DEPARTMENT OF HEALTH AND HUMAN SERVICES

PUBLIC HEALTH SERVICE

NATIONAL INSTITUTES OF HEALTH

NATIONAL CANCER INSTITUTE

NATIONAL CANCER ADVISORY BOARD

Summary of Meeting
December 5-6, 1994

Building 31, Conference Room 10
National Institutes of Health
Bethesda, Maryland

Department of Health and Human Services
Public Health Service
National Institutes of Health
National Cancer Institute
National Cancer Advisory Board
Summary of Meeting[1]
December 5-6, 1994

The National Cancer Advisory Board (NCAB) convened for its 92nd regular meeting at 8:00 a.m., December 5, 1994, in Building 31, C Wing, 6th Floor, Conference Room 10, National Institutes of Health (NIH).

NCAB Members

Dr. Barbara Rimer (Chairperson)
Dr. Frederick F. Becker
Dr. J. Michael Bishop
Mrs. Zora K. Brown
Dr. Paul Calabresi
Dr. Kenneth K. Chan
Dr. Pelayo Correa
Dr. Robert W. Day
Mrs. Barbara P. Gimbel
Dr. Alfred L. Goldson
Mrs. Marlene A. Malek
Ms. Deborah K. Mayer
Dr. Sydney Salmon
Dr. Philip S. Schein
Dr. Ellen V. Sigal
Dr. Vainutis K. Vaitkevicius
Dr. Charles B. Wilson

President's Cancer Panel

Dr. Harold P. Freeman (Chairperson)
Ms. Frances Visco
Dr. Henry C. Pitot

Alternate Ex Officio NCAB Members

Dr. Roy Fleming, NIOSH
Captain Bimal C. Ghosh, DOD
Dr. John Johnson, FDA
Dr. Hugh McKinnon, EPA
Dr. Lakshmi C. Mishra, CPSC
Dr. Sheila Newton, NIEHS
Dr. P. C. Srivastava, DOE
Dr. Ralph Yodaiken, DOL

Members, Executive Committee, National Cancer Institute, NIH

Dr. Samuel Broder, Director, National Cancer Institute
Dr. Edward Sondik, Acting Deputy Director, National Cancer Institute
Dr. Jerry Rice, Acting Director, Division of Cancer Etiology
Mr. Philip D. Amoruso, Associate Director for Administrative Management
Dr. Marvin Kalt, Director, Division of Extramural Activities
Dr. Bruce A. Chabner, Director, Division of Cancer Treatment
Dr. Peter Greenwald, Director, Division of Cancer Prevention and Control
Dr. Alan S. Rabson, Director, Division of Cancer Biology, Diagnosis, and Centers
Mrs. Iris Schneider, Executive Secretary, Assistant Director for Program Operations and Planning

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[1] For the record, it is noted that members absented themselves from the meeting when discussing applications (a) from their respective institutions or (b) in which conflict of interest might occur. This procedure does not apply to *en bloc* actions.

Liaison Representatives

Dr. Robert W. Frelick, Association of Community Cancer Centers
Dr. Elaine Locke, American College of Obstetricians and Gynecologists
Dr. James H. Brown, National Science Foundation
Ms. Michelle Cherry, American Cancer Society
Dr. Edward Gelmann, American Society of Clinical Oncology, Inc.
Dr. C. Michael Brooks, American Association for Cancer Education, Inc.
Mrs. Yvonne Soghomonian, Candlelighters Childhood Cancer Foundation
Dr. Edwin A. Mirand, Association of American Cancer Institutes
Ms. Sandra Lee Schafer, Oncology Nursing Society
Dr. Marston Linehan, Society of Urologic Oncology

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I. CALL TO ORDER AND OPENING REMARKS—DR. BARBARA RIMER

Dr. Rimer called to order the 92nd meeting of the National Cancer Advisory Board (NCAB). She began by introducing new Board members, Dr. Alfred Goldson, professor and Chair of the Department of Radiotherapy at Howard University Hospital in Washington, DC, and Dr. J. Michael Bishop, Nobel laureate professor at the University of California, San Francisco.

Dr. Goldson expressed his pride at becoming a member of the NCAB and his hope that his comments will be beneficial to the future of the National Cancer Institute (NCI). He thanked the Board for allowing him to participate and said that he looks forward to a positive working relationship.

Dr. Bishop stated that he was pleased to be present and expressed his hope that his participation on the NCAB will help direct cancer research toward prevention of the disease.

Dr. Rimer introduced the guests at the meeting, who represented a variety of cancer-related associations, foundations, and societies, as well as visitors from Federal agencies. Dr. Rimer announced that members of the public should communicate their comments on issues discussed during the meeting by writing to Dr. Marvin Kalt, Executive Secretary of the Board.

Dr. Rimer referred to future meeting dates listed in the agenda for 1995 and 1996 and pointed out the change to the Tuesday/Wednesday schedule, reserving Mondays for conducting business, as needed. She reminded the Board of the amendment to the quorum requirement that requires the presence of a majority of the appointed members. Dr. Rimer announced that two subcommittee meetings—the Centers Committee and Planning and Budget—would be held at 11:45 and that the bus to tour Frederick would leave at 1:10.

Dr. Rimer called for approval of the minutes of the October meeting, which were unanimously approved without change. She then introduced Dr. Samuel Broder, Director of the National Cancer Institute.

II. REPORT OF THE DIRECTOR, NATIONAL CANCER INSTITUTE—DR. SAMUEL BRODER

After welcoming the new members of the NCAB and offering the assistance of NCI staff in adjusting to their new duties, Dr. Broder noted the November 12th death of Dr. Guy Newell, who served as Deputy Director of the Institute between 1973 and 1979, serving as Acting Director from October 1976 to July 1977. Most recently, Dr. Newell was associated with the M.D. Anderson Cancer Center at the University of Texas. Dr. Broder stated that Dr. Newell was a very effective and dedicated leader during a difficult period in which proponents for alternative treatments generated considerable debate and criticism concerning NCI programs, particularly in the area of drug development.

Dr. Broder announced that the NCI held its annual awards ceremony on October 11th; he added that a handout listing all recent awards and staff changes would be distributed during

the meeting. In the Division of Cancer Biology, he stated, Dr. Michael Gottesman has been named Deputy Director for Intramural Research for the National Institutes of Health (NIH), and will remain as Chief of the Laboratory of Cell Biology, part of NCI's intramural program.

In the Division of Cancer Treatment (DCT), Dr. Broder announced that Dr. Bruce Chabner, the Division's Director, plans to resign in May 1995 to join the staff of the Massachusetts General Hospital. He thanked Dr. Chabner for his exceptional service and brilliant contributions to basic and clinical research.

In the Division of Cancer Etiology (DCE), Dr. Broder reported, Dr. Michael Sporn, Chief of the Laboratory of Chemoprevention, received the American Cancer Society's most prestigious award, the Medal of Honor, for his groundbreaking studies related to the growth of cells and chemoprevention. His early studies increased knowledge of how biologic reactive substances can regulate the growth of cancer cells.

Dr. Broder announced that the International Cancer Information Center's (ICIC) Information Associates Program will receive Vice President Al Gore's Hammer Award, given to Federal agencies that perform well while cutting costs. The Unified Information Associates Program provides "one-stop shopping" for all of NCI's information services, including the *Journal of the National Cancer Institute*, the Physician's Data Query (PDQ) data set, information about the Institute's programs, and technical assistance to electronic information services. Dr. Broder stated that Susan Hubbard, Director, ICIC and her associates have brought a great deal of honor to the Institute in winning this award. Ms. Hubbard, Dr. Broder added, has recently worn an additional hat at NCI by commuting to Pittsburgh as a mediator in the transition of leadership for the National Surgical Adjuvant Breast and Bowel Project (NSABP) trials.

Dr. Broder displayed the cover of an NCI booklet, produced by the Office of Cancer Communications, that flew aboard the October 1994 flight of the space shuttle Columbia, a mission focused on life science experiments. He stated that a transmittal letter from the National Aeronautics and Space Administration (NASA) to Dr. Bruce Wachholz, Chief, Radiation Effects Branch, described the inclusion of the booklet on that flight as a symbol of a common commitment to the betterment of human life. Dr. Broder suggested that the booklet, designed to help cancer survivors face an uncertain future, represents the efforts of NCI in facing challenges as formidable as those faced by the space program.

Dr. Broder commended Dr. Omar Yoder, director of NCI's liaison office in Brussels, and Ms. Clarissa Wittenberg of the Office of the Director (OD) for their efforts in fostering collaborations with European researchers, particularly in the area of clinical research. At the request of Stuart Eisenstat, U.S. ambassador to the European Union, Dr. Yoder and Ms. Wittenberg prepared a briefing paper on the American investment in European cancer research and the international importance of cancer clinical trials. The ambassador, Dr. Broder stated, is prepared to work with the economic agents of the European Union to ensure that stable core support is provided to the European Organization for Research and Treatment of Cancer. The United States has benefited from its investment in collaboration, he noted, and would benefit more if European cancer research had more stable support.

Dr. Broder reported that the President's Cancer Panel (PCP) met on October 5th at Tysons Corner, Virginia, to discuss "Lung Cancer: Clinical, Societal, and Governmental Challenges." He thanked Dr. Bishop for his letter to Congressman Thomas Bliley (R-VA) on the importance of the problem of smoking. When one considers not only the role of smoking in lung cancer but also the other health consequences of smoking, Dr. Broder stated, it becomes clear that the nation must pay attention to this problem. He called attention to the remarks of Dr. David Kessler, Commissioner of the Food and Drug Administration (FDA), on a recent episode of *60 Minutes*, in which Dr. Kessler indirectly represented all Government employees when he stressed the importance of the roles they play. Dr. Kessler has privately expressed, Dr. Broder added, his gratitude to the NCAB for its assistance in his efforts to deal with the problems of tobacco and nicotine addiction.

The President's Cancer Panel met again on November 30th in San Francisco, Dr. Broder continued, to discuss "Cancer and Cultures in America." Dr. Harold Freeman, PCP Chair, provided insights into aspects of the relationship between culture and illness, particularly among individuals who are underserved. The PCP examined the special impact of cancer in a number of cultural settings, including the different incidence and mortality rates among various populations. Dr. Broder noted that environmental and occupational factors are involved as well as diet and other lifestyle factors. He added that access to state-of-the-art approaches to prevention, diagnosis, and medical care varies among different populations in the United States.

The Cancer and Cultures in America meeting, Dr. Broder stated, provided an opportunity to stimulate certain hypotheses for basic research that otherwise might not have been available. For example, he said, it was learned at the November meeting that Alaskan natives have a very high rate of kidney cancer, hypernephromas, but do not have concomitant incidence of other urologic tumors, such as bladder cancer. He discussed this with Dr. Marston Linehan, head of the Urologic Oncology Section, who confirmed that this is an important research opportunity. One possibility, Dr. Broder suggested, is that a defined *p53*-type mutation is passed genetically, where there is a specified G-to-C transversion to a defined codon. Research in this area could enhance understanding of certain issues related to the pathogenesis of cancer.

For the benefit of new members, Dr. Broder reviewed the involvement of the NCAB in the evaluation of the NCI intramural program. A new NCAB ad hoc working group, referred to informally as the Blue Ribbon Panel, has been established to examine all in-house activities, including some that are not technically considered to be intramural research. This panel, which first met on October 28th and is scheduled to meet again on December 7th, plans to have a draft report ready for consideration by the NCAB in May 1995. The group is co-chaired by NCAB members Drs. Paul Calabresi and J. Michael Bishop; its other members are Dr. David Baltimore, of the Massachusetts Institute of Technology; Dr. Judah Folkman, of Harvard Medical School; Dr. David Livingston, of the Dana Farber Cancer Institute; Dr. John Minna from the University of Texas; Dr. Cecil Pickett, of Schering-Plough Research Institute; Leon Rosenberg, of Bristol-Myers-Squibb Pharmaceutical Research Institute; Dr. Louise Strong, a former NCAB member, of the M.D. Anderson Cancer Center; Dr. Bert Vogelstein, of Johns Hopkins Oncology Center; and Dr. Samuel Wells, also a former NCAB member, of Washington University. Dr. Marvin Kalt will serve as the NCI Executive Secretary for the Working Group.

The Working Group will make recommendations on the possible implementation of various findings concerning the structure and function of in-house programs, such as those in the so-called Marks-Casell report on intramural programs recently submitted to Dr. Harold Varmus, NIH Director. They will also look at the scientific and medical research priorities most central to the mission of the Institute that can be pursued intramurally, as well as alternatives for integration and consolidation of NCI scientific programs. The goal is to improve the focused coordination of basic and clinical research and to bring it in line with the Government streamlining process.

At the Panel's October meeting, Dr. Varmus reviewed the group's mission; Dr. Michael Gottesman led a discussion of the Marks-Casell report; Dr. Alan Rabson, Director, Division of Cancer Biology, Diagnosis, and Centers (DCBDC), provided a brief history of the National Cancer Institute; Mr. Philip Amoruso, Executive Officer, NCI, described the NCI decision-making process; Dr. Bruce Chabner discussed some of the Institute's clinical programs; and the group reviewed an evaluation of NCI's intramural laboratories.

Dr. Broder described a new research program on prostate cancer within the Cancer Centers Branch. This initiative will promote the development of new prostate cancer research programs by building interactive, multidisciplinary research bases at various NCI-designated Cancer Centers and at other institutions where there is an interest. Dr. Broder stated that research in prostate cancer has lagged behind research in other forms of cancer, and new scientists are entering the field in relatively small numbers. This is due in part, he said, to the lack of suitable *in vitro* and *in vivo* systems and the lack of accessible prostate tissues. Progress in developing new agents for treatment of prostate cancer has also been very slow.

NCI believes, Dr. Broder stated, that the reduction of prostate cancer incidence and mortality depends on a concerted research effort, and hopes to encourage participation by institutions with suitable expertise that have not previously been involved in prostate cancer research. It is hoped that research supported by this initiative will foster a mix of interactive basic, clinical, and prevention and control research, dealing with issues such as environmental and occupational carcinogenesis and high incidence rates among certain minority populations, although the specific scientific goals will be set by the applicants.

On October 11th, Dr. Broder reported, he spoke at a Capitol Hill briefing on "New Frontiers in Breast Cancer Imaging and Early Detection." The briefing summarized current research on early detection technology, such as magnetic resonance imaging (MRI), positron emission tomography (PET), and computer-assisted diagnosis; it also explored the potential of imaging technology from other fields, including defense, space, and computer graphics.

Dr. Broder also reported that NCI held a workshop on November 15th entitled "An Appraisal of Clinical Research for the Treatment of Early Breast Cancer." This workshop reviewed and updated information on clinical trials comparing conservative management, or breast-sparing surgery, versus mastectomy; examined the role of primary breast irradiation; discussed controversies in the conservative management of primary breast cancer; addressed barriers to appropriate utilization of breast-conserving surgery; and considered new approaches to adjuvant therapy. Dr. Broder stated that NCI is confident that for early breast cancer,

survival following total mastectomy and following lumpectomy with irradiation are equivalent.

On December 2, 1994, Dr. Broder met with the Executive Committee of the American Society for Hematology, who expressed interest in encouraging further initiatives in areas such as leukemia and stem cell research. Dr. Broder stated that the NCI will do its best to take their needs into consideration.

Turning to the subject of the NCI budget, Dr. Broder reported that Dr. Varmus has implemented his authority to transfer 1 percent of each Institute's budget for special projects. Dr. Broder noted that the limitation of this authority to emergency situations was removed during the current budget cycle. Dr. Varmus, he continued, is transferring $13.4 million to five Institutes to support four projects. NCI, which is not among the Institutes receiving transferred funds, is contributing approximately $2.6 million. The projects to be supported include aspects of the Human Genome Project, including development of new DNA sequencing technology, at a cost of $1.5 million; a National Heart, Lung, and Blood Institute project to construct a genetic map of the rat genome, at $3.2 million; a Child Health and Human Development Institute adolescent health study, at $5 million; and an assortment of other projects, including small-angle x-ray scattering and spectroscopy.

Dr. Broder showed a slide presenting the NCI total budget denominated in 1980 constant dollars. The year 1980 was chosen in order to look at changes in the Institute's "purchasing power" over one and a half decades. A deflator factor specifically designed to correct for cost inflation in biological research and development, which is slightly higher than the national inflation rate, was used to develop the slide. Since the Institutes recently transferred from the Alcohol, Drug Abuse and Mental Health Administration to NIH were not part of NIH in 1980, they were added to the baseline data in developing the slide.

During the 1980s, Dr. Broder stated, both NIH and NCI experienced a reduction in purchasing power. However, beginning in the late 1980s, NIH experienced a substantial growth in purchasing power, while NCI reached a plateau. In 1995, NIH purchasing power has grown 15 percent in terms of 1980 dollars, while NCI's, as a whole, has grown about 1 percent. Dr. Broder added that NCI would have shown a reduction compared with 1980 if it had not received its largest dollar increase in its history for fiscal year 1992, when the Institute funded close to 1,100 new and competing grants. Dr. Broder, acknowledging that multiple interpretations of these data are possible, offered his interpretation that in terms of purchasing power, there has been a fiscal reorientation toward activities that are not normally defined as being within the function of NCI.

Questions and Answers

Dr. Salmon asked what proportion of the increase in NIH funding during the 1980s was attributable to new AIDS initiatives. He compared this to the major increases in the NCI budget during the 1970s, which was accomplished without a negative impact on other Institutes. Mr. John Hartinger agreed that AIDS funding had a significant impact on the NIH budget, but he did not have specific data on hand.

Dr. Day suggested that a similar question could be asked concerning the Human Genome Project. He also stated that it would be helpful to see similar data comparing the budgets of other Institutes with the NIH as a whole. He observed that the rationale for major increases in the NCI budget for 1992 was the fact that the Institute had fallen behind other Institutes. If the same argument is being advanced today, he said, it would be important to have more detailed information, not only about NIH overall but about other Institutes and broad categories such as the research grant pool, the intramural programs, contracts, and others. He noted that the data presented are difficult to interpret because so much has changed in 15 years.

Dr. Rimer suggested that this type of information would be especially useful for the Planning and Budget Subcommittee's next meeting.

Dr. Sigal asked whether there had been any indication of what can be expected from the new Congress. Dr. Broder replied that it is too early to make any predictions.

Dr. Goldson stated that he had received numerous communications from the radiological and radiation therapy societies concerning the decision of the Executive Committee to consolidate the Radiation Research Program; a final decision is expected in February 1995. These groups expressed their opposition to this plan, suggesting that it will have a stifling effect on new initiatives and may create imbalances in future decision making. Dr. Goldson said the groups feel that the candidate being considered for the Associate Director's position is eminently qualified, both in radiation and diagnostic specialties, and they understand that restructuring is inevitable. Their only concern is that a strong multidisciplinary program remain in place. Dr. Goldson suggested soliciting the cumulative wisdom of constituent groups for the February meeting. Dr. Broder responded by emphasizing NCI's understanding of the importance of strong programs in radiologic research, both diagnostic and therapeutic. He agreed that there should be a discussion of this issue at the next NCAB meeting. Dr. Broder stated that downsizing is inevitable, adding that whatever steps are taken, someone will disagree with the decision. The realities of downsizing mean that some activities will have to be consolidated, and there will be limitations on hiring and promotions. Dr. Broder suggested that the Blue Ribbon Panel might expand its activities to look at this issue.

III. LEGISLATIVE UPDATE—MS. DOROTHY TISEVICH

Ms. Tisevich, legislative liaison for the NCI, presented a brief overview of changes in Congress and committee oversight of NCI programs resulting from the November 8th election. She mentioned that both the House and Senate have identified key issues on which they will focus when Congress convenes in January.

Ms. Tisevich described the pre-election composition of the Senate as having a Democratic majority of 54 to 46. In the incoming Congress, Republicans will have a 53 to 47 majority in the Senate. In the House, Republicans also gained a majority of 230 to 204, a slightly smaller margin than the Democrats held previously. She pointed out that there is one independent in the House, Representative Bernie Sanders (I-VT), who sponsored the Cancer Registry Act that mandated the study of breast cancer in the Northeast and mid-Atlantic States.

Ms. Tisevich reviewed the changes in the Senate committees that have jurisdiction over NCI and NIH programs. The full Appropriations Committee, which had been led by Senator Robert Byrd (D-WV), will likely be chaired by Senator Mark Hatfield (R-OR). The Subcommittee on Labor, Health and Human Services (HHS), Education, and Related Agencies, chaired by Senator Tom Harkin (D-IA), will probably be led by Senator Arlen Specter (R-PA). The authorizing committee in the Senate, Labor and Human Resources, is currently chaired by Senator Edward Kennedy (D-MA) and will be taken over by Senator Nancy Kassebaum (R-KS).

Ms. Tisevich also discussed changes in the leadership of the relevant House committees. The full Appropriations Committee, chaired by Representative David Obey (D-WI), will likely be led by Representative Robert Livingston (R-LA). The selection of Representative Livingston may be challenged because he ranks fifth in Republican seniority for appropriations. Ms. Tisevich said that Representative Neal Smith (D-IA), who chaired the Labor-HHS Subcommittee, lost his bid for reelection and will probably be replaced by Representative John Porter (R-IL). The authorizing committee in the House is a subcommittee of Energy and Commerce, the Subcommittee on Health and the Environment. The leadership of this committee will endure a dramatic change from Representative Henry Waxman (D-CA) to Representative Thomas Bliley (R-VA), who will take a very different position with respect to tobacco.

Ms. Tisevich pointed out that there are also reductions in the number of Democrats on each committee. On the Senate Appropriations Committee, there were no losses or retirements on Labor-HHS, but the power shift will likely result in the Democrats losing one or two of the most junior members' seats on the committee.

Ms. Tisevich noted that the changes to the House Appropriations Committee are significant. Representative William Natcher (D-KY) passed away and was succeeded to the chairmanship by Representative Neal Smith (D-IA), who was defeated. Assuming the same ratio for the 104th Congress as the 103rd, Ms. Tisevich remarked that the Democrats would have only four slots on this subcommittee, which will likely go to the four top remaining Democrats—Representative Obey, Representative Louis Stokes (D-OH), Representative Steny Hoyer (D-MD), and Representative Nancy Pelosi (D-CA). The three most junior members, Representative Nita Lowey (D-NY), Representative Jose Serrano (D-NY), and Representative Rosa DeLauro (D-CT), will probably be removed.

Ms. Tisevich reminded the Board of the "Contract with America," outlining the major initiatives that the Republicans of the 104th Congress plan to introduce both immediately and within the first 100 days. Ms. Tisevich listed the following items proposed for immediate implementation when Congress convenes in January: hire an independent firm to audit Congress for waste, fraud, or abuse; cut committees and committee staff; limit terms of committee chairs; ban proxy voting in committees; open committee meetings to the public; require a three-fifths majority for tax increases; and change the budgeting rules.

Ms. Tisevich listed the following House bills to be sponsored within the first 100 days: a balanced budget and tax limitation amendment and a legislative line item veto; sponsorship of a Crime Bill focused on sentencing, death penalty, increased law enforcement, and social spending cuts from previous Crime Bills to fund prison construction; prohibition of welfare to

minor mothers; cuts in Aid to Families with Dependent Children; cuts in welfare spending and requiring work-based welfare; enforcement of child support; enactment of tax incentives for adoption; strengthening of parents' rights in educating their children; tightening of child pornography laws; enactment of elder care and a $500 per child tax credit; repeal of the marriage penalty tax; prohibition of United Nations command of U.S. troops; increased defense spending, particularly on antimissile defenses; a raise in the Social Security earnings limit; repeal of tax increases on Social Security benefits; provision of tax incentives for private long-term care insurance; enactment of small business incentives, a capital gains cut and indexation; limits on punitive damages and reform product liability laws; and support of a vote on term limits.

Ms. Tisevich noted that the long-term Senate agenda is less ambitious than the House agenda: enact a balanced budget amendment to the Constitution; prevent crime without punishment with mandatory prison sentences; put prisoners to work in building less resort-like prisons; create work-based welfare; reward savings with Individual Retirement Accounts (IRAs); reduce capital gains tax and allow taxes on assets to be indexed for inflation; double the income tax exemption for children; reverse defense cuts and prevent future cuts in defense; reform health care by expanding consumer choices, promoting competition, reforming medical liability, and reducing bureaucracy; repeal the tax increase on Social Security, and repeal reduced benefits for the elderly who work.

Ms. Tisevich pointed out that a concurrent change in staff will occur with the change in the Congressional membership. According to Ms. Tisevich, 600 House staff will lose their jobs by the end of 1994. Jobs will be lost among Senate staff, not only due to the election, but to efforts to streamline Government. Ms. Tisevich hoped to have an update of the members of the subcommittees, chairs, and ranking members as well as more information on the direction of Congress at the next NCAB meeting.

