller, M. *et al.* (1996) *Int. J. Oncology* **9**: *in press.*

*Licensed from Geron Corporation. Patents pending.

†Purchase of this product is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) process in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Perkin-Elmer or as purchased, i.e., an authorized thermal cycler.

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AACR SPECIAL CONFERENCE IN CANCER RESEARCH

Basic and Clinical Aspects of Breast Cancer

March 7-12, 1997
The Keystone Resort, Keystone, Colorado

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Karen S. H. Antman / New York, NY
Mary-Claire King / Seattle, WA

SCIENTIFIC PROGRAM

Keynote Addresses
Mary-Claire King / Seattle, WA
Karen S. H. Antman / New York, NY

Basic Biology of the Breast
José Russo / Philadelphia, PA
Marc E. Lippman / Washington, D.C.
C. Kent Osborne / San Antonio, TX
Charles W. Daniel / Santa Cruz, CA

Molecular and Cellular Aspects of Breast Cancer
Jerry W. Shay / Dallas, TX
Mina J. Bissell / Berkeley, CA
Martha R. Stampfer / Berkeley, CA
Joyce M. Slingerland / Toronto, Ontario, Canada

Genetic Predisposition to Breast Cancer
David E. Goldgar / Lyon, France
P. Andrew Futreal / Durham, NC

Mechanisms of Hormone Action
V. Craig Jordan / Chicago, IL
Myles A. Brown / Boston, MA
Kenneth S. Korach / Research Triangle Park, NC

BRCA1 and BRCA2 Function/Biochemistry
Roy A. Jensen / Nashville, TN
Wen-Hwa Lee / San Antonio, TX
David M. Livingston / Boston, MA
Frank J. Calzone / Thousand Oaks, CA

Experimental Models of Breast Cancer
Tak W. Mak / Toronto, Ontario, Canada
Roger W. Wiseman / Research Triangle Park, NC
Michael N. Gould / Madison, WI

Epidemiology of Breast Cancer
Walter C. Willett / Boston, MA
Maureen Henderson / Seattle, WA
Malcolm C. Pike / Los Angeles, CA
Mary S. Wolff / New York, NY

Clinical Aspects of Breast Cancer
Judy E. Garber / Boston, MA
Jeffrey T. Holt / Nashville, TN
M. John Kennedy / Baltimore, MD

Applicants are encouraged to submit abstracts for poster presentation.

Application deadline: January 20, 1997

Information and Application Forms

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AMERICAN ASSOCIATION FOR CANCER RESEARCH SCIENTIFIC CONFERENCES

MARCH 7-12, 1997
Basic and Clinical Aspects of Breast Cancer
Chairpersons: J. Carl Barrett, Research Triangle Park, NC; Karen S.H. Antman, New York, NY; Mary-Claire King, Seattle, WA
Keystone Resort, Keystone, CO

APRIL 12-16, 1997
88th Annual Meeting
Chairperson: Frederick P. Li, Boston, MA
Co-Chairpersons: Clara Derber Bloomfield, Buffalo, NY; Stephen H. Friend, Seattle, WA
San Diego Convention Center, San Diego, CA

JUNE 7-11, 1997
Cancer of the Central Nervous System
Conference with Neurosurgery Joint Section on Tumors
Chairpersons: Peter McL. Black, Boston, MA; Webster K. Cavenee, La Jolla, CA
Loew's Coronado Bay Resort, San Diego, CA

SEPTEMBER 9-13, 1997
Molecular Genetics of Cancer
Conference with the European Association for Cancer Research
Chairpersons: Eric J. Stanbridge, Irvine, CA; Walter Bodmer, Oxford, England
Hertford College, Oxford, England

SEPTEMBER 26-30, 1997
Tumor Suppressor Genes
Chairpersons: Stephen H. Friend, Seattle, WA; Philip Branton, Montreal, Quebec, Canada
Victoria Conference Centre, Victoria, BC, Canada

OCTOBER 17-21, 1997
Gene Regulation in Differentiation and Development
Chairpersons: Robert Eisenman, Seattle, WA; Elaine V. Fuchs, Chicago, IL
The Sagamore Resort, Bolton Landing (Lake George), NY