After thanking Ms. Tisevich, Dr. Rimer explained that the December NCAB meeting is traditionally spent performing an annual review of NCI programs, using a 2-year cycle, and that this year's presentations would be from the Frederick Cancer Research and Development Center (FCRDC), the Division of Cancer Etiology, and the Division of Cancer Prevention and Control (DCPC). She noted that the directors of each program are responsible for setting their agendas and selecting speakers. Dr. Rimer added that the Divisions not providing presentations had assembled their relevant information in the Board members' books.

IV. INTRODUCTION AND OVERVIEW, FREDERICK CANCER RESEARCH AND DEVELOPMENT CENTER—DR. JERRY RICE

Dr. Rice, Associate Director of the Frederick Cancer Research and Development Center, referred Board members to the background material on the FCRDC in their Board books and the black meeting portfolio containing additional information relating to their afternoon tour. Dr. Rice explained that he would provide further background and details about FCRDC during the tour and confine his current presentation to an overview of FCRDC.

The FCRDC is a large facility, containing about 100 buildings located on 70 acres within an active Army base. The Center is functionally dependent on the Army and pays an annual fee for its supply of high-pressure steam heat and other utilities.

Dr. Rice described the FCRDC as a Federally funded research and development center (FFRDC). FFRDCs must meet rigid criteria and are reviewed by the National Science Foundation. FFRDCs enable agencies to use private sector resources to accomplish tasks that are integral to the mission and operation of the agency. The relationships between agencies and FFRDCs are usually long-term, providing the continuity to attract high-quality researchers.

FFRDCs are normally operated, managed, or administered by a university or other non-profit organization. The FCRDC is operated by a combination of five contracts with four different commercial firms. Four of these five contracts were awarded competitively under a recent recompetition. The largest contract, for operations, is currently under review in connection with NCI's and NIH's review of their changing needs. The primary sponsor for the FCRDC is the NCI, with responsibility for managing, administering, and monitoring the center. The National Institute of Neurologic Disorders and Stroke and the National Institute of Allergy and Infectious Diseases are other FCRDC sponsors.

The FCRDC is administered by a small NCI staff. The Office of the Director takes advice from the FCRDC Advisory Committee, which corresponds in its function to the Board of Scientific Counselors within the Divisions of the Institute. The OD also responds to decisions from the NCI Executive Committee and a semiannual NCI planning retreat. An NCI general manager and project officer work onsite at the Center and a group of contracting officials manage the financial functions of the Center, including its contracts.

Dr. Rice noted that FCRDC has both contract operations and intramural laboratories, the latter being managed, supervised, and evaluated by their sponsoring Divisions and corresponding Boards of Scientific Counselors (BSCs). Research conducted under the basic research program and under contract is reviewed by the FCRDC Advisory Committee.

On a diagram of FCRDC, Dr. Rice depicted the various intramural programs of NCI and other Institutes that are operated at the FCRDC, noting that NCI has laboratories at FCRDC from each of its four Divisions. Dr. Rice informed the Board that their tour of FCRDC would include a major component of the Division of Cancer Treatment, the Natural Products Branch, which focuses on new anticancer drug discovery and testing. A new building has been constructed at FCRDC to support the drug development effort.

Dr. Rice noted that FCRDC's only clinical program, the Biological Response Modifiers Program in the DCT, is operated offsite at the Regional Cancer Therapy Center in conjunction with Frederick Memorial Hospital.

Dr. Rice described FCRDC as home to the only supercomputing center used exclusively for biomedical research, which the Board would see on its tour. He qualified that the building's exterior is undergoing construction, but the interior is functional and will serve as the site for presentations about the center's activities in support of intramural and extramural research.

Dr. Rice reviewed the costs associated with operating the FCRDC. The direct cost of FCRDC's five contracts for fiscal year (FY) 1994 was approximately $56 million. The support contract, which provides support for intramural programs of the NCI, AIDS research efforts, construction, and other programs, is more expensive. The total expenditure through the operations contract at FCRDC in FY 1994 was approximately $128 million. Dr. Rice presented the operating budgets of the intramural programs, which amount to approximately one-half of the operations contract, the largest elements of which are in the DCT.

Dr. Rice pointed out that FCRDC, in addition to being a FFRDC, is also a Government-owned, contractor-operated (GOCO) enterprise. The advantage to this arrangement is that an understaffed agency can delegate activities to contractors on a competitive basis.

Dr. Rice highlighted the roles of the Advisory Committee that supervises FCRDC, including: providing scientific review of laboratories in the basic research program and contracted research that is not integral to an intramural research effort; reviewing technical support of all contracts at FCRDC; and providing advisory input for new support efforts and concept review for new efforts within existing contracts as well as during recompetition of FCRDC's basic operating contracts. The Advisory Committee also participates in strategic planning for FCRDC and its members serve on special issues committees, such as technology transfer or changes in a major contract's scope of work. Dr. Rice listed the members of the Advisory Committee for the Board and stated that the Committee would meet on December 8th and 9th.

Dr. Rice remarked on the posters commemorating World AIDS Day and raised the point that AIDS research is a major activity at FCRDC because of the Cancer Institute's expertise in the family of retroviruses, of which HIV is a member. Dr. Rice informed the Board that Dr. Arthur, director of the FCRDC vaccine program, would speak about that research during the FCRDC tour. AIDS research activities will soon be contained in a new five-story building at FCRDC, which Dr. Rice said should be constructed by April 1995 and occupied by next summer.

Dr. Rice closed by introducing the speakers who would present on behalf of FCRDC's basic research program: Dr. George Vande Woude, Director; Dr. Nancy Jenkins of the Mammalian Genetics Laboratory; and Dr. Stephen Hughes, Deputy Director.

Questions and Answers

Dr. Becker inquired about the $19 million construction budget for FCRDC. Dr. Rice explained that this figure includes funds for constructing the new building for AIDS research. The construction budget also covers expansions of the library conference center, the supercomputer center, and a laboratory building for the Biological Response Modifiers Program. Mr. Amoruso clarified that while the AIDS research building comprises the majority of the construction budget, at about $12 million, most of those moneys come from AIDS foundation research royalties from the Gallo patent, not appropriations.

Dr. Broder noted that the NIH has a buildings and facilities budget that covers building maintenance and repairs and is separate from moneys spent on construction. Mr. Amoruso

explained that the FCRDC must share NIH's repair and improvement fund with many other Institutes and facilities, resulting in a shortfall in maintenance funds which they supplement with moneys from their construction budget.

Dr. Ellen Sigal verified that the FCRDC's projected budget for FY 1995 is approximately $150 million, and that the multiyear contracts allow them the option to review the projected budget annually.

Dr. Sydney Salmon asked whether any of NCI's construction budget is used at FCRDC or is all used extramurally. Mr. Amoruso said that some of those moneys are used for maintenance and small construction projects at FCRDC; large projects, like the AIDS research building, are supported by foundation funds.

Dr. Rimer interrupted the presentations on FCRDC to introduce Ms. Visco to present her report on the President's Cancer Panel.

V. REPORT OF THE PRESIDENT'S CANCER PANEL—MS. FRANCES VISCO

Ms. Visco announced that she would be speaking on behalf of Dr. Harold Freeman, who was chairing an ad hoc meeting on the FTC cigarette testing method, and so could not deliver the report himself.

Ms. Visco informed the Board that the President's Cancer Panel met twice since the last NCAB meeting. The first PCP meeting dealt with the clinical, societal, and Governmental challenges of lung cancer, and included presentations from the Environmental Protection Agency (EPA), the Occupational Safety and Health Administration (OSHA), the Centers for Disease Control and Prevention (CDC), attorneys, the Association of State and Territorial Health Officials, affected cultural groups, and the Department of Education on air quality research, education, and regulatory efforts. Ms. Visco pointed to the significant absence of representatives from the Department of Agriculture and the Department of Commerce.

The PCP recognized cancer as a chronic disease process with a potential latency period of 20 years or more. The goal with respect to all cancers, particularly lung cancer, is prevention or intervention with a safe, effective chemopreventive agent that can alter the process of aberrant cellular differentiation. Ms. Visco noted that early detection and intervention with effective therapy is the next best alternative to successful prevention. However, current detection techniques are not sensitive enough, and there are few successful preventive or therapeutic strategies for advanced lung cancer, which will require the improvement and application of knowledge about disease induction and progression as well as the effective dissemination of that information to the public.

Ms. Visco reported that the likely points of clinical intervention will be components of autocrine growth regulatory systems, focusing on bombesine-like peptides, their receptors, development of antibodies, and exploitation of individual differences and possible gene mutations among patients. Ms. Visco noted that examination of genetic differences at the gross molecular biological level have led investigators to believe that certain populations are

affected more by behavioral, i.e., controllable factors, while others may have molecular differences in carcinogen metabolism, accounting for different incidence and mortality rates.

Ms. Visco emphasized that smoking cessation is the most dramatic and effective form of intervention and that detection strategies will need to focus on "fields of exposure" rather than confined organ system malignancies. Improved understanding of molecular and genetic mechanisms of lung carcinogenesis and identification of biomarkers of disease are needed to achieve effective chemoprevention. Ms. Visco noted that a better understanding of the factors contributing to onset and progression is needed to improve prevention, detection, and treatment of lung cancer. This entails study, not only of the molecular research, but of socio-cultural differences that may lead to smoking or offer intervention opportunities.

Based on the presentations made, the PCP considered the Government's approach to tobacco control inconsistent; while some agencies attempt to regulate tobacco, others (that declined to attend the meeting) support its growth and export. The disparity in resources available to the regulatory agencies versus the tobacco industry and the pro-tobacco sentiments expressed by new Congressional policy makers were also points of concern.

Ms. Visco mentioned the possibility that nicotine may be regulated as a drug and the devastating economic impact that the death of the tobacco industry would have on farmers and some urban groups. She predicted that recognition of nicotine's addictive quality could lead to development of safer cigarettes rather than a ban on tobacco. Ms. Visco reported that an effective deterrent to tobacco consumption is raising its cost through excise taxes. She also repeated a suggestion by Dr. Mitchell Zeller of the FDA that smoking should be treated as a pediatric disease with emphasis placed on prevention and early detection programs.

Ms. Visco reported that the PCP meeting, "Culture and the Cancers of America," held November 30th in San Francisco, echoed many of the same points and emphasized that intervention programs can only be successful if they are accepted by the population at which they are directed. The meeting focused on overcoming cultural barriers to reaching a cancer-affected population through understanding the population's norms, background, and attitudes toward disease and medicine.

Ms. Visco described the cultural impact of poverty as limiting options for screening and treatment, and the effect of insensitivity by caregivers, leading to mistrust and avoidance of doctors, which results in late-stage diagnosis and higher mortality. Ms. Visco mentioned the cultural beliefs and characteristics that help prevent cancer, developed by groups like the Seventh Day Adventists who treat the body as a temple. Presented in contrast was the Latino view that disease is God's punishment and death is inevitable. Ms. Visco stated a need for the study of cultural differences that affect lifestyles and may contribute to biological or molecular changes.

Ms. Visco noted the mistrust of the medical establishment by many special populations, and emphasized the importance caregivers should place on their patients' beliefs and behaviors with respect to medicine and disease, so that medical treatment can be accepted by patients without discord to their cultural values. She summarized the main points of the PCP meetings, including the importance of considering the patient and bringing him or her into the treatment on his or her terms to succeed in the war on cancer.

Dr. Rimer requested transcripts from the PCP meetings and congratulated Ms. Visco for being recognized by the National Women's Law Center for her work on breast cancer. Dr. Rimer turned the meeting back over to Dr. Rice, who introduced Dr. George F. Vande Woude, Director of the Basic Research Program at FCRDC.

VI. OVERVIEW OF THE BASIC RESEARCH PROGRAM, FCRDC— DR. GEORGE VANDE WOUDE

Dr. Vande Woude began his presentation by informing the Board that the recompetition for the 5-year, basic research contract at FCRDC was completed and awarded to Advanced BioScience Laboratories (ABL). The Basic Research Program consists of seven laboratories: the Macromolecular Structure Laboratory, the Mammalian Genetics Laboratory, the Chemistry of Carcinogenesis Laboratory, the Laboratory of Molecular Virology and Carcinogenesis, the Molecular Mechanisms of Carcinogenesis Laboratory, the Laboratory of Chromosome Biology, and the Laboratory of Eukaryotic Gene Expression.

Dr. Vande Woude mentioned that Dr. Steve Oroszlan will be retiring in 1995 and will become their first scientist emeritus. Dr. Oroszlan has made important contributions to the understanding of retrovirology and, in his role as scientist emeritus, will continue to have input into the program.

ABL will reorganize their investigators early in 1995 to be in line with related research interests, which will help facilitate the review process. ABL currently includes 24 research investigators as senior and junior staff. Looking back at the previous 7-year period, Dr. Vande Woude said that it had been very productive and that they are looking forward to the new contract period to exploit some of their earlier discoveries.

Dr. Vande Woude remarked that basic research has been responsible for the revolution in biology and biomedical research and that basic research is having a direct impact on understanding of the molecular basis for disease. This understanding, he said, can lead to new strategies for diagnosis and treatment. He added that it is remarkable to look back at how fundamental research has become focused on understanding diseases and mechanisms of cellular pathology, an area that has been termed molecular medicine. Dr. Vande Woude stated that this is not by accident, but because investigators are interested in solving problems that have a direct application in the diagnosis or treatment of disease.

Dr. Vande Woude said he would discuss three research projects: the *trk* family of nerve growth factors (NGF) receptors and the neurotrophin ligands, how benzylguanine is being used as a novel adjuvant to enhance cytotoxicity of alkylating agents, and studies on the relationship between antineoplastic drug specificity and the oncogenes responsible for the tumor.

In 1991, Dr. Vande Woude stated, Drs. David Kaplan and Luis Parada discovered that the *trk* proto-oncogene product was the receptor for nerve growth factor, which is required for the development and survival of nerves in the peripheral nervous system and some nerves in the central nervous system. The *trks* and neurotrophins are also of interest in studying mechanisms of cell growth control, because they often cause cells to stop growing as they

undergo neuronal differentiation. The signal pathway, Dr. Vande Woude said, used by *trk* to mediate survival, differentiation, and antiproliferative effects of nerve growth factor is being characterized in the laboratory. Multiple signaling pathways have been found to control NGF responses, including one in which the *ras* proto-oncogene is required for the maintenance and survival of neurons and a second pathway discovered by Dr. Kaplan, termed the SNT pathway, that is activated in cells that terminally differentiate.

Dr. Vande Woude said that Dr. Kaplan's group has also been investigating the growth inhibitory properties of *trk* and NGF as a therapeutic approach to treating human tumors of neuronal origin. The expression of *trk* A in human neuroblastoma cells includes neuronal differentiation and the cessation of cell growth, and *trk* A is currently used as a marker for good prognosis in human neuroblastomas. The goal of Dr. Kaplan's group, Dr. Vande Woude explained, is to enhance expression of *trk* A in human neuronal tumors. Several chemical inducers of *trk* A have been identified, including retinoic acid. The signaling differences between *trk* A and *trk* B were studied. Of all known proteins in neuroblastoma cells that respond to the neurotrophins, the only difference discovered between cells expressing *trk* A and *trk* B was that SNT tyrosine phosphorylation was evident only in cells expressing *trk* A. Agents that enhance expression or activity of SNT or *trk* A may be useful for inhibiting the growth of medulloblastomas, neuroblastomas, and glioblastomas.

Dr. Vande Woude then discussed studies from Dr. Robert Moschel's group. They discovered that O^6 benzylguanine, which is a substrate of alkyltransferase and is 2,000 times more effective than O^6 methylguanine in inactivating the alkyltransferase protein. Benzylguanine inactivation of alkyltransferase prevents the repairs of chloroethylated precursors to DNA crosslinking and greatly enhances the cytotoxic activity of alkylating agents. Dr. Vande Woude said that by inactivating the alkyltransferase repair system, a much higher degree of crosslinking can be obtained.

Dr. Vande Woude then discussed results from Dr. Moschel and his collaborators showing non-alkyltransferase inactivation by benzylguanine enhances treatment with 1,3-bis-(2-chloroethyl)-1-nitrosourea+Carmustine (BCNU). Athymic nude mice, he said, with intracranial medullar blastoma xenografts survive significantly longer with a single pretreatment of benzylguanine in BCNU compared with BCNU alone. Preclinical trials with benzylguanine and BCNU were completed in January and human Phase I trials have begun.

Dr. Vande Woude then discussed the studies of his laboratory correlating drug activity with specific cancer genes. He said that they had hypothesized that antineoplastic drug protocols may be specifically targeting tumor cells because the cancer genes responsible for the tumor were making the tumor cells more vulnerable than normal cells. To investigate this issue, his group collaborated with the Developmental Therapeutics Program (DTP). They asked whether the DTP cancer screen, which was developed for screening potential antineoplastic drugs, would be useful in identifying drugs that target cells containing specific oncogenes. The screen has approximately 60 cell lines from a variety of human tumors that have been tested with more than 40,000 compounds. For each cell line, the concentration of compound that inhibits cell growth 50% is displayed in a mean graph pattern, and, using pattern recognition computer software, algorithm COMPARE, the results of tests with one compound are compared with all drugs in the database, something analogous to fingerprint comparisons. Dr. Vande Woude said that COMPARE has been used successfully in

identifying compounds with similar structures or related mechanisms. It has also identified compounds with novel mechanisms.

Dr. Van Woude said, in wanting to determine if COMPARE could be used to identify drugs that correlate to specific cancer genes, he chose to test the cell lines for activating *ras* mutations. They chose the *ras* oncogene because it is the most frequently found oncogene in human cancers. Seventeen of the sixty cell lines contained *ras* mutations that clustered in three of a total of nine human tumor subpanels used in the screen. In the lung and colon subpanel, 7 of the 16 cell lines had *ras* mutations, similar to the frequency found in sporadic human tumors. They asked whether lung and colon cell lines with mutant *ras* genes were especially sensitive to certain compounds. A number of compounds were identified. COMPARE selected cytosine arabinoside, at the three different concentrations tested in the screen, as well as cyclocytidine, a cytosine arabinoside prodrug. The data showed a high correlation between the presence of *ras* genes and sensitivity to cytosine arabinoside sensitivity. Dr. Vande Woude illustrated his point with a slide that showed that at least five of the seven cell lines with *ras* oncogenes were very sensitive to cytosine arabinoside, while non-*ras*-containing cell lines were not sensitive.

Dr. Vande Woude remarked that this research has the potential to identify oncogene-sensitive pathways. It also has the potential for improving outcome by identifying a specific treatment with the presence of a specific cancer gene. Because cytosine arabinoside has been used clinically in combination with other drugs in the treatment of leukemias, it is possible to look retrospectively at acute myelogenous leukemia patients that have responded to Ara-C to determine whether there is a correlation between the responders and the presence of *ras*. He reported that this work is ongoing.

Questions and Answers

Dr. Schein asked about the long-term risk involved in stabilizing the mutation, especially in human bone marrow stem cells. Dr. Chabner responded that the amount of enzyme found in normal tissue is relatively small and the toxicity not great. The drug would show preferential enhancement of cytotoxicity in tumors, which have a high expression of the repair enzyme, with little effect on normal tissues.

Dr. Schein remarked that the fact that the dose needs to be decreased is an indication that subtle injuries are occurring that could result in long-term problems. Dr. Chabner responded that the patients undergoing this treatment are usually terminal, with a median survival time of 3 to 4 months in patients with glioblastoma mutliforme. Dr. Schein added that he is concerned that this approach will not be useful for other diseases.

Dr. Chan asked about the status of the *in vivo* system. Dr. Chabner answered that it is important to determine whether the candidate drugs are active in the whole animal. Xenograft systems have been developed for most of the cell lines; however, this has been a relatively expensive and laborious undertaking. An alternative is to test multiple tumors using a hollow fiber system. Dr. Chabner said that they are in the process of comparing the results of the hollow fiber studies to other *in vivo* systems to see if the results are comparable.

VII. MAP OF THE MOUSE GENOME—DR. NANCY JENKINS

Dr. Jenkins, head of the Molecular Genetics Development Section in the FCRDC's Mammalian Genetics Laboratory, indicated that her presentation would focus on the molecular genetic linkage map of the mouse genome that she and Dr. Neal Copeland have been working on at the Frederick Cancer Research and Development Center for the last 8 years. Dr. Jenkins stated that she would first describe the procedure that has been used to build the map; then the current status of the map; and, finally, the potential applications of the map in cancer research.

Genetic maps in any organism are built by performing genetic crosses and attempting to follow the polymorphism of a particular genetic trait in the progeny of the cross. Originally, meiotic maps in mice were built using crosses of inbred strains. These mice have been made genetically identical by extensive mating of individuals having closely similar genetic constitution. However, polymorphism was difficult to identify and mapping of the target gene frequently became burdensome, since all mice were very closely related to each other. In the mid-1980s, a new type of genetic cross ("interspecific cross") that substantially increased the polymorphic content of the offspring was introduced by three French scientists. They demonstrated that genetic variation could be maximized by crossing species that were very distantly related. Actually, progenitors of most interspecific crosses are separated by approximately 3 to 6 million years of evolution—a significant period of time for DNA sequence differences to accumulate. Consequently, identification of polymorphism for any gene of interest became a relatively simple procedure.

Dr. Jenkins indicated that the molecular genetic linkage map of the mouse genome was built using an interspecific cross composed of a prototypic inbred strain of mouse, C57BL16J, and *Mus spretus*—the most distantly related species of mouse that will still interbreed with the laboratory mouse and produce at least one class of fertile progeny. C57BL16J females were mated with *Mus spretus* males. The female F1 progeny of this cross is fertile and can be back-crossed with the C57BL16J inbred strain. Specifically, 205 independent back-crosses (N2 progeny) were made which served as the basis for the genetic map. Each N2 progeny inherits one copy of each autosome from C57BL16J and one from the F1 progenitor. Although genetic recombination during meiosis is of no consequence to C57BL16J animals themselves, N2 progeny will inherit the *Mus spretus* allele or the C57BL16J allele from the F1 hybrid parent.

Molecularly cloned probes were sequentially tested against the DNA isolated from the 205 animals of the N2 progeny. The segregation of either the *Mus spretus* allele or the C57BL16J allele was followed for each locus examined in the genetic map and the inherited allele was then determined. A map is built up by minimizing the number of double or multiple recombination events required to explain the allele distribution pattern.

Dr. Jenkins emphasized that the genetic map of the mouse available at the FCRDC is believed to provide the maximum amount of biological information possible, since it is a gene-based map. More than 1,700 genes have been mapped so far in one single set of DNA obtained from the set of 205 animals. The average distance between loci is approximately 1.0 centimorgan (cM), indicating that a molecular marker is placed every 1,600 kilobases across the genome. Presently, the map spans more than 95 percent of the entire mouse genome and it constitutes the most densely marked gene-based map available in the world.

Dr. Jenkins indicated that simultaneously with the development of the genetic map at the FCRDC, Dr. Eric Lander, Director of the Massachusetts Institute of Technology Genome Center, built a genetic map. This map was not gene-based, but was built on microsatellites, which are repetitive DNA sequences that appear approximately every 30 kilobases and are dispersed throughout the entire genome. Microsatellites are great markers for quick genetic map development and they are highly polymorphic even when inbred strains of mice are used. In addition, microsatellites can be typed by a polymerase chain reaction (PCR)-based approach, as opposed to a Southern blot approach for genes. The problem with microsatellites, however, is that they do not provide any biological information, since they are random pieces of DNA.

To provide a certain amount of biological information to the microsatellite map, NCI agreed to collaborate with Dr. Lander by fusing 250 of the microsatellites into the gene-based molecular genetic linkage map of the mouse genome. Results of this collaboration were published in the 1993 genome issue of *Science* magazine. The microsatellite map is currently being expanded to a total of 6,000 microsatellites; 1,500 of which will be mapped on the gene-based map at the FCRDC.

Dr. Jenkins stated that mouse maps play an important role as model systems for the identification of cellular genes causally associated with a disease process; a number of applications of the mouse maps relate to cancer research. Genetic maps of the mouse have provided the ability to identify human disease loci directly in the mouse.

In order to appreciate the importance of mouse maps, the relationship between mouse and human chromosomes must be well understood. Dr. Jenkins explained that during evolution of the mouse and human genomes, linked genes have tended to remain linked. Large segments of homology, called homology blocks or regions, exist between clusters of mouse and human genes within a vast number of chromosomes. From an estimated 135 homology regions that span the mouse genome, more than 80 percent have been identified. Since it is less complex and often faster to map a gene meiotically in mouse than to directly map it in humans, the loci where the gene will map in humans can be predicted on the basis of the homology regions.

Dr. Jenkins explained that the prediction of gene loci is particularly important for the identification of human disease. She stated that if a gene has been mapped to a particular region, the human genetic databases can then be analyzed to determine whether any human genetic disorder is known to exist that maps to that same region. If a human condition does exist, the candidate gene can then be cloned and sequenced from normal and afflicted human cells to determine whether a difference in the gene of the two populations is evident. This approach has been successfully applied on three different occasions using the gene-based molecular genetic linkage map of the mouse genome. One application refers to the mapping studies originally performed in collaboration with Drs. Richard Kolodner and Richard Fischel. These investigators cloned a human gene called *MSH 2*, which is involved in mis-match repair. This gene was then mapped in the mouse map and it localized to the distal region of mouse chromosome 17 in a region of human 2p21 homology. Shortly before mapping *MSH 2* in the mouse map, several groups of investigators reported that a set of patients that carried hereditary nonpolyposis colon cancer had a locus that appeared to map to human 2p21.

Human DNA was sequenced from both normal and affected individuals, demonstrating the involvement of *MSH 2* in colon cancer.

A second application of the mouse map relates to the cloning of mouse mutations. More than 800 phenotypic mutations exist in the mouse, many of which are important model systems for human normal and abnormal cellular processes. Dr. Jenkins referred to a mutation on a locus of mouse chromosome 2 that causes mice to exhibit obesity and a yellow overpigmentation. Under certain genetic conditions, mutations on this gene also result in the formation of tumors. The product encoded by this gene appears to be a hormone. The equivalent gene has recently been cloned in humans. Dr. Jenkins indicated that it will be interesting to determine whether this gene is involved in obesity and/or tumorigenesis in humans.

Dr. Jenkins explained that the cloning of mouse mutations has been accomplished through various approaches. The first is the candidate gene approach, which is similar to the identification of human disease loci and involves mapping a gene to a region of a chromosome where a mutation resides. Examination of the mutant phenotype to ascertain whether that phenotype would be expected for a mutation in the gene of interest will assist in determining whether the gene is a good candidate for mutations. If the gene is a good candidate, a more detailed molecular biology evaluation can be performed. Approximately 50 of the more than 800 mouse mutations that exist have been cloned, and about 20 percent of those cloned mutations have been obtained using the mouse map. The lymphoproliferation mutation, which is encoded by the *fas* receptor, as well as the generalized lymphoproliferative disease, which is encoded by the *fas* ligand, were molecularly cloned using the candidate gene approach in collaboration with a Japanese laboratory. This mutation is thought to be the best mouse model for human autoimmune disorders, particularly systemic lupus.

Another mutation cloned through the candidate gene approach has pigmentation and hearing effects, and the product encoded by this gene is a new member of the *myc* proto-oncogene supergene family of transcription factors. The mouse mutant phenotype is similar to that of humans exhibiting Waardenburg's syndrome—a syndrome which also affects pigmentation and hearing. Recently, investigators demonstrated a mutation in the *myc*-related transcription factor of subjects afflicted with a clinical subtype of Waardenburg's syndrome (Waardenburg's syndrome type 2). Dr. Jenkins explained that the mouse map was used to facilitate the cloning of the *myc*-related gene. This, in turn, provided the molecular basis for identifying the human disease. In addition, the mouse itself has served as a suitable model for the evaluation of this human disease.

Dr. Jenkins referred to a second approach for cloning mouse mutations. For positional cloning, a large genetic cross is established to segregate the mutation. The most closely linked molecular markers are identified and then used to build a physical map of the interval; eventually, an attempt is made to identify the affected gene. This approach is slower than the candidate gene approach and has not been extremely successful in the mouse; however, the fusion of maps such as the microsatellite map with the gene-based map should facilitate this type of studies in the future. Dr. Jenkins referred to a successful application of the positional cloning approach in the identification of the obesity mutation (OB). Dr. Jeff Friedman and coworkers have demonstrated that the product of the mouse obesity locus is a hormone produced and secreted by fat cells. This hormone may exhibit a feedback mechanism onto the

hypothalamus to regulate body weight. In the absence of a functional protein, the hypothalamus does not receive the correct signal that indicates the amount of fat cells in the body. Therefore, the animal continues eating and becomes obese. Dr. Friedman has also cloned the human gene and studies are currently ongoing to determine whether this gene is involved in human obesity.

Dr. Jenkins indicated that approximately one-third of all mouse mutations cloned so far represent an appropriate model for human disease. Since a larger number of mutant genes involved in human diseases are being cloned, the number of cloned mouse genes found to encode both a mouse mutation and the corresponding human disease locus will increase dramatically.

Future prospects derived entirely from the genome effort include the development of the physical map of the mouse genome, followed by the transcript map and, finally, the sequence of the mouse genome. Dr. Jenkins also indicated that future challenges include the cloning and characterization of polygenic disorders such as cancer. Identification and cloning of modifiers, suppressors, and enhancers is almost impossible in humans due to the lack of a sufficiently large human pedigree. In contrast, these studies are simple to perform in mice.

Dr. Jenkins concluded her presentation by referring to an example in which identification of a modifier was achieved. The gene for familial adenomatous polyposis was cloned; subsequently, the mutation in the affected pedigree was also cloned and called adenomatous polyposis coli (APC). APC has also been found to be involved in other types of familial colon cancer. The number of polyps within an individual family can vary dramatically, suggesting the presence of modifiers. Simultaneously with these studies, the multiple intestinal neoplasia (MIN) mouse mutation was characterized. The phenotype of MIN mice was almost indistinguishable from that of APC patients; subsequently, MIN was shown to be a mutation in APC. Under certain genetic conditions, the number of polyps was demonstrated to decrease dramatically, suggesting the presence of a modifier. Recently, the modifier was mapped to the distal region of mouse chromosome 4 corresponding to human 1q homology. Cloning studies are under way.

Questions and Answers

Dr. Broder congratulated Dr. Jenkins for her presentation and said she provides an excellent example of the capabilities of the Basic Research Program at the FCRDC as well as the value of the Advanced Bioscience Laboratories Program in relation to the entire National Cancer Program.

Dr. Broder commented that a number of genes have been first identified, cloned, and sequenced in humans rather than in mice. He then asked Dr. Jenkins about the cloning and sequencing status of BRCA-1 and BRCA-2 in the mouse and whether "knockout" mouse models are being used to characterize BRCA-1 and BRCA-2. Dr. Jenkins indicated that extensive efforts are under way, both in biotechnology and research laboratories, to clone the genomic locus of BRCA-1 and BRCA-2 for the mouse, since investigators are interested in developing mouse model mutations in this gene. Dr. Jenkins stated that future studies with BRCA-1 and BRCA-2 should focus on the identification of modifier genes that are highly important in breast cancer. A mouse mutant containing an aberration in the breast cancer gene

should be developed and crossed under different genetic conditions to determine whether a decrease in tumor number or a change in tumor type is attained.

Dr. Broder stressed that the priority status of BRCA-1 studies is based on published evidence that indicates that in every family known to carry BRCA-1, at least one woman has survived to age 80. These data suggest that a defined genetic or environmental system exists that could and should be identified to minimize the risk for breast cancer. Dr. Jenkins added that once the BRCA-1 wild type mouse gene is identified, it would have to be mutated to produce a model system that could be subsequently used for genetic crossing.

Dr. Day asked Dr. Jenkins whether the mouse genome program is being supported by the Human Genome Project. Dr. Jenkins replied that her work is being entirely funded by NCI. She explained that the original mission of the Human Genome Project was to sequence the genome, and no interest in gene-based maps existed. The only interest in developing crude and sturdy genetic maps was to build a physical map. Dr. Jenkins indicated that contrary to that view, she and her colleagues defended the idea that gene-based maps provide a maximum of biologic information, and requested support from the NCI. Dr. Jenkins added that substantial progress has been made in the identification of mouse genes that are consequential in human disease. In contrast, the long-term goal of the Human Genome Project to sequence the genome will not be accomplished in the near future.

Dr. Becker referred back to the example of the mutation on a locus of mouse chromosome 2 that produces obesity and yellow overpigmentation in mutant mice. He indicated that this gene is the only known promoter gene in mammalian biology. It is the only gene currently available that is known to accelerate almost any natural or induced tumor, including breast cancer in the mouse. This gene will not produce breast cancer if the mouse is not predisposed by chemical carcinogenesis or tumorigenic mouse mammary tumor virus (MMTV). The mutant mouse has a normal propensity for aberrations and immunological patterns. Thus, if a mutation is induced in the parallel mouse gene for breast cancer and transfected into the obese and overpigmented mutant mouse, a model system containing both the aberrant gene for breast cancer and the gene that accelerates tumorigenesis will be created. This combination model could be used to determine whether the young woman's syndrome of aggressive cancer can be paralleled in the mouse.

VIII. MECHANISMS OF RESISTANCE OF HIV REVERSE TRANSCRIPTASE—DR. STEPHEN HUGHES

Dr. Vande Woude introduced Dr. Stephen Hughes, Deputy Director of the ABL Basic Research Program and head of the Gene Expression in Eukaryotes Section at the FCRDC. Dr. Hughes received his doctorate degree from Harvard University in 1975, and then worked for several years at the University of California in San Francisco with Drs. Bishop and Varmus before he was hired at Cold Spring Harbor, NY. In 1984, he was recruited to work for the Basic Research Program. Dr. Hughes has been a key figure in establishing the Basic Research Program, and his research has focused on the characterization of HIV-reverse transcriptase (RT) and the specific oncogene called *ski*.

Dr. Hughes reviewed the HIV-RT studies performed in collaboration with Dr. Edward Arnold at Rutgers University. He explained that these studies have attempted to characterize the structure and function of HIV-1 RT in sufficient detail that this information could be used in the design and refinement of RT inhibitors. This approach has involved expression and purification of gram quantities of recombinant HIV-RT protein. The purified protein has been used for structural, immunological, and biochemical studies. Dr. Hughes indicated that his laboratory supplies purified material to drug-screening programs, including the one at the FCRDC, on a regular basis.

Dr. Hughes explained that finding an effective therapy for AIDS has been difficult because of the genetic variability of the HIV virus. Mutations in the HIV virus have provided an escape mechanism against RT inhibitors. Site-directed mutagenesis is being applied to define the essential elements in RT; more than 400 RT mutants have been generated and analyzed thus far. Part of this effort has involved the production of a panel of drug-resistant variants in an attempt to determine the mechanism by which the virus, and more specifically the RT molecule, evades known chemotherapeutic procedures.

Dr. Hughes described the function of RT. This enzyme is responsible for copying the single-stranded RNA genome that is found in virions into the double-stranded linear DNA form that is subsequently inserted into the genome of the infected cell. RT has two enzymatic components: a DNA polymerase and a ribonuclease H (RNase H). The DNA polymerase requires a nucleic acid primer. When the viral RNA genome is copied into DNA, a host tRNA is used to initiate reverse transcription. The RNase H activity of RT degrades the RNA template into short segments after it has been copied into DNA. The short segments of RNA serve as primers for initiating the synthesis of the second DNA strand. RT is a versatile enzyme capable of copying both RNA and DNA templates.

Dr. Hughes described the structure of RT. This enzyme is composed of two subunits that share a common amino terminus. The larger subunit, p66, contains 560 amino acids, while the smaller subunit, p51, contains 440 amino acids. The segment encoding RNase H is present only in the p66 subunit. The subdomains of RT have been given anatomical names based on RT's similarity to a human right hand (e.g., fingers, palm, and thumb). Although the two subunits share a common amino acid sequence, their three-dimensional structures are substantially different. Analysis of the structure showed that, while RT contains the amino acids for two polymerase active sites, only the one in p66 is active. The second site in the p51 subunit is buried in the structure.

Crystals of HIV-1 RT have been prepared with a bound 18, 19 double-stranded DNA. The DNA contains both A and B form segments. The DNA segment near the polymerase active site is in the A form, the DNA near RNase H is in B form. Dr. Hughes presented a slide of the polymerase active site which showed the high resolution currently attainable in the structural analysis. He referred to the catalytically relevant aspartic acid residues (110, 185, and 186), the tyrosine residues (181 and 183) that play an important role in conferring resistance to nonnucleoside inhibitors, and the specific site at the end of the double stranded DNA where a nucleoside inhibitor would attach to the growing DNA chain.

Dr. Hughes indicated that the acquisition of detailed functional and structural data on RT has been critical for the development of models that predict the ability of HIV-1 RT to distinguish normal nucleoside triphosphates from nucleoside analogs.

Dr. Hughes stated that as a consequence of the structural studies, RT has been found to exhibit not only genetic variability, but also physical variability. Dr. Arnold has obtained three different structures of RT. One of these structures is of a complex between HIV-1 RT and the nonnucleoside inhibitor alpha-APA. Dr. Hughes presented a number of slides illustrating the changes that take place in RT during the binding of the nonnucleoside inhibitor or the changes that take place in the structure of RT when a nonnucleoside inhibitor or double stranded DNA is bound. He explained that the variability in RT structure should be considered in the design of RT inhibitors.

Dr. Hughes explained that RT inhibitors can be classified into: 1) nucleosides such as ddI, ddC, and AZT, that act as chain terminators when they are incorporated into the DNA; and 2) nonnucleosides. All nonnucleoside inhibitors bind to the enzyme at the same site, which is an hydrophobic pocket that is near, but not at, the polymerase active site. Resistance to a nonnucleoside inhibitor arises when mutations in the enzyme occur at certain positions that interfere with the binding of the drug to the hydrophobic pocket. A combination of structural and biochemical analyses has demonstrated that most of the mutations that cause resistance to nonnucleoside inhibitors occur in the p66 subunit; the exception is the mutation E138K, which exerts its effect through a change in the p51 subunit. These results were predicted through structure analysis and were confirmed through biochemical studies. HIV-1 RT mutants were generated that had amino acid substitutions in only one of the two subunits. The mutant RTs were used to determine which subunit caused the mutation responsible for causing resistance to the nonnucleoside inhibitors.

Nucleoside inhibitors, which are more widely used in the clinical setting, are analogs of the precursors used by RT to make the DNA copy of the genome of HIV-1. Nucleoside inhibitors are incorporated into DNA by RT and, when incorporated, block the subsequent growth of the DNA strand. The polymerase active site is not exclusively composed of RT itself, but includes the template/primer. Nucleoside-resistance mutations are not clustered at the polymerase active site itself, but, instead, are clustered around the nucleic acid-binding groove. These data suggest that at least some of the mutations cause resistance to nucleoside inhibitors by altering the precise way the nucleic acid is bound, which, in turn, alters the geometry of the nucleic acid/protein complex that forms the polymerase active site.

Dr. Hughes presented a slide illustrating this model using a mutation at position 74—in which leucine is replaced by valine—that confers resistance to dideoxy compounds. Since this mutation lies a considerable distance away from the nucleoside-binding site, the issue is how the mutation confers resistance to nucleoside analogs. Computer modeling revealed that, if the DNA chain that is being copied is extended, it will pass very close to L74. Additional biochemical data indicate that the effect of mutation L74 is not evident unless the DNA template is long enough to make contact with the protein in the vicinity of L74. Similarly, evidence indicates that a mutation on position 89 has a specific effect on the exact position that the nucleic acid is held by RT.

Dr. Hughes concluded his presentation by referring to mutations in RT that give rise to resistance to nucleoside analogs (replacement of methionine by valine, isoleucine, leucine, or alanine at position 184). Although all of the mutant enzymes were resistant to the nucleoside analogs, two of the mutants were defective in their ability to move down the template without falling off ("processivity"). The mutations that were defective in processivity are not selected in response to therapy. This finding may have important implications for future strategies for the design of RT inhibitors.

Questions and Answers

Dr. Chabner asked Dr. Hughes whether the functional and structural research on RT has revealed combinations of nonnucleoside-resistance mutations that would be incompatible with viability of the virus. Dr. Hughes responded that resistant mutants have been generated against all RT inhibitors tested so far. The important question is whether additional constraints can be placed on the virus to render the development of resistance impossible. A research project has been recently initiated to investigate this question. The compatibility of various resistance mutations is currently being tested.

Dr. Chabner asked about the diversity of resistance mutations that arise in response to treatment with different nonnucleoside inhibitors. Dr. Hughes replied that his research relies on mutations published in the literature by other investigators. Single or multiple mutations are tested against HIV-1 RT with single, defined available agents to allow direct comparisons. In addition, the recombinant HIV-1 RT is distributed to other investigators to perform similar evaluations.

Dr. Broder asked Dr. Hughes to comment on the use of combination therapy (e.g., AZT and ddI) *in vivo* to potentially diminish the development of resistance. The combination of AZT and ddI was expected to reduce the emergence of escape mutants highly resistant to both AZT (mutation on codon 215) and ddI (mutation on position 74). However, only ddI-resistant mutants are restrained by the use of this combination of agents. Dr. Hughes indicated that current research under the direction of Dr. Arnold involves the development of Xray crystallographic structures to determine exactly what interactions occur between the extended template strand and the regions involving codon 215 and leucine 74. These studies are designed to determine the interaction of the extended DNA template with the wild type RT enzyme, and, ultimately, with RT enzymes specifically carrying the mutations that confer resistance to nucleoside inhibitors. Dr. Hughes stated that there are technical difficulties with these experiments and that the answers to these critical questions will not be obtained in the near future.

IX. INTRODUCTION AND OVERVIEW, DIVISION OF CANCER ETIOLOGY—DR. JERRY RICE

Dr. Rice began by stating the function of the Division of Cancer Etiology: to investigate the etiology of the various human cancers and utilize this knowledge in the development of effective cancer prevention programs in conjunction with the other Institutes at NIH. Dr. Rice added that the Division is also responsible for synthesizing all clinical, epidemiological, and experimental data regarding cancer etiology.

DCE is organized into three program areas, with oversight provided by an active Board of Scientific Counselors, chaired by Dr. Barry Pierce. Dr. Rice indicated that the first of these, the Chemical and Physical Carcinogenesis Program, is composed of eight laboratories and two extramural branches. In consideration of the ongoing review of NCI's intramural program, Dr. Rice stated that his presentation would focus only on the intramural activities of the Division. This decision was supported by Dr. Broder. Dr. Rice asserted that one of the primary purposes of the intramural research laboratories within NIH Institutes is to pursue high-risk, long-term studies that promise significant results. Advances achieved by the intramural research efforts within this program area include: initiating the use of human cells and tissues in carcinogenesis research; 2) devising the term "chemoprevention;" and 3) characterizing the transforming growth factor beta class of growth factors.

Dr. Rice continued by describing the Biological Carcinogenesis Program (BCP), which consists of six intramural laboratories and one extramural branch. The BCP is currently performing a critical review of its structure in conjunction with the BSC to allow for new initiatives during the current era of downsizing. He added that three of DCE's laboratories, as well as a portion of a fourth, are located at the Frederick Cancer Research and Development Center.

Dr. Rice emphasized the unique qualities of DCE's third program area, the Epidemiology and Biostatistics Program, including: 1) acting as one of very few epidemiological programs within the entire NIH intramural research purview; 2) maintaining an extremely effective research agenda; and 3) providing rapid response to inquiries from Congress, regulatory groups, and the public. Dr. Rice explained that within this program epidemiological and biostatistical research exploring cancer etiologies, particularly regarding environmental and host determinants, is performed to support the development of effective prevention strategies. He indicated that this program is also responsible for conducting studies that cannot be completed in extramural settings. Dr. Rice cited the epidemiologic program's work regarding non-Hodgkin's lymphoma, which was published as a supplement to *Cancer Research,* as an example of their ability to address emerging concerns in a coordinated manner.

Rapid-response inquiries within the epidemiologic program, Dr. Rice stated, have included examining the excess cancer risk associated with: 1) pesticides and other agricultural exposures; 2) residing near nuclear facilities; and 3) fluoridation of drinking water. Dr. Rice stressed the collaborative nature of responses to these inquiries that involve both intramural laboratories and one or more of the epidemiology branches, such as studies of cancer among minorities, which involve both the Environmental Epidemiology Branch and the Laboratory of Human Carcinogenesis groups, and studies of risk associated with occupational exposures, which involve the Environmental Epidemiology Branch and the Laboratory of Cellular Carcinogenesis and Tumor Promotion.

Dr. Rice continued by discussing budgetary issues. He informed Board members that DCE's 1994 estimated actual budget level is $373 million. The in-house and contract lines together comprise 28 percent of the total budget and include the intramural research effort which is funded at 26 percent of the total DCE allocation. Dr. Rice pointed out that the DCE budget includes funds for cancer and for AIDS; that 22 percent of the budget for cancer research is directed to in-house projects, while the remaining funds are allocated to

investigator-initiated grants, cooperative agreements, and grants issued in response to RFAs. He stated that 14 percent of DCE's budget is targeted to AIDS research, with a primary focus on vaccine development. Dr. Rice characterized the AIDS research budget as proportionately reversed to the cancer budget, with 70 percent allocated to in-house research. This is a result of the dearth of grant proposals that are directed to DCE from the Division of Research Grants (DRG), as well as the existence of a major center of retroviral research, which has become a primary focus of AIDS research, within the Division.

Dr. Rice also indicated that within DCE's three program areas there are differences in funding levels between intramural and extramural research, with the epidemiological area having the largest in-house allocation and the other two having approximately 20 percent of their budget directed toward intramural projects. He added that funding for the Office of the Director is primarily allocated to the in-house line because all professional staff within this Office are considered to be a part of the intramural research program.

Questions and Answers

Dr. Bishop asked whether Dr. Rice would prefer to have more extramural grants directed to DCE from DRG. Dr. Rice responded that DCE would rather receive more grants for cancer research than AIDS; however if the Division were to remain heavily active in AIDS research, then he would prefer more referrals of extramural grants for AIDS research.

Dr. Bishop requested that Dr. Rice characterize the AIDS research program of DCE. Dr. Rice indicated that he is satisfied with the current focus on AIDS-associated malignancies, as he believes this is the proper province of a cancer institute. He added that the Request for Applications (RFA) mechanism has had to be utilized to stimulate interest in these areas.

Dr. Salmon asked what laboratory-based research efforts regarding minority cancer have been initiated. Dr. Harris, Chief of DCE's Laboratory of Human Carcinogenesis, explained that the researchers explore susceptibility, which involves measuring genotypes associated with carcinogen activation and detoxification, and pathogenesis as it relates to different environments and cultural practices.

Dr. Bishop requested that Dr. Rice make available to the Board any documents generated during DCE's self-review process.

X. REPORT OF THE CHAIR, BOARD OF SCIENTIFIC COUNSELORS, DCE—DR. G. BARRY PIERCE

Dr. Pierce began his presentation by underscoring the partnership that exists between the Director of DCE and DCE's Board of Scientific Counselors, which acts to maintain the quality of in-house and extramural research. He outlined the functions of the BSC, including review of: 1) intramural researchers utilizing the site visit mechanism; 2) intramural research programs regarding allocation of resources, full-time equivalents (FTEs), space, money, and staffing decisions, such as promotions and tenure conversions; and 3) contracts and RFAs that are presented to the BSC, particularly in relation to overall program balance.

Dr. Pierce stated that while he believes there are adequate tenure track positions to ensure promotion of high-quality researchers, the BSC is limited in its actions in this arena. He explained that site visits, which occur within a year of project initiation, allow Board members to meet DCE staff and observe the research activities. Dr. Pierce commended what he characterized as a growing collaboration among researchers within the Division. Dr. Pierce reported that a recent site visit had resulted in an unfavorable review, which will necessitate another visit after 18 months to reassess the laboratory, as an indication of the effectiveness of the mechanism. He emphasized that "there are no villains in this situation." Resolution will require addressing complex scientific and administrative problems. He offered an anecdote in which he was handed 15 pounds of paper to read as background information for the visit to illustrate some of the changes that are necessary in the process. As an experiment, this material has been restricted to a maximum of three pages for portraying the lab's overall direction and two pages for each section to describe its current activities.

Dr. Pierce indicated that Dr. Susan Sieber is currently involved in preparing a plan for reorganizing the Division's laboratories to increase collaboration and communication. He mentioned that she is relying heavily upon the site visit reports to develop this plan. The group working on this project had originally hoped to present this plan at the October NCAB meeting; however, a future meeting is now being targeted. Dr. Pierce emphasized the importance of this project, particularly in view of the reorganization that is under way within the NCI as a whole.

Dr. Pierce informed members that intramural scientists are dissatisfied with their working conditions in terms of overcrowding, procurement problems, and other comfort issues. He reminded members that these scientists have little recourse to resolve these issues, as they cannot lobby against the Government. Dr. Pierce suggested that the 300 extramural scientists who comprise the BSC's of the various Divisions should act as advocates for in-house researchers.

The contract approval function and concept review process provides the BSC with the opportunity to examine program balance both in terms of in-house versus extramural research, as well as in regard to a scientific perspective. A major challenge to the fulfillment of this responsibility is the short term of appointment for BSC members. Dr. Pierce highlighted the ongoing reduction in intramural contracts, which have been redirected to R01 programs supported by the Division. He commented that this is an important indication of the attention directed to the extramural community by in-house staff.

Dr. Pierce indicated that the reason for the disproportionately high number of contracts issued by the Epidemiology Branch is that their research is necessarily conducted all over the world; if they were allocated a predetermined budget in the same manner as a laboratory, their effectiveness would be extremely hindered. He told members that any concept reports provided to the NCAB have already been carefully reviewed by the BSC. Dr. Pierce reemphasized his support of the unique manner in which this Branch operates and commended its efficacy.