DECEMBER 1997
Epigenetics of Cancer
Chairperson: Peter A. Jones, Los Angeles, CA
Location in Puerto Rico to be Announced

JANUARY 9-13, 1998
Colorectal Cancer — Biology, Treatment, and Prevention
Joint Meeting with the American Society of Clinical Oncology
Chairpersons: Robert J. Mayer, Boston, MA; One Additional Chairperson to be Announced
Renaissance Esmeralda Resort, Indian Wells (Palm Springs), CA

JANUARY 1998
Angiogenesis and Cancer
Chairpersons: Judah Folkman, Boston, MA; Michael Klagsbrun, Boston, MA
Location in Orlando, FL to be Announced

FEBRUARY 16-21, 1998
Innovative Molecular Biology Approaches to the Prevention, Diagnosis, and Therapy of Cancer
Joint Meeting with the Japanese Cancer Association
Chairpersons: Edward Bresnick, Worcester, MA; Kaoru Abe, Tokyo, Japan
Maui Marriott Resort, Maui, HI

AACR members will receive brochures on the above conferences as soon as they are available. Nonmembers should call or write:

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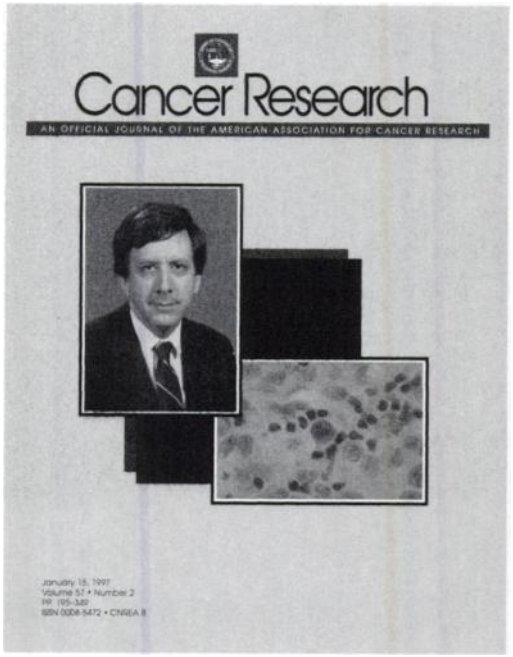

This issue's cover feature pays tribute to David Berd of the Division of Neoplastic Diseases, Thomas Jefferson University, a leader in the immunotherapy of melanoma and a contributor of many advances in the understanding and treatment of melanoma.

His interest in melanoma vaccines dates back to the late 1970s at the beginning of his tenure at what was to become the Fox Chase Cancer Center. Michael J. Mastrangelo had organized an immunotherapy group in the laboratory of Richmond T. Prehn, a pioneer in the early days of murine tumor immunology, who demonstrated in the 1950s that mice could be immunized to syngeneic, chemically induced tumors. Dr. Mastrangelo, along with Donald L. Morton at UCLA, had shown that intralesional injection of melanoma skin metastases with the adjuvant BCG caused regression not only of injected tumors, but sometimes of uninjected tumors as well. By 1977, Dr. Mastrangelo and J. Frederick Laucius had completed a clinical study of a vaccine consisting of autologous, irradiated melanoma cells mixed with BCG. A pivotal member of the group was Henry C. Maguire, a dermatologist with a research interest in contact sensitivity reactions. In 1967, he unexpectedly observed that the cytotoxic and immunosuppressive drug cyclophosphamide could actually *augment* cell-mediated immune reactions if given prior to immunization. Immunopotentiation by cytotoxic drugs is now a well established phenomenon, *e.g.*, as demonstrated by the work of Margalit B. Mokyr *et al.* (Cancer Res., *46:* 3313, 1986), but 25 years ago it was viewed with skepticism by the immunological community.