Establishing priorities among various cancer issues will become an important task of the BSC. Dr. Pierce praised the prior work of the BSC and asserted their dedication to maintaining the quality of the research performed under the purview of DCE.

Dr. Pierce outlined some of the administrative limitations that have complicated management within the Division. He reminded members that there has been a hiring freeze for 4 years, which is primarily a result of an earlier solution to the issue of low salaries. In addition, higher-level promotions were frozen in October 1993. He indicated that the regulations governing staff additions and deletions are extremely complex. Dr. Pierce suggested that a working group composed of members of the Administration, Congress, and senior members of NIH be formed to resolve these issues.

Questions and Answers

Dr. Bishop asked whether the reorganization of the Division's laboratories will be based on performance or theme. Dr. Pierce responded that it will reflect reassignment based on similar themes, which will facilitate better communication and collaboration. Dr. Bishop queried whether resource allocations will be adjusted according to performance. Dr. Pierce indicated that these reallocations are ongoing through the site visit mechanism.

Dr. Salmon asked whether the laboratories at FCRDC undergo the same review process as those on the Bethesda campus. Dr. Rice informed NCAB members that both campuses are subject to the same review by DCE's BSC.

Dr. Broder reintroduced the issue of purchasing power in constant dollars, which had surfaced during the previous day. He pointed out that every NIH institute has experienced a growth in purchasing power, with two exceptions, NCI and National Institute of Environmental Health Sciences (NIEHS). Dr. Broder explained further that if NCI and NIH allocations are adjusted to reflect only non-AIDS-related activities, NIH still experienced a 2 percent growth in budget overall, while NCI underwent a 10 percent decrease in purchasing power. Dr. Broder acknowledged that constant dollars are only one appropriate method for examining budget allocations. He asserted that this is not a 1-year change, but a long-term trend with which the Institute must deal. He continued by noting that if the NCI had not received a relatively large allocation in 1992, these figures would have been more dramatic. He commented that this trend is not a result of one specific event or policy, such as redirecting money to AIDS or the human genome effort. He suggested that the Board consider whether NCI is served by this reallocation and make appropriate recommendations based on their conclusions.

XI. UPDATE ON HUMAN T-CELL LEUKEMIA VIRUS-1— DR. GENOVEFFA FRANCHINI

Dr. Franchini, acting Chief of the Animal Models and Retroviral Vaccine Section of the Laboratory of Tumor Cell Biology, indicated that her presentation on human T-cell leukemia virus (HTLV) would summarize three different projects being performed in her laboratory. She explained that HTLV causes acute leukemia of CD4+ T cells, which is invariably a fatal malignancy. In addition, HTLV-1 causes the neurological disease known as HTLV-1-associated myelopathy, also called tropical spastic paraparesis because it was first described in the tropics. Both diseases occur in only 5 percent of HTLV-1-infected individuals.

Dr. Franchini mentioned that the genome of HTLV-1, although similar to the genome of other animal retroviruses, appears to be more complex in the 3' end region. The 3' end region of the HTLV-1 genome contains a gene that codifies a viral transactivating protein that affects cell growth and enhances transcription of several viral and cellular genes. Dr. Franchini indicated that her group, in collaboration with other investigators at the NCI, demonstrated that HTLV-1 can increase its genetic complexity and encode several additional proteins by alternative splicing. These proteins are denominated according to their molecular weights and include p30 and p13, which are nuclear proteins, and p12, which is a cell-membrane associated protein.

Dr. Franchini indicated that the mechanism of pathogenicity of HTLV-1 is poorly understood. Although the transactivating gene *tax* is believed to play an important role in the transformation of cells by HTLV-1, results from *in vitro* studies indicate that the transactivating protein enhances the expression of the alpha chain of the interleukin 1 (IL-1)-high-affinity receptor in T cells, and is capable of immortalizing, but not transforming, T cells. Dr. Franchini explained that transformation of cells by HTLV-1 *in vitro* involves a ligand-independent T-cell proliferation. Since ligand-independent cells can be selected from human T cell cultures infected with HTLV-1, other viral genes must be responsible for this transformation.

Dr. Franchini indicated that her research has focused on the membrane-associated p12 protein. Computer analysis has revealed sequence similarities between p12 and the E5 protein of bovine papillomavirus. Bovine papillomavirus is a relatively benign virus that contains a weak oncogene which encodes the E5 protein composed of 44 amino acids. Based on this sequence similarity, an experiment was designed to determine whether the gene encoding p12 could be classified as an oncogene. Cells obtained from C127 mice were transfected with either p12, E5, or a combination of both genes. The loss of cell growth arrest by contact inhibition was measured by foci formation. Transfection with a combination of p12 and E5 produced a potentiation of the foci formation induced by E5 alone, whereas transfection with p12 alone had no effect. These results suggest that the p12 gene might be a weak oncogene and might indeed be involved in the mechanism of cell transformation.

Dr. Franchini indicated that she and her collaborators have found that E5 binds to the 16-kb subunit of the vacuolar H+ ATPase, the platelet-derived growth factor (PDGF) receptor, and the epidermal growth factor (EGF) receptor. The binding of E5 activates the PDGF receptor, suggesting that E5 could function as an intracellular ligand to induce receptor activation. Binding of E5 to the 16-kb subunit of the vacuolar H+ ATPase was demonstrated by cotransfection of E5 with 16-kb and the subsequent coprecipitation of the E5-16-kb complex with either an antibody against E5 or an antibody anti-16 kb. Similarly, p12 and the vacuolar H+ ATPase 16-kb subunit were coprecipitated from cells cotransfected with p12 and 16-kb using specific antibodies. These results suggest that p12 also interacts with the 16-Kb subunit of the vacuolar H+ ATPase; however, further studies are necessary to understand the biological effect of this interaction. Dr. Franchini explained that the vacuolar H+ ATPase is present in lysosomes, endosomes, and the Golgi Apparatus, and that one of its functions is the degradation of the receptor-ligand complex after its cellular internalization. Dr. Franchini stated that she established a collaboration with investigators in Glasgow to study the effect of p12 on the vacuolar H+ ATPase in yeast, because its study in eucaryotic cells is difficult and

this enzyme appears to be critical for cell survival. Thus, testing the interaction between the vacuolar H+ ATPase and p12 in different systems could provide useful information.

Dr. Franchini explained that p12 was cotransfected with several receptors, and specific antibodies against either p12 or the receptor were used to determine whether the two molecules interact and therefore, coprecipitate. P12 did not coprecipitate with antibodies against PDGF-R, EGF-R, the alpha chain of the interleukin-2 receptor (IL-2R) or the erythropoietin (EPO) receptor. The reciprocal experiments showed that these receptors did not coprecipitate with an antibody directed against p12. Experiments with antibodies against the beta and gamma chains of the IL-2R showed interaction with p12. The same cotransfection experiment showed coprecipitation of the beta and gamma chains of IL-2R when anti-p12 antibody was used. Dr. Franchini added that her group has been studying the interaction of p12 with a newly described JAK protein kinase. JAK-1 is thought to bind to the beta chain and JAK-3 to the gamma chain of the IL-2R.

Dr. Franchini concluded that HTLV-1 interferes with T cell growth through the effects of the proteins encoded by the *tax*, *rex*, and, very likely, p12 genes. It has been demonstrated that the tax protein increases the expression of the alpha chain of the IL-1R. The rex protein regulates the expression and utilization of the viral messenger RNA (mRNA) and influences other cellular genes in concert with the tax protein. Dr. Franchini stated that the possible mechanism of action of HTLV-1 p12 is not novel, since it has been previously shown that a focus-forming virus that induces leukemia in mice, encodes for the truncated aberrant form of the envelope protein, which, in turn binds and activates the EPO receptor.

Dr. Franchini indicated that the second research project currently ongoing in her laboratory addresses the origin of HTLV. She stated that the determination of the origin of HTLV is not only important for establishing a phylogenetic relationship among viruses, but also could provide identification of new reagents that might be used in the study of other human retroviruses.

Dr. Franchini indicated that her group, in collaboration with the Mental Health Institute and Dr. Gajdusek, characterized a new HTLV that is present almost exclusively in Papua, New Guinea (Melanesia), and in Australia. Fifty percent of the people living in small villages in Papua display seropositive HTLV antigens. This Melanesian population has been isolated from the rest of the so-called civilization for several years. Dr. Franchini explained that the sequence of the envelope gene of this HTLV was analyzed, using polymerase chain reaction (PCR) to determine the genetic relationship between the Melanesian and the cosmopolitan prototype of HTLV-1. The cosmopolitan HTLV-1 infects mainly the Black population in most cities in the United States and in the equatorial region of Africa. Dr. Franchini explained that an HTLV-1 molecular variant was identified that is present mainly in Africa. This variant is spreading since some cases have been detected in Sicily. Dr. Franchini indicated that to elucidate the origin of these molecular variants of HTLV-1, her group studied the simian T-cell leukemia virus (STLV-1) in several populations of monkeys in collaboration with primate centers in the United States and Europe. The STLV-1 genetic sequences were obtained from 25 different species of monkeys. A 520 base pair sequence of the envelope gene of HTLV-1 or STLV-1 was analyzed in the supercomputer in collaboration with Dr. Steve O'Brien at the NCI in Frederick.

Dr. Franchini explained that mathematical analyses of the genetic relationships of the different viruses revealed that common chimpanzees have two different molecular variants of STLV-1. One of these variants is indistinguishable from the HTLV-1 from Zaire, while the other is closely related to the STLV-1 found in *Cercopithecus* monkeys, suggesting that more than one episode of transmission of the virus occurred. The transmission probably occurred from the *Cercopithecus* to the common chimpanzee, and, perhaps, from common chimpanzees to humans. Chimpanzees are the predators of *Cercopithecus* and are exposed to their blood when they kill them, and humans utilize these chimpanzees as a source of meat in Central Africa.

Dr. Franchini explained that her group searched for STLV-1 variants in other species. The pygmy chimpanzees (*Pan paniscus*) have been extensively studied because they are very close to humans, both genetically and in their social and sexual behaviors. Samples from pygmy chimpanzees were very difficult to obtain because it is an endangered species. Pygmy chimpanzees are kept and bred in captivity, in an effort to repopulate their natural habitat in Central Zaire. Pygmy chimpanzees live only between the Zaire River and the Lualala River, in an area populated by human pygmy tribes. The HTLV-1 serology found in the human pygmy tribes is indeterminant and very similar to the serology of the pygmy chimpanzee. The classical HTLV-2 serology on the commercial Western blot kit shows immunoreactivity with the p24 gag antigen of HTLV-1, the recombinant gp21, and the envelope polypeptide.

Dr. Franchini showed that the serology of the pygmy chimpanzee was not typical. The pedigree of the colony of pygmy chimpanzees shows that there is transmission from mother to progeny in approximately 50 percent of the offspring, suggesting that they might have a new variant of the virus. Human cord blood cells were used to isolate virus from peripheral blood cells obtained from pygmy chimpanzees. This virus is also capable of inducing cytopathic effects on human B cells. Partial sequences of the genome confirmed that the virus isolated from pygmy chimpanzees is related to HTLV-1 and HTLV-2 and to the bovine leukemia virus. The virus isolated from pygmy chimpanzees was provisionally designated as STLV-pan-p, in reference to the species of origin; however, the phylogenetic analysis showed that it is more closely related to HTLV-2. The reagents generated from STLV-pan-p can be used to produce kits that will help in the search of a similar virus that might infect humans.

Dr. Franchini stated that indeterminant serological reactivity against HTLV-1 and HTLV-2 has been found in a variety of neurological and other hematological human diseases, but these serological findings have never been confirmed by PCR or virus isolation.

Dr. Franchini concluded her presentation with a comment on the attempts to develop a vaccine against HTLV. She stated that HTLV-1 can provide a good animal model to develop a vaccine against not only SIV but also HIV. Dr. Franchini explained that a good vaccine against retroviruses has not been developed, with the possible exception of a vaccine for the feline leukemia virus. Dr. Franchini indicated that her group is using two different vectors, vaccinia virus and canary pox virus, both of which can infect humans as well as monkeys and do not produce viral progeny. Experiments performed in rabbits showed protection against cell-associated virus (5×10^4 cells) in animals vaccinated with the HTLV envelope. This protection was only observed in the absence of a booster immunization with the viral envelope. Dr. Franchini stated that it is possible that an HTLV-1 vaccine might never be useful in the United States, but will probably be important in other countries.

XII. BREAST CANCER: EMERGENT ETIOLOGIC HYPOTHESES— DR. LOUISE BRINTON

Dr. Brinton, Chief of the Environmental Studies Section of the Environmental Epidemiology Branch, began by explaining that researchers have faced many challenges in trying to determine the etiology of breast cancer, including its multifactorial nature. In addition, those factors that have been identified account for only a small percentage of the related cancer burden and do not suggest clear intervention strategies. She added that more recent research has resulted in promising information regarding etiologic agents and biochemical markers.

Dr. Brinton provided members with a brief statistical overview of the scope of breast cancer, noting that one in nine women will develop breast cancer and that some 44,000 deaths from breast cancer occur annually. She pointed out that since the early 1970s incidence rates have been rising by 1 or 2 percent each year; however, during the last decade this has increased to 3 or 4 percent. This increased incidence has primarily involved early-stage lesions, suggesting that the rise may be partly attributable to improved screening efforts. Dr. Brinton commented that as the increase in incidence is occurring among both older as well as younger women, who are less likely to be screened, it is probable that other factors are involved in the increase as well.

Recent trends toward having children later in life or not having them at all are contributing to the increase in breast cancer. Dr. Brinton said data show that women who have their first child after age 34 have a fivefold excess risk for breast cancer over those who give birth before age 18; however, research has revealed that delayed childbearing and intensive screening do not fully account for the increase in incidence. Dr. Brinton said that, as a result, recent efforts have begun to focus on a number of other hypothesized etiologic agents, including oral contraceptives, menopausal hormones, dietary factors, racial effects and environmental agents.

Dr. Brinton explained that since oral contraceptives were first issued to women in the 1960s, the initial users are just now reaching the age when breast cancer most commonly develops. Recent studies, primarily among women under age 45, correlate long-term use of oral contraceptives, as well as use at an early age, with an elevated risk for breast cancer. Dr. Brinton indicated that these results are not conclusive, as concerns have been raised about the role of bias and confounding factors. In addition, as most of the studies have targeted women under 45, it is not clear whether oral contraceptives are a true causal factor or merely act to advance the development of disease.

The Environmental Epidemiology Branch recently completed a large case-control study to elucidate the role of oral contraceptives in breast cancer. Dr. Brinton pointed out that the study's design included many unique components, such as: 1) covering three separate geographic areas; 2) involving participants up to age 54; 3) utilizing two control groups; 4) employing a process for validating exposure data; 5) conducting direct measurement of body fat distribution; and 6) utilizing biologic markers. She stated that the study concluded that among women under age 45, an excess risk of 1.3, or 30 percent, was found for those who reported ever using oral contraceptives. Among women who were diagnosed with breast cancer before age 35, there was a 70 percent excess risk for the disease associated with oral

contraception; this risk increased with length of use, with a twofold excess risk correlated with 10 years or more. Dr. Brinton noted that the study found no decreased risk among older participants, which would have been expected if oral contraception only advanced the diagnosis of breast cancer. In addition, observed relationships with tumor characteristics and screening history supported the notion of a biologic explanation for the correlation between excess breast cancer risk and oral contraception. Dr. Brinton added that breast cancer research is therefore focusing on potential explanations for increased susceptibility to early onset breast cancer among women who have used oral contraception, including determining the effects of specific types of oral contraception and their correlation with tumor characteristics.

Dr. Brinton related that menopausal estrogens, which are primarily used to mitigate symptoms of menopause, have been commonly used in the United States since the 1960s and that, in 1990, estrogen premarin became the fourth most commonly used drug in the nation. Recent trends are showing increased duration of estrogen use because of findings that its prolonged use reduces the risk of cardiovascular disease and osteoporosis. Dr. Brinton pointed out that concerns regarding this trend focus on the effects of long-term exposure to estrogen, which studies have revealed confer an elevated risk of breast cancer. Increasing risk with increasing duration of estrogen use was found in one study in which women who took estrogen for 20 years or more had a 50 percent higher risk than nonusers.

It is not yet clear whether progestin acts to offset the negative side effects of estrogen use. Dr. Brinton reported that there is some evidence suggesting that progestin may in fact induce breast cancer, despite its protective effect against endometrial cancer. This possibility has recently begun to be explored in the United States. A study comparing the effects of the drugs revealed that early-stage tumors in particular are affected by both forms of hormone therapy, with more than double the excess risk attributed to combined estrogen and progestin therapy. Dr. Brinton noted that this study is being extended to determine whether the association is a result of biological influences or increased screening efforts among hormone users. She emphasized the importance of such studies in helping women to make informed risk versus benefit decisions regarding use of hormone therapy. She also introduced other potential iatrogenic agents for breast cancer, including diethyl stilbestrol, infertility medications, and silicone gel breast implants, and indicated that three large studies have been initiated to explore their health effects.

Dr. Brinton turned to a discussion of the role of dietary factors in breast cancer development, during which she indicated that researchers have found it difficult to elucidate many of these associations. Epidemiologic studies have provided fairly conclusive evidence that higher levels of alcohol consumption can lead to an increased risk for breast cancer; for example, one study revealed a 70 percent excess risk among women consuming two or more drinks per day. Dr. Brinton said that research to identify the biologic mechanism involved in this association has been initiated.

Dr. Brinton informed members that despite the preponderance of descriptive evidence implicating dietary fat in breast cancer development, analytic research has not supported this conclusion. This phenomenon is potentially a result of the following factors: 1) lack of diversity in the American diet, which makes it difficult to assess risk associated with a wide range of fat intakes; 2) accurate dietary information is extremely difficult to obtain; 3) the role of childhood and adolescent diet has been underemphasized; and 4) the potential for risk to be

influenced not by one dietary factor, such as fat intake, but a complex interaction of consumption of nutrients. Dr. Brinton described a recent study designed to overcome these methodologic challenges by comparing breast cancer risk among Asian American women. The study revealed strong correlations between excess risk and migration patterns. A sixfold increase in risk was found among women whose families had migrated to the West at least three generations earlier, compared with those women who had recently come to the West. She indicated that various analyses are under way to determine what environmental factors may contribute to this increase. The study also revealed that among women in their 50s, a weight gain of 11 pounds or more during the previous 10 years doubled their risk of breast cancer, while weight loss appeared to be associated with a reduction in risk. Dr. Brinton noted that it appears that relative weight as an adult may have a significant and rapid impact on breast cancer risk. This study, as well as the study among younger women, also include components to better assess: 1) childhood and adolescent diet; 2) risk associated with intake of numerous nutrients; 3) the role of body fat in breast cancer; and 4) the protective effects of physical activity.

Dr. Brinton provided an overview of the differences in racial and geographic trends in breast cancer incidence. White women are more likely to develop breast cancer than African American women, except among those under age 40. There are large geographic variations in mortality as well, with extremely high rates in the Northeast. The reasons for both of these variations are not clear and, therefore, have become the focus of recent study.

A number of environmental agents are hypothesized contributors to geographic variations in breast cancer; however, the biologic evidence to support their role is limited. As a result, several studies have been initiated to examine the etiologic role of these agents, including the Long Island Breast Cancer Project. Dr. Brinton pointed out another study that is examining the attributable role of factors such as delayed childbearing and high socioeconomic status in geographic variations of mortality. She added that a telephone survey is currently being conducted in different areas to evaluate differences in the prevalence of both established and hypothesized environmental risk factors. The findings of this survey will be used to guide future research efforts regarding environmental carcinogenesis. One environmental agent targeted for study is organohalides, which some epidemiologic studies have shown to be associated with elevations in breast cancer risk.

Dr. Brinton informed members that most of her group's studies attempt to integrate epidemiological information and data on biomarkers of breast cancer. She added that genetic advances, such as the identification of the *p53* tumor suppresser gene, will facilitate the process of identifying high-risk populations. Future directions for research regarding etiologic mechanisms of breast cancer will include: 1) identifying hormonal changes that accompany delayed childbearing and increase risk of breast cancer; and 2) examining biochemical changes surrounding dietary patterns and exploring their impact on risk.

Questions and Answers

Dr. Bishop asked Dr. Brinton whether the term "epidemic" is an appropriate descriptor of breast cancer incidence. Dr. Brinton indicated that the steadily elevated incidence combined with the large increase experienced during the last decade qualify the disease as an epidemic. Dr. Bishop asked whether the factors responsible for the baseline incidence are a complete

enigma or are simply some combination of previously identified risk factors. Dr. Brinton replied that about 50 percent of the incidence is explainable by known risk factors and 50 percent has unknown causes.

Dr. Greenwald clarified that an epidemiologist defines an epidemic as a substantially elevated incidence. He stated that the international baseline is much lower than the incidence reported in the United States alone. The United States had approximately 46,000 breast cancer-related deaths this year; if the nation had experienced the same rate as Japan, this figure would have been approximately 11,000 deaths. He added that data showing that Asians who live in the United States for a few generations come to experience the same breast cancer rates as American women indicate that there is not a simple genetic baseline in operation. Dr. Greenwald asserted that the baseline would be about one-sixth of the actual incidence, which qualifies the situation as an epidemic. Dr. Bishop asked how much of the increase is a product of early detection efforts. Dr. Greenwald responded that there was a steady 1 percent increase in incidence for 20 years, followed by a 3 percent increase for the last 10 years. This elevated risk of the past 10 years is potentially a result of better screening efforts, but there is at least a 30 percent increase in true incidence over the past 30 years.

Dr. Salmon asked whether research regarding risk factors that may contribute to geographic patterns of breast cancer incidence are being explored. Dr. Brinton responded by mentioning a current phone survey to assess the prevalence of various risk factors according to geographic region. Dr. Broder emphasized the priority attributed to breast cancer research at NCI. He acknowledged that mortality rates due to the cancer are unacceptably high. He commented, however, that because the increased incidence is not being accompanied by a commensurate rise in mortality, and in light of the fact that no definitive advances in treatment have been made, the rise is probably due to diagnostic improvements. Dr. Broder suggested that any person who uses the term "epidemic" should state the meaning they ascribe to the term. He added that the large geographic variations in breast cancer incidence provide hope for treatment in that they suggest that breast cancer is not biologically unavoidable but, more accurately, a result of external factors that may be intervened with. Dr. Broder also recommended that migratory studies focus on the loss of protective dietary behaviors as well as the adoption of negative eating habits.

Dr. Sondik asked for Dr. Brinton's conclusions regarding the unique breast cancer incidence curve in Japan, which steadily rises until women reach age 50, at which point it flattens. Dr. Brinton responded that this curve was one of the reasons for launching their study among Asian American women. In response to Dr. Sondik's question regarding the flattening of the mortality curve in the United States, she stated that this effect is primarily a result of the increase in early-stage tumors, which are treatable.

Dr. Bishop clarified that within the field of microbiology, "epidemic" specifically indicates a dramatic increase in incidence that is confined in time and space. He warned that the term has very dramatic connotations for the public and that it should be used with caution. Dr. Day indicated that within chronic disease epidemiology, the definition is different because, typically, there are no sudden or dramatic increases and decreases in incidence. Dr. Greenwald commented that what is being argued are varying timeframes. Dr. Rimer pointed out that Dr. Greenwald may be adding a new dimension to the definition by discussing an international baseline.

Dr. Chabner advised members that they were disallowing the role of adjuvant therapy within the decrease in mortality. He commented that until it has been determined whether the increase over the last decade is a result of better diagnostics, the term "epidemic" should not be used. In response to Dr. Rimer, Dr. Brinton indicated that the most profound effects of weight gain have been found among older women. Dr. Rimer asked how resources and research efforts are allocated between Dr. Brinton's branch and DCPC's surveillance branch. Dr. Brinton responded that the two branches are extremely collaborative; however, her branch tends to be more analytically focused. Dr. Greenwald added that DCPC's surveillance branch has been primarily focused on developing cancer interventions and processes for facilitating the identification of biomarkers.

Dr. Calabresi asked whether a decrease in the growth of the breast cancer incidence rate is occurring, as would be expected with the detection of more cases of breast cancer. Dr. Sondik indicated that a flattening is beginning to become apparent. Dr. Sondik supported Dr. Chabner's advice and commented that while the incidence of breast cancer has definitely increased during the last 10 years, it is important to remember that it started out high. He added that "epidemic" has connotations of increases that are out of control. He added that the more recent elevated rise in incidence is probably primarily a result of changes in health care delivery, which has begun to focus on screening efforts.

Dr. Salmon asked whether there are data that correlate geographic variations in incidence with frequency of mammography screening in those areas. Dr. Sondik indicated that the geographic variations precede the popular use of mammography. Dr. Day concluded the discussion by conveying his concerns about the conclusions the public may draw from the preceding debate. He suggested that these issues be clarified and then redressed at the next meeting, primarily to allay any of the public's concerns.

XIII. MELANOMA: WHO GETS IT AND WHY?—DR. MARGARET TUCKER

Dr. Tucker, Chief of the Genetic Epidemiology Branch, began by presenting melanoma incidence data, which were compiled primarily through the use of the Surveillance, Epidemiology and End Results (SEER) Program. She indicated that melanoma has one of the most rapidly increasing incidence curves, as well as being one of the five most common cancers among young adults. Dr. Tucker commented that mortality is not rising as quickly as incidence, primarily because of increasing survival time. Survival rates vary by stage and are higher among individuals diagnosed with thinner tumors, which have almost no chance of metastasizing and confer a 99 percent survival rate. Dr. Tucker asserted that since most melanomas are visible, virtually no one should die from this form of cancer. She explained that a simple surgical procedure eradicates early melanomas and leaves minimal scarring.

Dr. Tucker informed members that a study conducted in Sydney, Australia, revealed that before 1960 only 10 percent of the melanoma lesions diagnosed were early stage (thin), while by the late 1980s this had increased to nearly 50 percent. Conversely, the proportion of thick lesions being diagnosed, associated with lower survival rates, has decreased from 25 to 10 percent. She attributed the large rise in detection of early-stage tumors to increased public awareness about the warning signs of the disease.

Among White men, Dr. Tucker continued, there are much higher incidence rates of melanoma in the southern and western regions of the United States. She added that while the elevations have been hypothesized to be attributable to a sun-related element, the mechanism through which this factor acts is unclear. In contrast to nonmelanoma skin cancers, melanomas do not occur in the more commonly sun-exposed areas, but are more likely to develop on the torso in males and the legs in females. Dr. Tucker pointed out that case-control studies have associated intense intermittent exposures resulting in sunburn with melanoma development. She stated that changes in sunbathing behaviors and clothing styles probably contribute to increased risk of developing melanomas over time.

Dr. Tucker summarized the family studies of melanoma, which began in 1976. An advantage of family studies, she stated, is that the participants have similar genetic material as well as environmental exposures. By using multidisciplinary approaches, hypotheses about disease etiology can be generated from family data and then tested among the general population. Dr. Tucker explained that the study of melanoma-prone families requires: 1) clinical examinations to count and classify all nevi, as well as to document other host characteristics such as hair, skin, and eye color; 2) questionnaire completion regarding known and suspected environmental risk factors, including sun exposure and hormone use; 3) collection of biological specimens to allow functional assays and DNA isolation for genetic studies; and 4) pathological review of all pigmented lesions. She reported that over 700 individuals from 26 different families have participated in this study, and that 17 new melanomas were diagnosed during the participants' first visit. She commented that many of the lesions of family members diagnosed prior to this study were intermediate and thick lesions, but melanomas diagnosed during the study have been very thin.

When Drs. Green and Clark examined the first families in 1976, they discovered that some had very unusual nevi, now termed "dysplastic nevi." Physical characteristics of dysplastic nevi include large size, flat surface, variable pigmentation, an irregular outline, and indistinct borders. They occur on both sun-exposed and protected areas. Dr. Tucker emphasized that lesion characteristics vary, even within the same person. She stated that these family studies have established dysplastic nevi as precursors of melanoma and allowed characterization of the lesions' natural history.

Dr. Tucker pointed out that participants with dysplastic nevi are 80 times more likely to develop melanoma than individuals in the general population, and those participants under age 20 who have these nevi are 1,000 times more likely to develop melanoma. The excess risks are reduced by half after 5 years of adherence to skin care guidelines and initiation of protective behaviors, including complete blockage of sunburn through sunscreen use, the use of protective clothing, and avoidance of midday sun exposure. In addition, family members with dysplastic nevi are advised to examine their skin once a month by comparing their nevi to clinical photographs. Dr. Tucker explained that the frequency with which participants are seen by health care professionals is determined by the lesional activity of their nevi. If the nevi are rapidly changing, then the participant may be examined by a doctor every 3 months; otherwise, they are seen every 6 to 12 months. She added that a special focus is placed on those participants experiencing hormonal changes, particularly during puberty and pregnancy.

Dr. Tucker reported that melanoma susceptibility appears to be a genetic trait within the families being studied. Linkage analyses have revealed at least two loci which appear to be

important in melanoma susceptibility 1p36 and 9p21. Dr. Tucker noted that additional melanoma susceptibility loci are likely and that this area is being actively studied.

A candidate gene, p16, has been located on chromosome 9, Dr. Tucker reported. In collaboration with Dr. Nick Dracopoli from NCHGR, mutations in this gene have been evaluated among 38 melanoma-prone families. Of the 11 germ line mutations that have been evidenced in 19 of the families, seven are potentially disease-related. Dr. Tucker said that only families with linkage to chromosome 9 are showing mutations in p16.

Dr. Tucker highlighted the results of a population-based case-control study of melanoma, which was completed in conjunction with a group of investigators from Denmark. Consistent with the findings of other studies, fair skin and red or blonde hair were found to confer twofold excess risk. She added that freckles act as a measure of sun exposure as well as host susceptibility, and were associated with a threefold risk. Other factors associated with excess risk for melanoma include: 1) sunbathing (twofold risk); 2) sunburns, particularly at an early age (threefold risk); and 3) an elevated number of nevi (fivefold risk).

The methodologies used to count nevi are subject to significant error, Dr. Tucker continued, and few studies have attempted to quantify dysplastic versus common nevi. In addition, previous research has evidenced contradictory results regarding the risk associated with various types of nevi. Due to these shortcomings, a large case-control study was launched in collaboration with the University of California at San Francisco and the University of Pennsylvania to evaluate the roles of both dysplastic and common nevi in melanoma development. Large numbers of participants were recruited to allow evaluation of the interaction between numerous exposure variables and various types of nevi, as well as to explore the association between hormonal exposures and nevi in melanoma risk.

The study included 716 participants newly diagnosed with melanoma and 1,014 control participants some of whom had never been previously diagnosed with melanoma. Components of the study included: 1) full-skin examination to record the number and type of nevi, the extent of freckling, solar damage, number of excision scars, and skin, hair, and eye color; 2) completion of a questionnaire exploring sun exposure, family history, medical history, and hormonal exposure; 3) an interview; 4) photographic recording of skin; and 5) an optional nevus biopsy. Preliminary results indicate that: 1) an increased number of common nevi doubles the risk of melanoma; 2) half of the melanoma patients had dysplastic nevi; 3) multiple dysplastic nevi confer a 10-fold excess risk of melanoma; 4) freckles are correlated with a threefold excess risk of melanoma; and 5) individuals with both dysplastic nevi and freckles have more than a 20-fold increased risk of melanoma.

Dr. Tucker summarized the data she presented by indicating that people with light hair, freckles, many common nevi, or multiple sunburns at an early age have approximately two to three times the risk of developing melanoma compared to people without these risk factors. She said that half of all melanomas occur in individuals with dysplastic nevi, who comprise about 5 to 10 percent of the population. Adherence to protective measures can halve excess risk for those at highest risk. Furthermore, early detection of lesions leads to diagnosis of thinner tumors, which have almost no chance of metastasizing. Dr. Tucker concluded by suggesting that the high-risk groups that have been identified who would benefit from education and screening interventions.

Questions and Answers

Dr. Becker asked whether family members who are prone to dysplastic nevi typically display melanoma in sun-shielded areas of their bodies. Dr. Tucker replied that while dysplastic nevi occur more commonly in sun-exposed areas, these nevi also occur in areas not exposed to the sun. In terms of melanoma incidence, distribution in these families is virtually identical to the general population, with the majority of melanomas appearing in heavily sun-exposed areas on the trunk and back. Dr. Tucker continued by noting that although these families may be alerted not to expose themselves to sunlight, for many, a substantial proportion of the damaging sun exposure has already been experienced (i.e., prior to age 20).

Dr. Becker asked if it is correct to conclude that exposure to ultraviolet (UV) radiation promotes transformation from dysplastic nevi to melanoma. Dr. Tucker explained that the sun's role in developing melanoma is more complex than this statement suggests. At first, sun exposure was believed to only induce development of nevi; however, researchers now hypothesize that the sun also has a late promotional effect. Dr. Becker asked whether dysplastic nevoid syndrome has been observed in African Americans. Dr. Tucker responded that the only case she has seen is a child of a White and African American couple who has an increased number of nevi; however, it is too early to determine whether the nevi will become dysplastic.

Dr. Bishop asked whether lesions that occur after sun-protective measures have been initiated still provide molecular evidence of UV damage. Dr. Tucker replied that it is extremely difficult to acquire this evidence because of the infrequency and small size of these lesions—the entire lesion is typically required for pathological analysis of prognosis.

XIV. MOLECULAR EPIDEMIOLOGY: THE *p53* TUMOR SUPPRESSOR GENE—DR. CURTIS HARRIS

Dr. Rice introduced Dr. Harris as the Chief of the Laboratory of Human Carcinogenesis in the Chemical and Physical Carcinogenesis Program. Dr. Harris, Dr. Rice stated, has pioneered in an area of study that combines laboratory and epidemiologic studies and coined the term "molecular epidemiology."

Dr. Harris began his presentation by explaining how he decided to work at the NCI. He said that when he started his clinical training he developed a research strategy that would use *in vitro* and *in vivo* studies to test hypotheses generated in the clinic or in epidemiology. These *in vitro* and *in vivo* studies would investigate the activation and detoxification of carcinogens and the functional studies of activated proto-oncogenes and tumor suppressor genes, and ensure the mechanistic investigations. Dr. Harris said that the place to pursue such investigations was the NCI.

Dr. Harris remarked that classical epidemiology has been successful in identifying high-risk populations and risk factors. The goal of molecular epidemiology, he stated, is to identify individuals in these high-risk populations who are at the highest risk. This strategy has two facets: 1) molecular dosimetry or carcinogen exposure; and 2) increased susceptibility due to inherited and acquired host factors. The molecular dosimetry aspect, he explained,

involves carcinogen macromolecular adducts, cytogenetic endpoints, and mutational spectrum and frequency of cancer-related genes. Inherited cancer predisposition includes genetic polymorphisms of enzymes involved in activation and detoxification of carcinogens, genomic instability in DNA repair conditions, and germ line mutations in tumor suppressor genes.

Dr. Harris then focused his presentation on *p53*, which is a transcription factor that is involved in replication, programmed cell death, and DNA repair. *p53* was identified as a cellular protein in 1979 and was cloned in the early 1980s from mouse and human tumors. In 1989 it was discovered that researchers were investigating not the normal or wild type gene but mutant forms of the gene. When the wild type gene was inserted into cancer cells with the endogenous defect, the wild type gene was shown to be a tumor suppressor gene. At that time the *p53* gene was discovered to be a recessive tumor suppressor gene in colon cancer and this was later shown to be true for other cancers.

The functional data on *p53*, Dr. Harris continued, involve the DNA response pathway once there are single- or double-strand breaks that are accumulated by the *p53* protein. This, he said, transactivates certain genes, including p21, cyclin-G, and GADD-45. p21 is a cyclin-dependent kinase inhibitor and inhibits enzymes, causing cell cycle arrest. He added that *p53* can also modulate the expression of genes called BAX and BCL-2 that are involved in apoptosis. *p53* also interacts with transcription repair and replication factors to modulate their function. Dr. Harris reported that his group has recently shown the *p53* protein to form a complex with XPB and XPD, which are involved in transcription coupled repair. The formation of this complex can lead to cell cycle arrest and modulation of DNA repair capacity, and is a pathway for apoptosis.

Dr. Harris then posed some more practical questions, including: Does the somatic mutational spectrum of the *p53* gene reflect the exposure to environmental carcinogens? Is the *p53* mutation early or late in carcinogenesis? and Do *p53* mutations or elevated levels of protein correlate with survival and treatment response?

In answer to the first question, Dr. Harris said the rationale for investigating the *p53* mutational spectrum is that it is mutated in approximately half of human cancers, and its size is reasonable to work with in the laboratory. The main value, he said, of these types of studies, is to generate new hypotheses, such as: Do different mutant forms of the protein have different biological forms of activity? and Does the cellular context influence the selection of certain mutants? He added that studies have revealed evidence to support both these hypotheses.

Dr. Harris commented that *p53* is different from other tumor suppressor genes in that it incurs missense mutations, whereas other tumor suppressor genes incur frame shift mutations. Missense mutations still make proteins, albeit abnormal, while frameshift mutations cause a protein to be truncated or not to be made at all. Missense mutants of *p53* lose suppressor activity and gain oncogene activity.

A schematic representation of *p53* was shown by Dr. Harris. It has 393 amino acids and the n-terminal region is involved in transactivation while the carboxy-terminal region is involved in oligomerization of the protein. The central part of the gene product is involved in binding the consensus sequence in DNA. Mutations are nonrandom and there are hot spots at codons 175, 245, 248, 249, 273, and 282—the majority of them arginines. These mutational

hot spots, such as 248, which encodes for arginine, are important in the interface between the protein and the DNA double helix. The 248 arginine fits into the minor groove of the double helix. The other hot spots, Dr. Harris reported, are either directly involved in the interface or at the scaffolding maintaining the interface.

Dr. Harris said that the mutational spectrum of lung cancer versus colon cancer is quite different. The majority of mutations found in lung cancer are G to T transversions, while the majority of mutations found in colon cancer are C to T transitions at CpG sites. These CpG sites may be a source of endogenous mutagenesis, because approximately 3 percent of the human genome is 5-methyl-C and most of the 5-methyl-Cs occur at CpG sites. The 5-methyl-C would spontaneously deaminate, losing its amino group to change from a C to T. If there is insufficient GT glycosylase for repair, this will lead to a C to T transition at CpG sites. Hydroxy radicals will also enhance the rate of deamination.

Dr. Harris then discussed the association between environmental or lifestyle causes of cancer and changes in the *p53* gene. He and his colleagues are using the footprint of the *p53* mutation and attempting to link that to environmental causes of cancers, including aflatoxin in hepatocellular carcinomas, sunlight in skin cancers, cigarette smoke and radon in lung cancers, and vinyl chloride in hepatic angiosarcomas.

Dr. Harris pointed out that one of the most striking associations was seen in liver cancer. In a study done in Quidong, China, which has a high incidence of liver cancer, the vast majority of mutations were found to be transversions from G to T at a particular codon. Aflatoxin and hepatitis viruses were known to be risk factors. When liver cancers in other parts of the world (where aflatoxin was not a problem) were studied, the mutational spectrum was quite different. There is a positive correlation between the estimated amount of aflatoxin intake and the percentage of *p53* mutations at that site.

Other studies, Dr. Harris reported, show not only an association between dietary exposure and *p53* mutations at codon 249, but that it can be an early event in liver carcinogenesis. Also, mechanistic studies related to hepatitis-B virus have shown that the X protein of hepatitis-B virus complexes with the *p53* protein and inhibits the sequence-specific binding and its transcriptional activity. Recently, it has been shown that the X protein will inhibit *p53*-dependent apoptosis.

Dr. Harris stated that they were interested in comparing the mutational spectrum of *p53* in breast cancers from Caucasian Americans with African Americans, and Japanese and Chinese populations. He noted there are some interesting differences, including: a higher frequency of G to T transversions in Caucasians and a preponderance of C to T transitions at the CpG dinucleotides in African Americans. This information, he said, is used to generate hypotheses. One such hypothesis Dr. Harris mentioned is that the *p53* mutational spectrum in breast cancer reflects the exposure to chemical carcinogens found in the diet and in tobacco smoke, and the influence of cancer susceptibility genes. A number of breast cancer case-control studies are testing this hypothesis.

Data from one ongoing study suggest that there is a relationship between cigarette smoking and breast cancer in certain individuals. Dr. Harris displayed a graph depicting individuals who are rapid acetylators (have normal N-acetyl transferase) and slow acetylators.

The graph showed an increase in the odds ratio among cigarette smokers who are slow acetylators. He noted that about half of Caucasian women, one-third of African American women, and about 10 percent of Japanese women are slow acetylators. These studies were all done in postmenopausal women, and premenopausal women are now being studied.

Dr. Harris said his group is also studying whether there are gender differences in lung cancer risk. Current studies suggest that women are at an increased risk for cancer per amount of smoking compared with men. These studies show that women have an increased frequency of carcinogen-DNA adducts in their nontumorous lung for an equivalent amount of cigarette smoking compared with men. Recent research has also shown that women have a high frequency of GST M1 null genotype, which is involved in detoxification, if they have lung cancer.

In conclusion, Dr. Harris stated that in the past, cancer risk assessment has traditionally been driven by cancer epidemiology and laboratory animal studies, but it is now time to add molecular epidemiology to the paradigm. He added that he believes the susceptibility genes will be determined to play a very important role in who gets cancer and who does not. The distribution of these genes needs further elucidation, as well as their function in human populations.

Questions and Answers

Dr. Broder asked whether Dr. Harris believes that uranium miners are inhaling a form of aflatoxin. Dr. Harris responded that he does not think that is the case, because dock workers in the Netherlands exposed to high levels of inhaled aflatoxin did not have a high frequency of 249 serine mutations. However, it might be due to hydroxyl radicals, which also cause G to T transversions. Dr. Harris added that his group is trying to reproduce the study in the laboratory by exposing cultured human bronchial epithelial cells to alpha particles in a manner to determine whether there are changes in the *p53* mutations that occur.

Dr. Chabner asked if there are specific mutations of *p53* that are associated experimentally with GST inactivation. Dr. Harris said that in that situation one would observe mutations caused by bulky, electrophilic carcinogens. He added that there is some specificity, but it is more toward class and type of carcinogen than a specific carcinogen.

XV. FOOD-DERIVED HETEROCYCLIC AMINE MUTAGENS— DR. ELIZABETH SNYDERWINE

Dr. Snyderwine, acting head of the Chemical Carcinogenesis Section of the Laboratory of Experimental Carcinogenesis, began her presentation by noting that there is a general consensus among epidemiologists that environmental factors play an important role in the incidence of human cancers. Many of these environmental factors extend from lifestyle habits such as smoking, exposure to sunlight, and alcohol consumption.

Dr. Snyderwine noted that Doll and Peto have estimated that of the environmentally related cancers, tobacco contributes 30 percent, radiation exposure (from UV light and x-rays) contributes 10 percent, and occupational exposures contribute from 1 to 10 percent. The single

most important factor, Dr. Snyderwine said is diet, which has been estimated to be responsible for 30 to 60 percent of environmentally related cancers.

Breast cancer, Dr. Snyderwine stated, is one example of a cancer that appears to be associated with lifestyle factors. This conclusion is based on studies that show a wide difference in breast cancer incidence rates in different countries, as well as studies of the offspring of migrants who have acquired the same incidence rates of their host country.

Some of the largest differences in incidence rates, Dr. Snyderwine reported, are between the United States and Japan, which has a fourfold lower breast cancer incidence rate. Distinct dietary differences exist between the two countries, with the United States consuming more calories and a higher percentage of calories from dietary fat. Thirty to forty percent of total calories are from dietary fat in a typical American diet, a diet which is also rich in cooked meat. In contrast, the traditional Japanese diet is rich in carbohydrates and contains just 15 percent of calories from the consumption of dietary fat. Dr. Snyderwine added that in urban areas of Japan, an increase in risk and incidence of breast cancer is being seen, and this appears to be due to a Westernization of their diet.

Dr. Snyderwine remarked that the way diet and nutrition influence carcinogenesis is complex because a variety of components of the diet can affect steps in the carcinogenic process. For instance, diet can be the source of the carcinogen, and dietary fiber can affect the uptake of the carcinogen, and, therefore, affect the internal dose. Dr. Snyderwine added that cruciferous vegetables can affect the metabolic activation of the carcinogen and, therefore, the subsequent biologically effective dose. Also, dietary fat may influence the subsequent promotion and progression of the initiated cells to the neoplastic cancer.

Dr. Snyderwine said there are two types of dietary components that influence carcinogenesis: macronutrients and micronutrients. Macronutrients include total calories, fats, protein, fiber, and alcohol. Micronutrients include vitamins, minerals, food additives, and food contaminants, such as DDT. Mutagens and carcinogens such as aflatoxin and ethyl carbamate can also occur naturally in food, and cooking food can introduce additional mutagens and carcinogens. Dr. Snyderwine said that she would focus the rest of her presentation on food-derived heterocyclic amines, which are formed in food during the cooking process.

According to Dr. Snyderwine, heterocyclic amines comprise a family of approximately 20 different compounds that have been purified from a variety of proteins, pyrolized amino acids, and cooked meats. The five most prevalent heterocyclic amines in the human diet are PhIP, a(α)c, 8-MeIQX, 4,8- diMeIQX, and IQ. They all possess a structural similarity with multiple aromatic rings and the presence of an exocyclic amino group (the critical feature with regard to metabolic activation, genotoxicity, and carcinogenicity).

Dr. Snyderwine said that when meats are cooked by frying, broiling, baking, and barbecuing at ordinary cooking temperatures, heterocyclic amines are formed. When hamburger is cooked well-done, it becomes mutagenic because of the formation of the heterocyclic amines from the precursors found in the meat. Studies have shown the major mutagen in cooked meats to be PhIP, which contributes 80 percent by mass of the mutagenic heterocyclic amines. The quinoxalines contribute another 15 percent, while TMIP contributes approximately 5 percent. All of these compounds are formed from precursors in the muscle

meat, specifically creatine and amino acids. The reaction necessary to form these compounds occurs at temperatures achieved during cooking.

Dr. Snyderwine explained that creatine is responsible for the formation of the amino imidazole portion of the PhIP and phenylalanine contributes to the phenyl and the pyridine parts of the PhIP. The longer the cooking time and the higher the cooking temperature, the more the reaction occurs and the more heterocyclic amines are formed. Heterocyclic amines are found in beef, chicken, fish, and pork, with the highest levels of heterocyclic amines found in barbecued foods, because of the high temperatures achieved on the grill.

Dr. Snyderwine said that daily exposure to heterocyclic amines is estimated to range between 1 and 20 micrograms per person among consumers of cooked meat. A lifetime dose is approximately 2 milligrams per kilogram of heterocyclic amine. All heterocyclic amines that have been tested have been shown to be carcinogenic in rodents, she added.

Dr. Snyderwine reported that studies conducted in Tokyo have shown that when IQ is given in the diet of mice or rats at .03 percent for 1 year, it induces hepatocellular carcinoma. IQ induces multiple primary tumors in both species and, in the rat, adenocarcinoma of the small intestine and colon as well as squamous cell carcinomas of the zymbals gland, clitoral gland, and skin. Studies done at NCI have shown that IQ is also a potent hepatocarcinogen in cynomologous monkeys when given by gavage at 10 to 20 milligrams per kilogram per day. In contrast, PhIP has not been shown to be a hepatocarcinogen, but does induce lymphoma in mice when given in the diet. In the rat, dietary PhIP induces adenocarcinoma of the mammary gland and colon in female rats, and colon in male rats. Dr. Snyderwine noted that these are two sites that might be associated with a carcinogen prevalent in the Western diet.

Dr. Snyderwine remarked that her group's studies have shown that heterocyclic amines are procarcinogens that require metabolic activation for genotoxicity. If not detoxified, this metabolic activation to reactive electrophiles can lead to the formation of DNA adducts, which, upon replication, can lead to mutations and an initiated cell and then, under promotion and progression factors, can lead to neoplasia.

Dr. Snyderwine then described metabolic activation, which occurs via a two-step process. The first step is the cytochrome P450-mediated N-hydroxylation of PhIP to the N-hydroxy-PhIP and the second step is the esterfication by O-acetyltransferase to the N-acetoxy-PhIP. The N-acetoxy-PhIP is directly reactive. The ester group can be removed, forming a guanine adduct in DNA; the predominant adduct is N^2-(deoxyguanosine-8-yl)-PhIP. Studies have shown that the guanine adducts of PhIP cause G to T transversion mutations and, to a lesser extent, G to A transition mutations in certain vector systems. Collaborative studies investigating PhIP-induced mutations in the endogenous dihydrofolate reductase gene of Chinese hamster ovary cells have further supported these claims.

Dr. Snyderwine then presented data on studies of the mammary gland carcinogenicity of PhIP. She and her colleagues wanted to address three questions: 1) Does PhIP form DNA adducts in the mammary epithelium, which is considered to be the target for mammary gland carcinogenesis? 2) How are the PhIP-DNA adducts generated in the mammary gland, i.e., what enzymatic pathways account for the formation of these adducts? 3) Can an animal model be established in which to evaluate the human condition and to determine whether dietary

promotion factors influence the mammary carcinogenicity of this food-derived heterocyclic amine?

To answer the first question, Dr. Snyderwine said, they used the 32p-postlabeling method. Rats were dosed with PhIP and the mammary epithelial cells were analyzed for DNA adducts. Three guanine adducts were observed, with the major adduct being N^2-(deoxyguanosine-8-yl)-PhIP. The levels of adducts were quantitated and found to be the highest 1 day after the last dose. The adduct levels then declined within the first week, but then stayed constant from week 1 until week 6, suggesting an opportunity for mutagenesis and the initiation of carcinogenesis to occur. In looking at the metabolic activation capacity of mammary gland cells, a very low capacity for formation of the N-hydroxy PhIP was observed; however, the mammary gland had a 10-fold higher capacity than the liver to perform the second step to the N-acetoxy PhIP. Thus, a possible route of PhIP-DNA adduct formation in the mammary gland might be the metabolism of PhIP to N-hydroxy-PhIP by cytochrome P450 in the liver, with the subsequent transfer of N-hydroxy-PhIP to the mammary gland, where the N-acetoxy PhIP is formed *in situ*, leading to DNA adduct formation and the initiation of carcinogenesis at that site.