Dr. Berd reasoned that pretreatment of patients with low dose cyclophosphamide prior to administration of autologous melanoma vaccine mixed with BCG would produce cell-mediated immunity to melanoma cells. In tests begun in 1982, he demonstrated that patients with metastatic melanoma developed delayed type hypersensitivity to their own tumor cells following treatment with cyclophosphamide plus vaccine (Cancer Res., *46:* 2572, 1986), although in retrospect some of these responses had been due to the enzymes used for tumor dissociation rather than to melanoma antigens. To everyone's amazement, several patients exhibited regression of metastases (J. Clin. Oncol., *8:* 1858, 1990). However, a small trial of this regimen as a postsurgical adjuvant in patients with bulky regional node metastases was clearly negative, so disappointment quickly replaced euphoria.

By the time Dr. Berd's group moved to Thomas Jefferson University in 1984, it was clear that a new approach was necessary. With Dr. Maguire's encouragement, Dr. Berd rediscovered the phenomenon of autoimmunity induced by immunization with cells modified by a hapten. Several investigators, especially Gene M. Shearer at the NIH, had shown that treatment of murine cells with trinitrophenyl (TNP) rendered them highly immunogenic in syngeneic hosts. Remarkably, immunization with TNP-modified cells sometimes resulted in the generation of T lymphocytes that recognized *unmodified* syngeneic cells as well. This phenomenon was recently elucidated on the level of cell-bound peptides by Hans Ulrich Weltzien's group in Freiburg, Germany (Eur. J. Immunol., *25:* 2788, 1995).

In September 1988, Dr. Berd's group was the first to treat a patient with a vaccine consisting of irradiated autologous melanoma modified with the hapten dinitrophenyl (DNP). Dr. Berd retained elements of his previous program, specifically the use of BCG as an immunological adjuvant and pretreatment with low dose cyclophosphamide. The result was unexpected and, to their unprepared minds, quite wondrous. Over a period of 4 months, the multiple skin metastases in this patient developed an inflammatory response: the tumors became erythematous, warm, and tender, and eventually ulcerated with drainage of necrotic material. Biopsy of these inflamed tumors showed infiltration of lymphocytes with focal tumor cell necrosis (Cancer Res., *51:* 2731, 1991).

It soon became apparent that this initial patient was not an anomaly, as the development of tumor inflammatory responses was observed in about half of the patients with superficial metastases. The photomicrograph on the cover, taken by George F. Murphy, then of the University of Pennsylvania School of Medicine, illustrates a typical histology of lymphocytes exhibiting satellitosis around a central degenerating melanoma cell (Cancer Immunol. Immunother., *39:* 141, 1994).[1] Subsequently, Dr. Berd determined that the lymphocytes were T cells, predominantly CD8+, that expressed cell surface markers indicative of activation. A very productive collaboration with Giorgio Parmiani and Marialuisa Sensi in Milan has demonstrated that these T cells have been clonally expanded (J. Clin. Invest., in press, 1997) as a result of stimulation by an as yet uncharacterized tumor antigen.

Despite these tumor inflammatory responses, autologous DNP-vaccine only rarely causes clinically defined regression of metastases. Recent work, in collaboration with Edmund C. Lattime and Takami Sato, suggests that production of the anti-inflammatory cytokine IL-10 by melanoma cells might be responsible by suppressing the proliferation of T cells in the tumor site (Clin. Cancer Res., *2:* 1383, 1996). On the other hand, autologous DNP-vaccine has produced promising clinical results in melanoma patients with micrometastatic disease (Ann. NY Acad. Sci., *69:* 147, 1993). Although these trials are still in progress, 5-year survivals of about 60% in patients with stage 3 melanoma with large, resectable regional lymph node metastases are being seen. This compares to an expected survival of 20–25% with surgery alone (J. Clin. Oncol., *14:* 7, 1996). A multi-institutional, randomized trial of the DNP-vaccine as postsurgical adjuvant therapy will be initiated this year, sponsored by AVAX Technologies, a biotechnology company based in Kansas City, MO.

Dr. Berd has published over 57 original research articles and 26 reviews. He has served on many government advisory groups and belongs to several professional societies, including the American Association for Cancer Research (AACR), of which he has been an active member since 1977.

Sidney Weinhouse

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