Dr. Snyderwine then discussed her group's attempts to find an animal model. She said that they based their work on a model described by Dr. Charles Huggins in 1959. Dr. Huggins showed that, in the Sprague-Dawley rat, between 35 and 55 days of age is the period in which the mammary gland undergoes its most rapid differentiation and proliferation. Histologically, she added, this is when the terminal end bud epithelium in the mammary gland is differentiating into the alveolar buds, making it highly susceptible to chemical carcinogens. Using this model, they administered doses of PhIP to rats between the ages of 35 and 55 days. The doses were then stopped and the animals put on either a low-fat or high-fat diet. The high-fat diet had a striking effect on the weight of the tumors and the incidence of palpable tumors induced by PhIP. The incidence of palpable tumors was 16 percent in the low-fat-diet rats and 55 percent in the high-fat-diet rats. A difference in the malignancy was also seen. In the low-fat-diet animals, all the tumors were histologically benign, while the animals on the high-fat diet had a high percentage of tumors that were histologically malignant showing, for example, infiltrating duct carcinoma.

In summarizing her findings, Dr. Snyderwine said that only benign lesions were found in animals treated with PhIP and put on a low-fat diet, while animals on the high-fat diet were found to have a high percentage of their tumors to be histologically malignant. She added that none of the control animals, neither those on the low-fat nor on the high-fat diet, developed any mammary tumors.

In conclusion, Dr. Snyderwine stated that she believes that PhIP, in combination with dietary fat, is necessary for the step-wise progression of normal breast epithelium to the precancerous state and then to malignant cancer. In their model, PhIP alone did not appear to cause the conversion of normal tissue to malignant cancer. She added that PhIP induces the formation of DNA adducts in the mammary gland. Given that O-acetyltransferase is a polymorphic enzyme with individuals displaying rapid, intermediate, and low rates of acetylation capacity, this raises the possibility that individuals who have a high acetylation rate and also consume a substantial amount of cooked meat might be at increased risk for developing breast cancer. Recent studies, she mentioned, have shown that there is a higher

risk of colon cancer among individuals who eat well-done meat and have a rapid acetylator phenotype. Also, a high-fat diet enhances the malignancy of PhIP-induced tumors, and promotional factors in the diet appear to substantially influence the mammary carcinogenicity of PhIP. It also appears unlikely, Dr. Snyderwine concluded, that DNA adducts alone are responsible for the tumors, but in the presence of dietary fat they enhance the malignant conversion of these tumors.

Questions and Answers

Dr. Chan asked about the genetic polymorphism of N-acetyltransferase compared with O-acetyltransferase and Dr. Snyderwine responded that both N- and O-acetyltransferase appear to be part of the same enzyme. Dr. Chan also asked if there is any urinary metabolite that can be used for determining exposure to PhIP. Dr. Snyderwine responded that methods are being developed to identify urinary metabolites, as well as the parent compound, in urine. She added that the state of the art involves GC mass spectrometry analyses. These analyses have shown that the amount of parent compound that spills into the urine may be a reflection of the amount that is not metabolically activated. Therefore, by looking at intake and comparing that with urinary output, an assessment to the degree of metabolic activity can be made. She said that another group is looking at the detoxification products of MeIQX in humans and their findings indicate that substantial detoxification is occurring. She also added that the urinary metabolite indicative of *in vivo* metabolic activation is the N-glucuronide conjugate of the N-hydroxylamine, which has not yet been identified in urine of humans consuming cooked meat, but has been identified in animal models, notably nonhuman primates.

Dr. Schein asked about the acid stability of the procarcinogens, since most of them are ingested and subject to stomach acid. Dr. Snyderwine stated that she does not think that the acidity of the stomach would influence the stability of the compounds. Dr. Schein then followed up with a question about the formation of nitrosamines in the stomach. Dr. Snyderwine responded that this is an active area of investigation.

Dr. Correa asked how the high-fat diet acts. Dr. Snyderwine said that the high-fat diet acts as a promotional factor; however, precisely how it works is still under investigation. Some possible ways, she added, are that it increases radical damage, alters signal transduction, or alters cell-to-cell communication. In humans, it is thought that a high fat diet might change estrogen metabolism, specifically, by increasing the ratio of 16α-hydroxyestrone to 2-hydroxyestrone, the former metabolite being genotoxic. In answer to an additional question from Dr. Correa, she said that they have not done an analysis of hormonal changes in rats.

Dr. Salmon asked how meat could be cooked to lessen the amount of heterocyclic amine produced. Dr. Snyderwine answered that eating meat that is not well done and removing the charred portions of the meat might decrease an individual's exposure. There are also some studies that suggest that by microwave cooking the meat first and discarding the juices that form, and then cooking the meat in a conventional manner, exposure to heterocyclic amines might be reduced.

Dr. Broder asked if chlorophyll could in any way interfere with the formation of heterocyclic amines. Dr. Snyderwine said studies are under way that suggest that chlorophyllin, a derivative of chlorophyll found in green leafy vegetables, can affect the

absorption and metabolism of heterocyclic amines. Studies in nonhuman primates are being conducted to measure hamburger-equivalent doses of PhIP and determine whether a salad's equivalent of chlorophyllin could then inhibit the absorption and metabolic processing of PhIP that lead to DNA adduct formation.

XVI. TRANSFORMING GROWTH FACTOR BETA (TGF-beta)—DR. ANITA ROBERTS

Dr. Rice introduced Dr. Anita Roberts, Deputy Chief of the Laboratory of Chemoprevention, Division of Cancer Etiology at NCI.

Dr. Roberts indicated that her presentation would focus on research conducted in collaboration with Dr. Michael Sporn to determine the role of transforming growth factor-beta (TGF-ß) in carcinogenesis and its prevention. Dr. Roberts acknowledged her coworkers, who have generated many of the concepts regarding the role of TGF-ß in chemoprevention, elucidated the mechanisms of transcriptional regulation of the peptide, and developed the first antibodies against TGF-ß.

TGF-ß is a dimeric peptide linked by a single disulfide bond. It plays a critical role both in normal physiology and disease processes, and is localized in many tissues of the body; its highest concentrations are found in blood platelets.

Dr. Roberts indicated that the technique for purification of TGF-ß from platelets was developed in her laboratory and is still being used by pharmaceutical companies to provide the natural peptide. She also stated that TGF-ß was first characterized and described by her group in 1983. Initial cloning and description of the transcriptional regulation was also conducted in her laboratory. Dr. Roberts emphasized that much of the credit in the advancement of the understanding of this growth factor is due to the worldwide availability of the peptide, its antibodies, the promoter constructs, and the clones provided by her group.

The cloning of TGF-ß has revealed that this peptide is a member of an extended superfamily of structurally related molecules (e.g., inhibins, activins, mullerian inhibitory substance, and all the bone morphogenetic proteins), all believed to have evolved from a common ancestral gene. Of the five identified isoforms of TGF-ß, only three are found in mammals (TGF-ß-1, -2, and -3). The structure of TGF-ß's isoforms was recently described through x-ray crystallography analyses and nuclear magnetic resonance studies.

TGF-ß receptors are unique since they exhibit a serine/threonine kinase activity as opposed to most other growth factor receptors which have tyrosine kinase activity. While many of the intermediate steps in the signaling pathways of the tyrosine kinase-mediated receptors have been elucidated, little is known about the signaling pathways involving receptors linked to serine/threonine kinase activity. Elucidation of these pathways is critical for the overall understanding of the mechanisms of cell regulation, since effectors of tyrosine kinase receptors are considered—in the most simplistic sense—accelerators of cell growth, whereas TGF-ß and its related peptides should be considered blockers or obstructors of cell growth.

Dr. Roberts indicated that receptors for TGF-ß-related peptides—activin, bone morphogenetic proteins, and mullerian inhibitory substance—have been recently described and found to be all homologous serine/threonine kinase receptors. Thus far, only members of the TGF-ß family are known to act through serine/threonine kinase receptors.

Characterization of the promoters for the three mammalian isoforms of TGF-ß reveals that TGF-ß-1 is induced in response to many factors as opposed to TGF-ß-2 and TGF-ß-3, which are more developmentally regulated. TGF-ß-1's transcription can be activated in response to injury through immediate early genes; in carcinogenesis through a variety of oncogenes, acting directly or indirectly on the promoter; and in viral diseases through the HTLV-1 *tax* protein, the hepatitis-B virus X-transactivator protein, and cytomegalovirus IE-2 protein.

TGF-ß exhibits a variety of biological activities, including regulation of cell migration, which is critical for a variety of developmental processes and for response to injury; inhibition of cell growth; suppression of immune cell responses; and regulation of the extracellular matrix and tumor stroma. TGF-ß is involved in both synthesis and degradation of matrix proteins and tissue repair.

Dr. Roberts described the role of TGF-ß and its receptor in carcinogenesis. TGF-ß is a negative regulator of the growth of most epithelial and lymphoid cells; these cells, in turn, form the basis of most human cancers. Dr. Roberts explained that during carcinogenesis, a cell can change its response pattern to TGF-ß. Fully malignant cells might be stimulated or inhibited by TGF-ß, or might become refractory to the peptide. Since loss of sensitivity occurs late in the carcinogenesis process, most cells are sensitive to inhibition by TGF-ß for a significant period of their latency. Dr. Roberts indicated that under this circumstance, the TGF-ß ligand-receptor system and each of its constituents (i.e., TGF-ß, its receptor, and its signaling intermediates) might be considered tumor suppressors, since loss of this system as a whole or its parts can lead to increased tumorigenesis.

Dr. Roberts referred to the work of Dr. Allen Bradley who described alpha-inhibin as a secreted protein exhibiting tumor suppressor activity in the development of gonadal stromal tumors. This investigator also suggested that other peptides of the TGF-ß family, including TGF-ß itself and mullerian inhibitory substance, should also be considered tumor suppressors.

Dr. Roberts indicated that evidence in support of the notion that expression of the TGF-ß receptor is critical in carcinogenesis was derived from studies performed in her laboratory using various human gastric carcinoma cell lines. These studies demonstrated that all malignant cells that had lost sensitivity to TGF-ß contained aberrant type II receptors—one of the two TGF-ß receptors that is most implicated in cell growth. These results indicate that loss of expression of TGF-ß receptors increases the tumorigenicity of cells. Conversely, studies carried out by Michael Brattain's laboratory have shown that transfection of the expression vector for TGF-ß type-II receptor into human breast cancer cells that lacked expression of this receptor, but secreted TGF-ß, significantly decreased tumorigenicity in an *in vivo* model.

Dr. Roberts stressed that as a general conclusion drawn from these studies, tumorigenicity is reduced by differentiating agents that have been shown to induce expression

of the TGF-ß receptor in *in vitro* systems; whereas loss of this receptor during carcinogenesis leads to an increase in tumorigenicity.

Dr. Roberts referred to a study performed by Dr. Adam Glick using keratinocytes derived from the TGF-ß "knockout" mice—the TGF-ß-1 gene has been deleted in these animals by homologous recombination. In this system, keratinocytes form mainly squamous carcinomas, whereas keratinocytes derived from wild type litter mates, which have two functional copies of the TGF-ß-1 gene, form only benign papillomas. Moreover, keratinocytes derived from animals containing only one functional copy of the TGF-ß-1 gene exhibit dysplasia of the papillomas. Dr. Roberts indicated that this study clearly shows that expression of the ligand is also critical in carcinogenesis; loss of the TGF-ß-1 ligand increases propensity to tumorigenicity.

Dr. Roberts described a second study with transgenic mice that demonstrates that expression of TGF-ß suppresses tumorigenicity. In this model, one set of animals overexpressed TGF-ß-1 in the mammary gland. Another set of mice were induced to overexpress TGF-alpha; these animals developed mammary carcinomas. Development of the mammary lesions was completely suppressed when the two sets of animals were crossed.

Dr. Roberts focused on the role of TGF-ß in chemoprevention and its relation to other known chemopreventive agents. She stated that retinoids, vitamin D, tamoxifen, and gestodene, which are agents known to have chemopreventive activity, all have been demonstrated in a variety of systems to increase the expression of either TGF-ß or its receptor. In contrast, estrogens and androgens, which are known to promote tumor formation in hormonally sensitive cancers, decrease the expression of TGF-ß or its receptor.

Dr. Roberts stated that data from a variety of *in vitro* systems using steroids or retinoids suggest that many of the actions produced by retinoids, particularly on cell growth and extracellular matrix production, are mediated through their ability to either enhance secretion of TGF-ß or expression of its receptor. Antibodies against TGF-ß have been used to confirm this notion. Dr. Roberts described a study in which TGF-ß antibodies blocked the inhibition of cell growth produced by retinoids. This study was conducted using a rat prostate cell line developed in her laboratory which was sensitive to growth inhibition by retinoids, deltanoids, and TGF-ß, and stimulation by androgens.

Dr. Roberts indicated that TGF-ß mediation of the activity of chemopreventive agents has also been demonstrated *in vivo*. In animal models, retinoids have been shown to increase the expression of TGF-ß in epithelial tissues. In both animal models and humans, tamoxifen has been shown to increase expression of TGF-ß in stromal cells; whereas androgens have been shown to decrease the expression of TGF-ß in the prostate.

Dr. Roberts pointed out that a chemopreventive trial is being initiated to assess TGF-ß both systemically and in specific directed biopsies in women at high risk for development of breast cancer. These individuals will receive treatment with tamoxifen and the synthetic retinoid 4-hydroxyphenylretinamide (4-HPR).

Dr. Roberts noted that an overall conclusion drawn from studies performed with chemopreventive agents is that these agents are capable of inducing expression of different

TGF-ß isoforms in specific target cells. She added that a priority research project in the last few years has been the development of the combined use of two or more chemopreventive agents in an attempt to induce TGF-ß in complementary cell types. One example has been the use of 9-cis-retinoic acid in combination with tamoxifen in a mammary carcinogenesis model in rats. The average tumor burden is significantly reduced in animals treated with both agents as opposed to animals treated with either one by itself. Whether this effect is due to complementary expression of TGF-ß or its receptor will have to be determined. Meanwhile, the combined use of a vitamin D analog and TGF-ß-1 in an *in vitro* system using human monocytic U937 cells has demonstrated a marked stimulation in the expression of TGF-ß receptors (type I, II, and III) as opposed to the expression obtained with the use of either agent alone.

Dr. Roberts indicated that the identification of agents that enhance TGF-ß activity and their use as chemopreventive agents would be a strategy for applying clinically the concepts developed in her laboratory regarding the role of TGF-ß in carcinogenesis. Determination of TGF-ß levels would be a rational approach for the development of novel chemotherapeutic agents, and this effort should be undertaken by pharmaceutical companies.

Dr. Roberts pointed out that the role of TGF-ß in other disease processes has also been investigated. Similar to its role in carcinogenesis, systemic application of TGF-ß decreases the severity of autoimmune diseases and administration of antibodies against TGF-ß increases their severity. Dr. Roberts noted that the use of TGF-ß for treatment of autoimmune diseases is an extraordinary example of translational research by which the basic research observation that TGF-ß exhibits profound immunosuppressive activity in animal models can be applied in the clinical setting. A Phase I clinical trial with systemic administration of TGF-ß-2 (using an intermittent schedule) is being conducted in patients with multiple sclerosis.

Dr. Roberts indicated that a number of pharmaceutical companies are interested in the clinical application of TGF-ß; this molecule is being used as a topical formulation for wound healing, ophthalmologic applications, and oral mucositis.

Dr. Roberts concluded her presentation by stressing that the position of her laboratory as an international leader in this field has been largely due to the unique atmosphere at the NCI that has allowed investigators to switch from one area of research into another, and to the financial support that has allowed NCI investigators to provide the TGF-ß molecules, the clones, and the antibodies to other investigators around the world, generating, in turn, an immense amount of information in this area.

Questions and Answers

Dr. Goldson referred back to a study he was conducting in 1980 to evaluate the use of retinoids and keratinoids as chemopreventive agents in albinos. He suggested that this study could be used as translational research for a Phase I clinical trial, since some albinos die from skin cancer.

Dr. Sclar commented that another application of TGF-ß that is under investigation is its use to reduce toxicity of various therapeutic modalities, including chemotherapy and radiation therapy. The prevention of cell proliferation in a temporary fashion may protect cells from the

toxicity produced by chemotherapeutic agents. He asked Dr. Roberts for the current status of this research. Dr. Roberts replied that her laboratory has not been involved in that type of research; however current data regarding oral mucositis suggest that rather than enhancing repair, pretreatment with TGF-ß results in the placement of the oral mucosa in a non-proliferative state in which subsequent treatment with 5-fluorouracil induces a reduced number of lesions.

Dr. Chan asked Dr. Roberts whether the stimulation of TGF-ß's expression by retinoids and tamoxifen has a molecular basis. Dr. Roberts replied that this issue has been highly pursued by her group without much success. It is clear that a direct transcriptional effect is not involved; no response elements for these agents are present in the TGF-ß promoters. Instead, a posttranscriptional effect is thought to be involved in the induction of TGF-ß expression by these agents. However, a specific mechanism has not yet been identified. Moreover, evidence also suggests that a translational mechanism or stabilization of the mRNAs might contribute to the observed effects.

Dr. Becker made an historical remark on the pioneering work of Dr. Michael Sporn on this field and acknowledged his intuitiveness and perseverance. Dr. Broder joined Dr. Becker in the acknowledgment of the administrative leadership of the Division of Cancer Etiology and NCI in general, that provided Dr. Sporn enough independence and resources against great criticism.

Dr. Broder pointed out that the clinical applications of TGF-ß and its isoforms transcend the jurisdictional boundaries of a categorical institute. While the use of TGF-ß for the healing of decubitus ulcers and related disease processes may not necessarily be within the jurisdiction of the NCI, these are important areas of clinical application and the NCI should attempt to pursue noncancer as well as cancer applications of TGF-ß isoforms. Dr. Broder stressed that the Institute will do its best within its structure to pursue some of these non-cancer clinical applications.

XVII. SUBCOMMITTEE REPORTS

Cancer Centers

Dr. Day reported that the Cancer Centers Subcommittee focused on the amount of money available for the Cancer Centers Program. Dr. Margaret Holmes, head of the Cancer Centers Branch, reviewed budget expenditures for the last 5 fiscal years and discussed ways to manage shortfalls in the budget for approved applications. Dr. Becker offered a resolution that passed unanimously to use the priority score as the primary guide for funding decisions in cases of a shortfall.

Dr. Day reported that the Subcommittee also discussed ratios, a topic raised in 1992, when the Subcommittee recommended applying a ratio between the total amount of NCI funding and the size of a core grant at any given time. This discussion will be continued at the next Subcommittee meeting. Also to be covered at the January Subcommittee meeting, at Dr. Broder's request, is a review and recommendations on the relative priorities of the Specialized Programs of Research Excellence (SPORE) program, which is part of the Cancer Centers

budget. Dr. Day added that they had intended to discuss the Subcommittee to Evaluate the National Cancer Program (SENCAP) report as it refers to Cancer Centers, but ran out of time, agreeing to cover it in January.

Planning and Budget

Dr. Sigal reminded the Board that it had been decided at the last meeting to create a user-friendly companion document to the Bypass Budget to explain the challenges and accomplishments of the NCI in layman's terms. She referred members to a handout that was a draft of this document. Dr. Sigal requested the Board's comments on the draft, which would be submitted primarily to Congress and constituent groups that review the Bypass Budget. Acknowledging that the Planning and Budget Subcommittee's agenda does not include communications, she stressed the need for communicating NCI's challenges and future needs. She thanked Ms. Eleanor Nealon, Mr. Paul Van Nevel, and Dr. Judith Karp for their help on the draft.

Dr. Sigal mentioned other issues on the Subcommittee's agenda—the crisis of FTEs, possible expansion of the use of GOCOs, and specific recommendations for the SENCAP report that have budgetary influences. She also sought suggestions for issues the Board feels the Subcommittee should address. Dr. Sigal asked that comments on the draft and other suggestions be sent to herself or Dr. Nealon as quickly as possible. Dr. Nealon's fax number was announced—301-402-2594—as reflected on page two of the draft handout.

Activities and Agenda

Dr. Rimer reported that the Activities and Agenda Subcommittee agreed that Dr. Paul Calabresi should chair an oversight committee for implementing the recommendations of the SENCAP report, to include Ms. Ellen Stovall and members of the report-writing team. In addition, Subcommittee chairs will review the SENCAP recommendations, selecting the most feasible and practical for implementation. Dr. Rimer announced plans to meet with members of the Senate to seek support for the NCI's agenda.

Dr. Rimer reported that there was a unanimous recommendation to set aside time at the January NCAB meeting for discussing the role of the Board. There was also sentiment that the Board needs to have a more balanced discussion of cancer issues in conjunction with AIDS research issues. The Subcommittee requested that AIDS topics scheduled for January's Board meeting be postponed to allow focus on cancer issues, particularly on updates of the NSABP and BRCA-1.

Dr. Rimer reported that there were requests for budgetary information from FCRDC and for more information about the epidemiology program and its allocation of resources. Subcommittee members also suggested that future annual program reviews have more active involvement by the Board in development of the agenda. Dr. Rimer proposed future discussions between the Board and relevant Division Directors to ensure the Board's input into the review process.

Dr. McKinnon clarified the written minutes presented by the Activities and Agenda Subcommittee, noting that an *ex officio* committee would be an official body, if the Board

created it, but that the *ex officio* members would have no voting power and their actions would be confined to making recommendations to the Board.

The Board unanimously approved the minutes of the three subcommittee meetings.

Questions and Answers

Dr. Broder welcomed the Board's involvement in the program review process and encouraged dialogue with Division leaders. With respect to BRCA-1, he explained that the investigators were unable to come and present at this meeting, but promised that this topic would be addressed soon. Dr. Broder also reported that there would be an update on the NSABP soon, but that negotiations are under way in a related lawsuit, effectively limiting what can be publicly discussed about the project. He noted that accrual is starting again in the chemoprevention tamoxifen studies and expressed his pleasure with the progress being made.

Dr. Broder urged the Board to reconsider and clarify the message it is sending with respect to AIDS. He reminded them that AIDS is a formidable cause of cancer and that Congress has given the Board responsibility to exercise oversight in this area. He pointed out that DCE's interest in retroviruses and viral oncology has led to that Division's emphasis on AIDS research.

Responding to Dr. Broder's concerns, Dr. Becker explained that on its tour of FCRDC, the Board was told that many projects were mandated and that funds were directed toward AIDS. They were shown a product laboratory of natural products aimed at AIDS, but did not see Dr. Vande Woude's laboratory or the Chemical Carcinogenesis Center. Dr. Becker stated that the concentration of presentations on AIDS did not assist the Board in evaluating other work at FCRDC.

Dr. Rimer expressed her desire for a continuing dialogue to achieve a balance in presentation topics. Dr. Rice relayed the great difficulty faced by the DCT screen operators in finding active agents to screen both for tumors and AIDS; only 1 in 10,000 natural product isolation agents get to the Investigational New Drug (IND) stage and even fewer are useful. The tour of the natural product lab was intended to show the Board how those resources are utilized.

Dr. Rimer thanked Dr. Rice and broke for lunch, noting that Dr. Day would start the meeting at 1:20 p.m., because she, Dr. Broder, and Dr. Calabresi would attend a meeting with Dr. Varmus.

XVIII. INTRODUCTION AND OVERVIEW, DIVISION OF CANCER PREVENTION AND CONTROL—DR. PETER GREENWALD

Dr. Greenwald began with an overview of the organization of the Division of Cancer Prevention and Control. DCPC is organized into four program areas, each of which is composed of approximately four Branches. There are four intramural Branches and one lab, Dr. Greenwald continued, and the intramural program is actively involved in advising and assisting extramural investigators. For example, intramural researchers are members of the

DCPC Director's Committee, assist in improving the overall quality of clinical trials, and help develop methods (i.e., work on factorial designs) for use in clinical trials.

The early detection program area consists of four Branches: Early Detection, Community Oncology and Rehabilitation, Preventive Oncology, and the Intramural Biomarkers and Prevention Research Branch. The prevention program Branches include: Diet and Cancer, Chemoprevention, the Intramural Cancer Prevention Studies Branch, and an intramural laboratory of Nutritional and Molecular Regulation.

Two program areas, Cancer Control Science and Surveillance, are in the process of being merged into one area, to be headed by Dr. Brenda Edwards. Within this area, the Computer Science Branch has been disbanded and moved into the Applied Research and Cancer Statistics Branch. The other three Branches involve special populations, community research, and behavioral research.

Dr. Greenwald expressed his belief that changes in the DCPC organization will strengthen the Division and, because of the current downsizing, it is increasingly important to consolidate to ensure a critical mass of people in each program area in order to work most effectively.

Periodic site visits, Dr. Greenwald continued, are conducted as part of the review of DCPC's extramural programs. Two site visits are being planned currently, one for the Special Populations Studies Branch, because of its importance and to ensure that meaningful work is being performed, and one for Cancer Statistics, because of the renewal of the SEER Cancer Registry Contract, a cost item exceeding $11 million per year.

As part of providing an overview of DCPC, Dr. Greenwald stated that he would try to frame its current activities in a historical context. He began by recounting that the definition of cancer control—the reduction of cancer incidence, morbidity, and mortality—was developed over a 2- year period in the early 1980s in conjunction with input from the Board of Scientific Counselors. This definition took a new approach to cancer control, focusing on rates (i.e., incidence, morbidity, and mortality) and how to impact these rates in defined populations through research on interventions and the systematic application of research results. The focus of cancer control has expanded, Dr. Greenwald continued, to include some applied epidemiology, preclinical work in chemoprevention, and nutritional science research.

Dr. Greenwald noted that several distinct phases were identified in the process of defining cancer control, including: hypothesis development (i.e., a particular intervention will reduce incidence or morbidity); methods development (i.e., development and testing of an intervention in a manner that people will comply with and adoption of successful methods to special populations); controlled intervention trials, when feasible; defined population studies (i.e., using research to show how an approach is meaningful for a community, State, etc.); and, finally, demonstration and implementation of particular interventions.

Dr. Greenwald offered tobacco control efforts as an example of the phases of cancer control. In the early 1980s, studies confirmed that tobacco causes lung cancer and other disease, but few studies examined methods to intervene to prevent smoking. The hypothesis, then, was "what are the causes and means of preventing smoking." Methods development

included development and testing of interventions using the media, in the school setting, and directed towards minorities and women. Next, a controlled intervention trial, COMMIT, was implemented among 11 paired communities randomized to intervention with a control community that is roughly the same demographically to evaluate the impact on quit rates of heavy smokers. Dr. Greenwald stated that this is a difficult undertaking and that the project is in its final evaluation phase. Finally, demonstration and implementation is being conducted through the American Stop Smoking Intervention Study (ASSIST) involving 17 contracts, mainly to State health departments, with a partnership with the American Cancer Society and other groups. The target population is 95 million Americans living in these 17 areas. The goal is to develop coalitions to use the methods that have been shown to be effective in reducing smoking rates. The ASSIST project will operate through 1998.

Dr. Greenwald emphasized that an important future question will be where to turn after ASSIST, pointing out that policy development will have the largest impact. He noted that the NIH has been ambivalent about aggressively pursuing policy development, acknowledging that it is not always a natural fit with science. He reflected, however, that it is an important area, and one in which the NCAB and the President's Cancer Panel may be able to assist and provide leadership.

As another example of the phases of cancer control research, Dr. Greenwald described efforts in early detection of colon cancer, the hypothesis being how to use a particular marker (i.e., *ras* gene) as a screening test to improve the detection of the cancer. Methods development involves both translating diagnostic techniques, such as fiberoptics, into a screening test, and characterizing the sensitivity, specificity, and predictive value of biomarkers so that they can be used effectively for early detection. Dr. Greenwald noted that although myriad biomarkers exist, almost none have been adequately characterized in order to be of use in predicting cancer incidence.

Dr. Greenwald continued by stating that the largest controlled intervention trial under way is the prostate, lung, colon, and ovarian trial, which is just beyond the first year of accrual. A report will be presented in May 1995 to the DCPC Board of Scientific Counselors reviewing the trial and making recommendations on its continuance. A different trial related to cervical cancer (atypical squamous cells of unknown significance and low-grade squamous intraepithelial lesions) is examining whether papillomavirus testing or cervicograms can assist in determining whether cervical ablation, associated with morbidity, is a necessary procedure.

Defined population studies include a pooled analysis of mammography trials internationally to determine if more information can be obtained; use of SEER and Medicare databases to study particular populations; and examination of early detection issues in defined populations.

Demonstration and implementation involves informing the public. Dr. Greenwald stated that the PDQ system provides information on the efficacy of early detection methods. In addition, DCPC is entering into an interagency agreement with the Agency for Health Care Policy Research (AHCPR) to attempt to define how mammography information should be used by physicians in the clinical setting.

Dr. Greenwald referred back to the morning's discussion on the definition of "epidemic," citing the definition used in the *Dictionary of Epidemiology*, "Epidemic: the occurrence in the community or region of cases of illness, specific health-related behavior, or other health-related events, in excess of normal expectancy." He stated his belief that while technically correct, it could be misleading to use this term in regard to breast cancer because it might imply to some people the idea of infectiousness, giving the wrong impression about the increase of breast cancer incidence.

Dr. Greenwald stated that he would move on to a discussion of research in the areas of breast, colon, and prostate cancers. There are a number of hypotheses related to diet, particularly dietary fat, and its association with breast cancer. Dating back to the 1940s, Tannenbaum studied the effect of caloric restriction and fat intake on mammary tumors in mice. Those on a high-fat diet, keeping calories constant, had a higher tumor incidence than those on a low-fat diet. In addition, restricting calories resulted in a marked reduction in tumor occurrence. In the past year, investigators in DCPC's intramural lab have been studying *p53* knockout mice in relation to lymphomas and sarcomas. Similar to the former study, when the mice are placed on a restricted calorie diet, there is a delay in tumor onset and an increased survival of approximately 60 percent. Dr. Greenwald stated that at least this one finding suggests that modifications in diet affect gene expression, and that further study regarding the interrelation of diet and cell genetics is needed.

Dr. Greenwald continued discussing the relationship between fat intake and breast cancer by noting that a summary of epidemiologic studies in western countries, comparing the lower fifth and the upper fifth of fat intake in menopausal women, indicated that women on a high-fat diet had approximately a 50 percent higher breast cancer rate than women with the lower fat intake. Dr. Greenwald acknowledged that there is continuing controversy regarding dietary fat and its relation to breast cancer, but that two trials in progress should provide better information. The first is the NIH Women's Health Initiative, which will test whether a low-fat diet combined with higher intake of fruits and vegetables and fiber lowers breast cancer risk. There is also a trial being conducted by Dr. Wynder's group at the American Health Foundation looking at whether a lower fat diet improves outcome in early-stage breast cancer patients.

Dr. Greenwald summarized findings regarding antioxidant vitamins. An epidemiologic study in Boston suggested that those individuals who ate foods high in vitamin E had lower breast cancer rates. This led to a current study being conducted by Dr. Julie Buring of Harvard, in which 41,000 women are being given beta carotene, vitamin E, or aspirin in order to determine the potential impact on breast cancer, other cancers, and heart disease. Dr. Greenwald briefly noted that the Breast Cancer Prevention Trial, which is looking at the effects of tamoxifen, has just restarted accrual. The protocol was changed so that every woman with an intact uterus now receives an annual endometrial aspiration, and planning is underway for side studies, such as the potential benefits of progestins in reducing endometrial cancer risk.

Dr. Greenwald stated that the study of biomarkers is one of the most difficult areas to address in breast cancer research. Workshops are being held on markers that appear most promising, may be on the causal pathway, and are measurable. Examples of these biomarkers include ductile carcinoma *in situ*, lobular carcinoma *in situ*, atypical hyperplasia, and some

markers of cell growth. In the past year, a prevention trial Decision Network has been established in this area, and approximately 75 biomarkers have been registered and corresponding data entered into the computer network. Dr. Greenwald revealed that biomarker studies have reached a point at which clinical trials can be conducted, and that the Board may be hearing results from those trials in several years.

In regard to the prevention of colon cancer, Dr. Greenwald pointed out that multiple opportunities exist for intervention research and chemoprevention to build upon the knowledge base that has been accumulated on mutations and molecular events leading to this disease. First, the pathology of the disease indicates that the adenoma progression occurs in middle age (40s and 50s), which means intervention might be effective during that period. Second, because there are multiple mutations, there should be multiple opportunities to intervene using anti-initiators as well as antipromoters.

Agents being tested by the Chemoprevention Branch, in coordination with the DCT drug development group, include compounds that block or suppress mutation, including N-acetylcysteine, a derivative of the amino acid cysteine that may deactivate electrophilic chemicals and enhance glutathione-S-transferase; and Oltipraz, a drug used for schistosomiasis that is a synthetic dithiolthione similar to chemicals found in cruciferous vegetables. At this point, it is not known with any specificity the precise mutations these agents may block, only that they could act at multiple points along the causal pathway. Agents that may suppress promotion at some point in the pathway include calcium, non-steroidal anti-inflammatory drugs, lovastatin, antioxidants, and differentiating agents (vitamin A- and vitamin D-related compounds). Finally, Dr. Greenwald noted the potential for folic acid to lessen the hypomethylation that occurs in the development of colon cancer, thereby reducing the incidence of this disease. He cited a study in Boston of foods high in folate that found that individuals at the high end of folate intake had a reduced rate of colorectal adenomas.

Dr. Greenwald continued by summarizing studies related to colorectal cancer. An epidemiologic study conducted by Dr. Cedric Garland of 2,000 people followed-up 19 years later found that those individuals who took the most calcium and vitamin D had the lowest rate of colorectal cancer. Dr. Lipkin, in a series of studies, showed that the cell proliferation rate in the colon crypts was higher before feeding calcium than after feeding calcium, suggesting that calcium reduces proliferation in the bowel. Dr. Baron is conducting several studies to determine whether calcium, aspirin, and/or folate prevent new polyps in patients who have already had one polyp removed. A report by the American Cancer Society on a large prospective epidemiologic study indicates that taking at least one aspirin every other day may have an effect in reducing colon (not rectal) cancer deaths. Similarly, Dr. Greenberg, at Dartmouth, compared colonoscopy at baseline and 1 year later and found that consistent users of aspirin had a lower incidence of colorectal polyps. Contradicting this finding, Dr. Greenwald stated, are initial findings from the Physicians' Health Study, a randomized trial of 22,000 doctors who took aspirin or placebo. At the 5 year follow-up, there appears to be no significant benefit from taking a low dose of aspirin every other day; however, there may be a dose effect or a longer follow-up required, which is planned. There is ongoing work being done, particularly in cancer centers across the country, to characterize histologic markers of colon cancer.

Next, Dr. Greenwald reviewed progress in the study of prostate cancer. Dr. Ross, at the University of Southern California, studied serum-5 alpha reductase levels, the enzyme that converts testosterone to dihydrotestosterone in the prostate, in different cohorts of men. He found that Japanese men had lower levels of metabolites in the urine than American men and that the metabolites found in White and Black men were about the same, a surprising and as yet unexplained outcome. A trial is under way of finasteride, a drug that blocks the formation of dihydrotestosterone, in which 18,000 men are randomized to finasteride or placebo and the endpoint will be a 7-year biopsy. Dr. Greenwald noted that biomarkers of interest related to prostate cancer are being developed and researchers are close to developing intervention studies.

In conclusion, Dr. Greenwald stated that he would like to highlight two collaborative studies with the intramural group. The first was a study of use of antioxidants in 20,000 people in China in an area where esophageal and stomach cancers were common. A combination of beta carotene, vitamin A, and selenium was found to cause a 21 percent decline in stomach cancer deaths, leading researchers to conclude that these antioxidants appear to reduce stomach cancer in this nutrient-deficient population. A Finnish study of 29,000 male heavy smokers randomized men so that half received beta carotene, half vitamin E, one-quarter both, and one-quarter neither. Surprisingly, those taking beta carotene had an 18 percent higher occurrence of lung cancer, a result that has not been explained. Another finding in the same study was that vitamin E appeared to reduce incidence of prostate cancer. This is an important lead for future study.

In closing, Dr. Greenwald reflected that prevention research must move in two general directions—public health and medicine—both of which are important and require attention. Questions to be answered in regard to public health include: What can we do about smoking? What policies do we have? Can we better understand diet? and How can we affect the population at large? Medically, there is research on biomarkers that are on the causal pathway to cancer and chemopreventive research to determine how to modulate biomarkers or other factors to lower the cancer occurrence rate. Both approaches need to be developed in a coordinated, integrated manner.

Questions and Answers

Dr. Sigal asked if Dr. Greenwald could address environmental tobacco smoke. Dr. Greenwald stated that there are a number of studies that together indicate a 30 percent increase over the risk of a nonsmoker. He clarified that while 30 percent of all cancer deaths are attributable to smoking and tobacco, only a very small proportion of cancer deaths are associated with environmental tobacco smoke.

XIX. DCPC BOARD OF SCIENTIFIC COUNSELORS, REPORT AND NUTRITION SUBCOMMITTEE REPORT—DR. CULBERTO GARZA

Dr. Garza, Director of the Division of Nutritional Sciences at Cornell University, began by highlighting the function and operation of the DCPC Board of Scientific Counselors. The function of the BSC, like most others at NCI, is to provide scientific advice and review the progress of various programs, in this case within DCPC. Dr. Garza stated that the Board takes

all official action as a committee of all of its members. However, preparatory work is often performed by the four subcommittees of the Board: Cancer Prevention, Early Detection and Community Oncology, Cancer Control, and Cancer Surveillance.

Next, Dr. Garza briefly reviewed the schedule for intramural site visits in 1995. One, on biomarkers and prevention research for the Prevention Research Branch, has already occurred. The next site visit will focus on intervention studies of the Cancer Prevention Studies Branch. Dr. Garza noted that several of the large studies referred to by Dr. Greenwald are within this category. The third site visit, scheduled in May 1995, is in the Laboratory of Nutritional and Molecular Regulation, which is responsible for trying to define the basic mechanisms by which nutrients regulate either signaling mechanisms or gene expression in order to better understand the link between diet and disease. The fourth site visit, Dr. Garza mentioned, is for the Biometry Branch, scheduled in November 1995. The Biometry Branch provides epidemiological support for a number of studies as well as focuses on mathematical modeling of the processes that are relevant to cancer prevention and control.

The BSC, Dr. Garza continued, also reviews and approves extramural program RFAs and RFPs. Subcommittees usually first conduct an extensive review and try to address any concerns. This facilitates approval when the projects are presented to the Board for approval.

Dr. Garza spent the remainder of the presentation discussing a specific function that the Board has paid particular attention to over the past year, a report of the Nutrition Subcommittee, a subcommittee of the Cancer Prevention Subcommittee. Dr. Garza noted that he chaired this Subcommittee, and went on to list the other members. The charge was to develop a plan to identify the scope and nature of nutritional science to be supported by DCPC. The context of this charge was DCPC's mission to delineate the areas of diet and nutrition most relevant to cancer prevention and control.

The Nutrition Subcommittee, Dr. Garza continued, began by reviewing a variety of studies and reports from the past 15 years. It was discovered that certain themes were repeatedly found in reports of studies in this area. These themes included: a need to identify foods and food constituents that modulate cancer risk; a need to look at the mechanisms underlying those effects; a need to identify early biomarkers of diet-related processes in order to modulate cancer risk; and a need for methods for assessing human exposure to relevant dietary components. In addition, reports uniformly stressed the need to understand the role that nutritional factors play in the expression of genes known to increase the risk of development of certain cancers, the way in which nutrients determine normal growth and development, and the significance of those interactions in the etiology of various degenerative diseases, including cancer.

The Subcommittee next tasked itself with determining why the same recommendations had been made in report after report without a more aggressive response from the scientific community. Looking at funding, it was determined that for NIH, the ratio of intramural funding to extramural funding was 1:7; the ratio for NCI was about 1:4; and about 1:24 for DCPC. The funding ratio for nutritional research was 1:20 within both DCPC and NCI. The Subcommittee also looked at absolute dollars, noting that only about 2 percent of the NIH's budget was being spent on nutrition research. From this, the Subcommittee concluded that a strong constituency for nutrition research does not exist within the NIH system, or within NCI

or DCPC. The Subcommittee felt that looking at the total amount being spent together with how those sums were being distributed intramurally and extramurally likely explained much of the lack of progress in addressing the same recommendations.

Dr. Garza continued by outlining the recommendations of the Nutrition Subcommittee, both for the intramural and extramural program. Intramurally, it was recommended that a nutrition research facility be established that addresses basic, biological, and behavioral research in an integrated manner. The facility should ideally be on the main NIH campus in order to ensure its success. A nutrition research facility would achieve an orientation within the NIH to prevention research as well as foster excellence in the extramural programs because of the leading role that intramural research plays within the NIH scientific structure. The roles of the facility, as envisioned by the Subcommittee, would be fourfold: to improve both the quality and intensity of the research in nutrition; to build a definitive database for developing quantitative dietary recommendations; to act as a focal point within NCI for nutrition research, training, and public education, and also for other agencies within the NIH since nutrition is important not only in terms of cancer, but many other disease processes; and to serve as a model for other institutions or Federal agencies that have a mandate in this area.

Recommendations for the extramural program, Dr. Garza continued, were targeted to four priority areas. The first area is basic studies of nutrition and diet modulation of human genomic expression. Studies show that cancer rates can change dramatically as populations migrate from one geographic area to another, where diet and health practices may differ. It is, therefore, important to understand the basic mechanisms that determine the development of diverse phenotypes from very similar genotypes across time and geographical residence. The second priority area is to focus on human metabolic studies that explore mechanisms by which diet and nutrition alter cancer risk and cancer control. Third, is to take advantage of the opportunity to perform substudies, or supplementary studies, as part of ongoing intervention trials, particularly in regard to development of early or intermediate biomarkers. The fourth priority area is to collaborate with other NIH Institutes to ensure that adequate basic behavioral research is performed.

Following preparation of the report, the Subcommittee recommended that it be presented to the NCI Executive Committee, the National Cancer Advisory Board, and the NIH Director's Office. The Subcommittee also requested that DCPC draft a plan to be implemented by DCPC after review by the Subcommittee and full Board. Dr. Garza stated that it had been hoped that this plan would be completed by September 1994 and presented to the DCPC BSC in October 1994. He noted that a full plan could not be presented at the DCPC BSC meeting in October, but that part of a plan, relating to extramural activities, had been implemented at that meeting. The BSC approved four concepts for the extramural program: to focus on nutritional dietary manipulation of human genomic expression for cancer prevention; assessment of dietary exposure to, and human metabolism of, constituents of plant foods; human metabolic studies for the modification of dietary fatty acid intake for the prevention of breast, prostate, and colon cancer; and nutrient modulation of cell integrity and repair mechanisms.

Dr. Garza, speaking for the Nutrition Subcommittee, expressed his belief that approval of these four areas represents a substantial accomplishment in implementing the Subcommittee's recommendations for the extramural program, as well as his hope that there

will be a similar response from DCPC toward building a stronger infrastructure in nutrition within NCI and NIH.

Questions and Answers

Dr. Day began by asking what is planned in terms of developing a nutrient database and how that relates to what is available through the Department of Agriculture. Dr. Garza responded that this is an underfunded, but necessary area of work, domestically and internationally. As more foods are imported, it will become increasingly important to have a stable database of nutrient constituents beyond the classical nutrients. There is some coordination with the USDA. Dr. Greenwald noted that he was on an advisory committee to the USDA 1 year ago and it was suggested that the best way for establishing or improving the existing system be studied.

Dr. Day also requested clarification on how genomic modulation of dietary intake would be performed. Dr. Garza explained, for example, that several substrates of vitamin A metabolism play a key role in modulating gene expression. Other nutrients, such as vitamin E in prostate cancer, may be involved in dynamic regulation. He acknowledged that researchers will have to deal with complex genetic traits and the interaction of diet with multiple genes. However, as progress is made in mapping the human genome, patients will want advice on how to minimize their risks. Dr. Day asked whether the state of the art in genomic analysis is at a point where these types of metabolic studies can be performed. Dr. Garza responded that the state of the art exists with colon cancer, and may with breast cancer in the near future. Dr. Greenwald qualified this statement by noting that although there are a few examples in which cell systems can be modulated, this type of research is in an early stage, and thought needs to be given to how this research can be approached and whether new methods can be developed.

Dr. Day continued by asking how large the Nutrition Subcommittee envisioned the nutritional laboratory to be. Dr. Greenwald responded that it is difficult to specify a precise dollar amount at this conceptual stage, but that one view is to reallocate 1/10, or $10 million, of the NCI budget currently spent on nutrition science ($90 million) to the complex.

Before moving on to the remaining presentations, Dr. Rimer stated that she would like to raise an issue regarding the DCPC reorganization. She informed the Board that she has received, in the last several weeks, letters from approximately 30 senior people in behavioral sciences and medical oncology, as well as numerous phone calls, representing substantial concern on the part of the behavioral research community that the reorganization would weaken the ability of the Division to adequately address social and behavioral science issues. Dr. Rimer proceeded to read portions of letters received from Kaiser Permanente, the American Society of Addiction Medicine, the Appalachian Leadership Initiative, Brown University, individual scientists, and a member of the DCPC Board of Scientific Counselors.

Dr. Greenwald expressed his appreciation for the concerns presented. He emphasized that the intent of the consolidation is to make the behavioral program stronger, not weaker, in that surveillance is a natural component of cancer control and will enhance endpoint measures. He noted that there are also complementary staffs that will work closely together and provide the needed depth to work effectively. He observed that this may be an opportune time to have the BSC perform site visits of some extramural branches, and invited interested NCAB

members to participate in these site visits. He also pointed out that approximately $45 million of the $190 million cancer control budget is allocated toward behavioral research, including smoking and some communication research. In addition, several major trials have in their intent a behavioral endpoint. Dr. Greenwald did acknowledge the need for dialogue, however, and expressed his willingness to discuss these issues.

In terms of personnel, Dr. Greenwald noted that several branch chiefs in leadership roles in DCPC have a behavioral science background, and that Dr. Barry Portnoy, who works with Dr. Greenwald and Dr. Sondik, is a behavioral scientist. Dr. Greenwald offered to have researchers in this field provide a presentation of their work to the NCAB, if that would be helpful.

Dr. Greenwald reflected on the difficulty of the review process. Some reviewers focused on the impact of behavioral studies in the areas of smoking, diet, or use of early detection techniques, as the important issues to address. Others focused on what builds the field of behavioral science and the methods of behavioral science research. A balance needs to be found. Dr. Greenwald stated that DCPC is willing to help build the field, but that researchers in the field need to help establish relevance, quality and importance.

Dr. Bishop asked whether DCPC is the major locus for behavioral research in NIH. Dr. Greenwald responded that the National Institute on Mental Health does behavioral research, and it is also performed in other areas. He said he does not consider DCPC the lead group on fundamental basic research on behavior, but does consider it to be the lead group in applications to cancer control. He also observed that the work of the Office of Cancer Communications is connected to DCPC's work, and that providing resources to this office to learn how to focus, evaluate, and refine their messages could be a part of the behavioral field—the part that connects most with the public.

Dr. Schein, as a nonbehavioral scientist, expressed his opinion that behavioral science has perhaps the most potential to affect incidence and mortality from cancer. He cited AIDS as an example of the effectiveness of behavioral modification; the primary means of reducing incidence of this disease. Other examples related to cancer include tobacco, asbestos, vinyl chloride, and radiation exposure. He concluded that importance of behavioral research should not be underestimated so long as there is a need to influence large populations with valid information, which, if implemented, could seriously impact the epidemiology of cancer.

In conclusion, Dr. Greenwald expressed his openness to potential partnerships with other groups in this field, such as the American Cancer Society, and that appropriate relationships could be discussed with the BSC and boards such as NCAB.

XX. BIOMARKERS FOR CHEMOPREVENTION OF BREAST CANCER—DR. KAPIL DHINGRA

Dr. Dhingra, from the Department of Breast and Gynecologic Medical Oncology at the M.D. Anderson Cancer Center, began by informing the Board that he would discuss the problems and challenges in designing chemoprevention trials, and how these problems are being addressed in the field of breast cancer. He noted that the state of the art for identifying

someone at high risk for breast cancer involves epidemiologic risk factors and that in reality, three-fourths of all breast cancers will occur in women with no identifiable conventional high-risk factors. Furthermore, the majority of women in epidemiologically high-risk groups will not develop breast cancer, with the exception of women in families with hereditary risk factors, such as BRCA1. The Breast Cancer Prevention Trial, he continued, is the best example of an attempt to randomize women based on what is known of assessing risk; 8,000 (out of 16,000) women considered to be at high risk will be administered tamoxifen, and an absolute decrease of only 60 cancers is projected based on original projections of risk in that population.

Dr. Dhingra described some biomarkers that could be used for breast cancer risk assessment and efficacy of chemopreventive agents, including: conventional histologic markers such as ductal carcinoma *in situ* (DCIS) and lobular carcinoma; markers of proliferation and differentiation that can predict neoplasia; markers of genetic and epigenetic instability; and specific genetic changes associated with breast cancer.

An important aspect of developing biomarkers, Dr. Dhingra continued, is quantifiability. There are also specific considerations that apply to development of biomarkers of breast tissue. First, unlike some other organ systems, such as aerodigestive tract cancers and colon cancers, there are no premalignant external lesions that can be seen. Some lesions that have been identified, such as atypical hyperplasia, may be associated with a higher risk of malignancy. However, Dr. Dhingra noted, there is continuing controversy over whether these lesions are truly premalignant or only indicative of a subsequent risk of neoplasia.

Another special consideration is the cyclical physiology of normal breast tissue, particularly in premenopausal women. Dr. Dhingra provided an example that during the menstrual cycle, the proliferation rate in the breast epithelium changes significantly. Therefore, if one were to use proliferation as a marker, one would have to examine tissue in the context of the menstrual cycle physiology. Dr. Dhingra stated that researchers are also limited by lack of knowledge of defined genetic changes in what are currently considered to be premalignant lesions. Finally, he emphasized that there will be some markers that will correlate with risk of neoplasia, and that these need to be differentiated from markers that are potentially indicative of the efficacy of a chemopreventive agent.

Dr. Dhingra indicated that the failure or limitation of histologic and phenotypic markers has led researchers to consider genetic changes as potential biomarkers. He noted that the molecular advances of the last two decades have increased this possibility. He continued by explaining that known genetic changes can be broadly divided into two categories: those that are associated with predisposition to breast cancer and those that have a potential role in tumor formation, such as oncogenes that are either amplified or over-expressed in breast cancer and tumor suppressor genes (i.e., *p53* and possibly, TGF-beta) that appear to be inactivated or downregulated in breast tumors.

In terms of predisposition, the BRCA1 gene is the most recent discovery and it is suspected that there is a BRCA2 on chromosome 13. Li Fraumeni Syndrome, with hereditary *p53* mutations, and several other syndromes confer a very high risk of breast cancer. The limitation of this knowledge is that it only applies to approximately 10 percent of all breast cancers; 90 percent of breast cancers occur in women who do not have any of these genetic

factors. Dr. Dhingra noted that an important area for research is ataxia telangiectasia, a classic syndrome of genetic instability. Epidemiologic evidence indicates that women with this syndrome have a high risk of breast cancer, and between 8 and 20 percent of White women are suspected to be carriers of the ataxia gene. However, the gene has not yet been cloned.

Dr. Dhingra explained that one way to view the utility of genetic changes in the second category as potential biomarkers is to learn when they develop in the evolution of breast tumors. Dr. Dhingra presented a slide depicting a sequential evolution of genetic changes for breast cancer, as interpreted by some researchers. According to this diagram, many of the oncogenes and tumor suppressor genes appear to be altered at the stage of *in situ* carcinoma, or later. However, there is a large gap in knowledge related to what occurs between normal breast epithelium and the *in situ* carcinoma stage; thus, this is an active area of investigation. Dr. Dhingra referred to the next slide to summarize other known genetic changes, many of which seem to occur at the stage of invasive cancer; a stage that is probably too late to be testing preventive strategies or identifying biomarkers.

To address some of these problems, Dr. Dhingra continued, a workshop of approximately 200 people was organized by the Chemoprevention Branch in order to exchange information on the state of the art of knowledge of various risk factors and genetic changes, and to reach consensus on how to develop biomarkers. Following this meeting, a consensus statement was issued, followed by an RFP and a competition for contracts to pilot a study of chemopreventive agents, using DCIS lesions as a target. The DCIS lesion, Dr. Dhingra explained, is nearly 100 percent curative with definitive local therapy, but when incompletely removed, has a significant risk of subsequent invasive carcinoma or recurrent carcinoma. A limitation of using DCIS as a chemoprevention target for a long-term trial is that until a whole lesion is removed, researchers cannot be certain there is no invasive component. However, the opportunity for short-term trials exists.

Dr. Dhingra described the protocol that was submitted following the RFP announcement. The protocol would involve recruiting women who present with DCIS, performing an initial core biopsy and a fine needle aspirate, and designating this as specimen number one. These women would then be randomized to one of four interventions: placebo, tamoxifen, 4-hydroxyphenylretinamide (4-HPR), or a combination of the two. Following a minimum intervention period of 2 to 4 weeks, the women would proceed to definitive surgery and a second specimen would be obtained at that time. These two specimens would then be used for comparative biomarker assessment. Dr. Dhingra noted that the intent of developing the protocol is to minimize extra intervention that may be required for a trial that does not have a true therapeutic benefit for participants.

Next, Dr. Dhingra referred to a slide listing the biomarkers proposed for study in this trial. He noted that a host of proliferation markers could be used, but Ki67 has been chosen. Dr. Dhingra digressed briefly into a discussion of nuclear morphometry, noting that it is a known indicator of either neoplasia or risk assessment for neoplasia. He stated that in this specific instance, technology is driving research—with the development of new computer hardware and using an image analysis system, researchers can now characterize the morphology of lesions to a level not possible with visual examination. The significance is that use of this technology may enable identification of nuclear features (i.e., texture, form, shape) and detection of subtle changes before the development of neoplasia. No data are yet

available, however, on the effectiveness of this technique. Dr. Dhingra acknowledged Dr. Charles Boone for his efforts in quantitating nuclear morphometry.

Returning to a description of the markers chosen for this protocol, Dr. Dhingra stated that they were chosen to meet certain categorical needs. The first, are markers of growth factors or genes that are essential for determining whether there will be a response to the proposed chemopreventive agents (tamoxifen and 4-HPR). Another set of markers were chosen whose modulation indicates whether the chemopreventive agent made its way into the breast tissue and whether downstream response pathways are intact. A third set of markers are more phenotypic of neoplastic change.

Dr. Dhingra described an example of the potential interactions expected to have an interplay in this study. Tamoxifen's action in the chemoprevention of breast cancer is usually mediated through an estrogen receptor. Administration of tamoxifen leads to upregulation of estrogen receptor within a few days of treatment. Since tamoxifen administration also induces upregulation of the progesterone receptor, except when mutations in the estrogen receptor exist. Therefore, upregulation of progesterone receptor would indicate that the estrogen receptor function is intact, and that downstream response pathways are also intact.

TGF-beta, another biomarker, is induced both by tamoxifen and retinoic acid and is one of the strongest inhibitors of proliferation of breast cells to date. It also inhibits neovascularization, which is essential to development of neoplasms. Dr. Dhingra stated that expression of the *neu* oncogene will also be studied, in part because of its frequent upregulation described in *in situ* carcinoma. More specifically, in estrogen receptor-positive cells, tamoxifen induces upregulation of *neu* oncogene, a stimulator of proliferation. Yet, when breast cells are exposed to tamoxifen it decreases proliferation. In several systems, retinoic acid has been shown to downregulate transcription of the *neu* oncogene, so a number of counteracting influences are present. The trial will help determine which influences prevail.

Dr. Dhingra briefly described the retinoid receptor. Dr. Reuben Lotan, in a preliminary study of 22 breast tumors and adjacent normal tissue, examined the expression of retinoic acid receptors (RAR). He observed that while RAR-beta was expressed on all the adjacent normal tissues, it was lost in more than half of the breast tumors. Dr. Dhingra noted that Dr. Anita Roberts had also discussed the loss of RAR-beta expression during the development of neoplasia in her presentation to the Board that morning. Several laboratory systems have shown that retinoic acid can induce RAR-beta expression, and this expression coincides with a senescent phenotype.

Addressing the concern that a short-term trial may not show modulation of any biomarkers, Dr. Dhingra referred to several pilot studies that have been performed. These studies showed that estrogen receptor upregulation following tamoxifen administration was seen within 8 to 10 days, and, in fact, reached a plateau effect. Thus, the biomarkers that are transcriptionally regulated can be modulated in a short time period of several days. Dr. Dhingra acknowledged that fixed genetic lesions probably could not be modulated in a short term trial and that longer term trials would be needed.

Dr. Dhingra indicated that while DCIS will enable pilot studies to be performed, the vast majority of women do not have DCIS and tissue will have to be obtained by random

needle aspirate or, perhaps, blood tissue. Dr. Dhingra addressed the use of specific genetic changes (morphologic changes) as biomarkers. He alluded to an example of genetic instability in breast epithelium. A tissue section from a patient with breast cancer that was monosomic for chromosome 17 was displayed. In addition, a cluster of normal cells was shown containing three copies of chromosome 17, suggesting the presence of an underlying genetic instability. Dr. Dhingra mentioned that a drawback in the use of tissue sections instead of needle aspirates for determination of genetic changes, is that polysomy can be underestimated. Referring back to the same example, Dr. Dhingra stated that needle aspirate was then performed on adjacent normal tissue of breast cancer patients. Results of this study indicated polysomic cells could be detected in randomly obtained needle aspirates of adjacent normal tissue. However, because of concern that there may be tumor cells that may have spread along the ducts, needle aspirates were performed on contralateral breast tissue with no tumor. These studies showed the presence of polysomic cells, representing genetic instability, in "normal" breast epithelium.

Dr. Dhingra concluded that the next step is, therefore, to identify markers that can be studied in a random aspirate. Dr. Dhingra stated that he believes these markers will indicate a process that is going on in the whole breast tissue, or the "field at risk," with specific genetic changes being superimposed on the background.

Questions and Answers

Dr. Day questioned the projection that there would be an absolute decrease of only 60 cases of breast cancer in the Breast Cancer Prevention Trial. Dr. Dhingra clarified that this represents the minimum number of breast cancers that are expected to be prevented based on the model of projected risk. Dr. Day also asked how postponing surgery for these types of lesions would be addressed with human subjects review boards. Dr. Dhingra responded that medically and ethically, most of the medical oncologists who treat patients, as well as the surgeons at M.D. Anderson, do not feel that delaying surgery for 2 weeks in any way increases the risk of either developing a lesion or metastasis during the natural history of breast cancer. In addition, there is often a natural delay in obtaining surgery, based on how much trauma the initial biopsy may have induced, the time the patient may take to decide between lumpectomy versus mastectomy, or the need to see a plastic surgeon for consultation prior to surgery. He did acknowledge that this was, however, a subject of intense discussion. Dr. Dhingra stated that, ideally, he would like to do a 4-week intervention because of the cyclical physiology referred to previously. If an initial biopsy is performed, and the 28 days elapse, most women will be back to their original phase of the menstrual cycle.

Dr. Dhingra also clarified for Dr. Day that since larger tumors are likely to be faster growing, they will probably be excluded. Also, the researchers would focus on DCIS and earlier lesions as much as possible, because they are more likely to be amenable to chemopreventive strategies.

XXI. BIOMARKERS FOR CHEMOPREVENTION OF PROSTATE CANCER—DR. DAVID BOSTWICK

Dr. Bostwick, from the Department of Laboratory Medicine and Pathology at the Mayo Clinic, indicated that data he would present had been agreed upon at a number of prostate

cancer and chemoprevention consensus meetings. He commented that the lack of conflict regarding knowledge about prostate cancer is probably a result of newly gathered, more definitive information. It is known that prostate cancer kills approximately 1 person every 15 minutes and experiences a 2 to 3 percent rise in incidence each year. This increase has been attributed to: 1) an increase in detection through serum prostate specific antigen (PSA); 2) a decrease in other causes of death; and 3) an elevation in the average age of the population, who experience more prostate cancer as they become older.

Dr. Bostwick pointed out that a primary challenge to imaging prostate cancer is that it is not always possible to detect the entire cancer, particularly when it develops bilaterally. He noted that interventions attempt to prevent the cancer from metastasizing to the lymph nodes. Once the cancer leaves the boundaries of the prostate gland, the prognosis becomes poor. It is, therefore, extremely important, Dr. Bostwick stated, to detect the cancer before it metastasizes. He suggested that reduced mortality may be achieved through prevention.

Dr. Bostwick summarized his presentation as addressing three primary questions: 1) What are the goals of the prostate cancer chemoprevention trials? 2) What are the best target populations for these trials? and 3) What biomarkers should be used to assess the outcome of the intervention?

Dr. Bostwick indicated that the goals of the prostate cancer chemoprevention trials, which are similar to the goals of any chemoprevention trial, are the reduction or elimination of precancerous lesions and the reduction of the cancer's incidence. He contrasted prostate cancer with other forms of cancer based on its extraordinarily high incidence, 80 percent in men older than 80 years, and its slower growth rate. Dr. Bostwick informed members that during the last 8 years, researchers have achieved consensus regarding the description of some of the precancerous lesions of the prostate. He added that there is virtually complete international agreement that prostatic intraepithelial neoplasia (PIN) is the most likely precursor of prostatic adenocarcinoma, and that PIN is a more appropriate term than carcinoma *in situ* or dysplasia of the prostate. He clarified that there may be other precursors, but PIN is the one most likely to lead to cancer.

Dr. Bostwick described the criteria for identifying PIN lesions, which are classified as low or high grade according to nuclear and nucleolar characteristics, including: 1) enlarged nuclei; 2) more than 10 percent of cells with prominent nucleoli; 3) intact basal cell layer; and 4) cell proliferation. He remarked that PIN primarily is associated with cytologic abnormalities, which are similar to those found in adenocarcinoma, but are confined to preexisting structures by the basal cell layer.

Dr. Bostwick noted that he would focus his discussion on high-grade PIN, as researchers have the most information about this condition and believe it is the premalignant lesion with strongest links to cancer. He pointed out that PIN and cancer are both primarily localized to the peripheral zone of the prostate. Due to their spatial association, lesions found in this region are most probably precancerous. Dr. Bostwick explained that in most cases of high- and low-grade PIN as well as adenocarcinoma, multiple foci develop. Moreover, the multifocality of PIN and adenocarcinoma frequently overlap.

Dr. Bostwick presented data that were revealed in a compelling and unique study regarding the association between PIN and prostate cancer. He explained that Wael Saka and colleagues in Detroit autopsied the prostates of more than 300 men who had died traumatically. It was found that incidence of PIN among these men followed an upward curve, with its presence detected among men in their 20s and 30s and rapidly rising among those in their 30s and 40s. PIN lesions in this age group overlapped with cancerous lesions. Dr. Bostwick indicated that the curves were similar among African American and White males, except that among African Americans, the increase began slightly earlier. Dr. Bostwick pointed out that the parallel increase between PIN and cancer was further support of the lesion's precancerous nature.

Dr. Bostwick summarized the findings regarding DNA ploidy in PIN. Three earlier studies found that most cases of both low- and high-grade PIN were diploid, but that some were aneuploid. He commented that the highest levels of aneuploidy were found among participants in O'Malley's study, which was conducted using flow cytometry. In addition, Dr. Bostwick contrasted the data from earlier studies with information from more recent studies, which revealed that approximately 50 percent of PIN cases are actually nondiploid. A recently completed study that utilized fluorescence *in situ* hybridization (FISH) for ploidy analysis, confirmed that about 50 percent of PIN cases display trisomy or another type of aneuploidy.

Dr. Bostwick then presented a morphologic continuum of prostate cancer development that assumes PIN to be a precursor to prostate cancer. The morphologic continuum, which has received international support, begins with a normal prostate structure and proceeds through low-grade PIN, high-grade PIN, and ends with early invasive cancer. He emphasized that the model does not presuppose a step-wise progression toward malignancy, as there are no data to support this assumption. He indicated that the basal cell layer of the prostate, which defines the boundaries of the gland, remains intact until the highest grades of PIN, at which point fragmentation begins and the basal cell layer is entirely lost. Dr. Bostwick noted that while early data suggested that the basement membrane disintegrated during the early stages of prostate cancer development, more recent data indicate that the membrane is not lost until moderately to poorly differentiated cancers are found. Dr. Bostwick allowed that the membrane which these studies are observing might not be the normal basement membrane, but stated that the markers they have used, including heperan sulfate, proteoglycan, and type IV collagen, have shown that it is preserved until the later stages of carcinogenesis.

Dr. Bostwick reported that two recent studies revealed that there is almost 90 percent diagnostic concordance among pathologists for high grade PIN; however, the concordance for identification of low-grade PIN is much lower. Dr. Bostwick informed members that a study conducted in his laboratory found that among participants in which PIN was detected through needle biopsy, the likelihood of cancer development was 38 percent on repeat biopsy. Dr. Bostwick interpreted these findings to indicate a sampling error during first biopsy, as it is not currently believed that most men with PIN will "develop" cancer within a year. It is more likely that the cancer was missed during the first biopsy.

Dr. Bostwick underscored that there is a consensus among researchers that detecting PIN in an individual does not qualify the individual for therapy initiation, which primarily involves radical prostatectomy. Instead, PIN should be used as an ideal lesion for chemopreventive therapy. He added that a study conducted this summer concluded that, in

most cases, prostate cancer follows high-grade PIN detection, which is the prevailing belief among scientists. However, he qualified this conclusion by noting that at this point, sequential studies that show a lack of coexistence of PIN and cancer are not available since this is extremely difficult to prove.

Dr. Bostwick reported that he is not aware of any published results of chemopreventive trials for prostate cancer in humans. He stated that his group recently published the results of a retrospective study that compared the frequency and prevalence of high-grade PIN among patients undergoing prostatectomy with data from individuals who had received androgen deprivation therapy as a preoperative procedure; they found that the rates were much lower in those receiving intervention. Dr. Bostwick noted that similar effects were found among those who are treated for 3 weeks or 3 months. While this is becoming more accepted as an effective form of therapy, it is not yet standard practice.

Dr. Bostwick addressed the issue of the frequency with which PIN is observed without simultaneous detection of cancer. He indicated that his group just completed a study pursuing this question, since there was no literature regarding this topic. The study concluded that a comparison of the biopsies of two distinct groups of 200 men indicated that one group evidenced 10 percent of its cases as PIN only, while it was determined in the other cohort that 16 percent of participants displayed PIN. Dr. Bostwick characterized these rates as a small amount. He added that these are the cases that usually cause urologists to perform a second biopsy, despite their noncancerous classification, because of their highly suspicious nature. He reported that cancer was also identified in similar numbers of participants in both cohorts. Based on these data, Dr. Bostwick concluded that there is a population of individuals that have PIN without invasive cancer who are potential participants for chemoprevention trials.

Dr. Bostwick summarized this section of his presentation by stating that while high-grade PIN is the most likely precursor of cancer, the supportive evidence is not direct. He noted that because PIN has been detected in young men, intervention should be initiated much earlier than the average age of prostate cancer development. Dr. Bostwick also pointed out that while researchers know that incidence increases with increasing age, the natural history of the disease is unknown.

Dr. Bostwick turned to a discussion of appropriate target populations for chemoprevention trials. He asserted that appropriate participants include those individuals who have PIN but not invasive cancer, which is probably about 10 to 16 percent of the total population of men who have PIN. He suggested that a second biopsy should be obtained before chemoprevention is initiated.

Dr. Bostwick indicated that another possibility for a target population is to include individuals from high-risk groups. He remarked that the challenge with this approach is the difficulty involved in defining high risk. While genetic linkages potentially account for 9 percent of prostate cancer incidence, these individuals are not identifiable preoperatively at this time. In addition, while family history indicates a higher likelihood of developing prostate cancer, the consensus group concluded that individual risk could not be determined with enough precision to base a trial on family member participation. Dr. Bostwick added that utilizing recruits from the general population requires an extremely large number of participants and a long follow-up period, and introduces a number of confounding factors.

Dr. Bostwick suggested that PIN, particularly high-grade, can act as a biomarker. He explained that during a recent meeting of pathologists, a list of other promising biomarkers was generated. He characterized the list as manageable. Dr. Bostwick clarified that the list is not reflective of definitive data, but represents merely the best possibilities based on the current state of information. The only serum marker on the list is serum PSA, which Dr. Bostwick asserted is an excellent marker that should be included in chemoprevention trials. Other types of markers, which can all be observed in needle biopsies, include morphometric markers, proliferation markers, and DNA ploidy assessment. Expression of the C-*erb* B-2 oncogene and microvessel density are also believed to be strong biomarkers. He indicated that a problem with these biomarkers is that they are prognostic factors for prostate cancer which are being applied as biomarkers for a surrogate endpoint (i.e., PIN) in chemopreventive therapy.

Dr. Bostwick cited that one advantage to using morphometry to explore biomarkers is that it is microscope based, which supplies quick and effective results that are subject to little user variation in input procedures due to impending standardization of practices among many laboratories. By capturing the image with a camera or digitizing tablet, the computer can separate out individual cells or groups of cells. Researchers can then assess the nuclear roundness and chromatin patterns as well as explore the nucleus and nucleolus for numerous abnormalities that can be quickly and objectively detected. Dr. Bostwick indicated that the main problem with morphometry, according to the consensus group, is that at least 100 nuclei are needed for accurate assessments to be made.

Dr. Bostwick reported that the pathologists reached a consensus on some issues regarding tissue fixation processing, cutting, and staining for examination of biomarkers. Standards that were agreed upon include having: 1) internal age- and procedure-matched controls; 2) external batch controls; 3) rigorous definitions for classifying results; 4) minimum percentages required for flow cytometry gating to label DNA as nondiploid versus diploid; and 5) large enough population groups to perform valid statistical analyses. He suggested that these standards are appropriate for examination of morphometric and immunohistochemical markers as well as DNA ploidy when Feulgea stains are used.

Dr. Bostwick concluded by asserting that chemoprevention trials are feasible, practical, and potentially cost-effective through the use of surrogate endpoint biomarkers. He described the best target populations as those individuals with precancerous lesions. In addition, if effective mechanisms for identifying high-risk individuals are developed, they would also be appropriate trial participants. Dr. Bostwick remarked that the surrogate endpoint biomarkers for prostate cancer are very similar to those cited for breast cancer.

Questions and Answers

Dr. Calabresi asked whether finasteride has been shown to reduce PIN. Dr. Bostwick replied that only one unpublished study, which was conducted in Canada, has supported the role of finasteride in reducing PIN in humans. In addition, an additional study among rats evidenced a similar effect of finasteride; however, it is not universally accepted that human and rat lesions are similar. Dr. Bostwick remarked that the histologic changes associated with the use of finasteride suggest that this drug will elicit similar results as androgen deprivation

therapy. He concluded by noting that while many researchers agree that finasteride will reduce PIN, there are currently no published human data available to support this conclusion.

Dr. Calabresi queried whether anyone has measured beta FGF in blood serum or urine. Dr. Bostwick responded that most growth factors have been examined to some extent and that while many are interesting prospects for biomarkers, the pathologists at the consensus meeting felt that the data were too limited to recommend their inclusion on the list. Dr. Bostwick stressed that the panel concluded that if biomarkers are to be examined, it is important to look at them in combination to explore possible interactions.

Dr. Bishop asked Dr. Bostwick to describe the correlation between serum PSA and PIN. Dr. Bostwick replied that in individuals with PIN but no invasive cancer, serum PSA counts are not elevated. He indicated that this was shown through a definitive study conducted by Johns Hopkins, which his group has confirmed. He added that a few studies concluded that PIN can increase serum PSA; however, these were confounded by the presence of cancer. He reasserted that it is currently agreed among most researchers that PIN cannot increase serum PSA. He remarked that this is a logical conclusion, since PIN secretions would most likely be carried out through the urine.

XXII. EFFICIENCY AND COST-EFFECTIVENESS RESEARCH ON CANCER SCREENING—DR. NICOLE URBAN

Dr. Urban began by informing members that she has worked for 10 years with the Fred Hutchinson Cancer Research Center, which housed the first NCI-funded cancer control research unit. She commented that she works with an extremely diverse team of researchers, including a statistician, gynecologists, an economics student, and the NCI Breast Cancer Screening Consortium. Dr. Urban indicated that the goal of her presentation would be to provide new perspectives regarding cancer control research.

Dr. Urban introduced three primary questions that she would address. The first is whether ovarian cancer screening can be cost-effective. She suggested that some researchers immediately reject this possibility because ovarian cancer is so rare, but noted that she would offer data to challenge this reaction. Another question is whether simulation models are useful tools for evaluating screening modalities. Dr. Urban asserted that while she encounters many people who believe simulation models to be completely useless, she does not agree. The final question explores what can be learned from research regarding the promotion of breast cancer screening efforts within communities.

Dr. Urban told members that to decrease mortality among a defined population through screening efforts, three things are necessary: 1) effective therapies; 2) screening protocols that can detect early-stage cancer, which is more treatable; and 3) good participation among members of the target population.

Dr. Urban presented the rationale supporting early detection efforts. Before individuals develop invasive cancer, which eventually becomes symptomatic and can be detected clinically, they will have asymptomatic, noninvasive cancer. The challenge to research on early detection is that the duration of this earlier stage is not directly observable. Dr. Urban

explained that screening efforts attempt to detect cancer earlier than clinical diagnosis can occur. The term "detectable preclinical phase" is applied to the period when a cancer can be diagnosed preclinically. Dr. Urban asserted that preclinical diagnoses are vital to early detection. She added that lead time, the difference between screen-detected and clinically detected cancer, is dependent not only upon the modality that is employed—for example, mammography versus clinical breast exam—but also the frequency of the procedure. More frequent mammography, for example, typically confers longer lead times.

Dr. Urban defined a screening strategy as cost-effective if it produces results that are worth the additional cost attributed to its use. This necessitates that an appropriate baseline be determined to act as a point of comparison. In addition, appropriate measures must be determined for the equation of cost-effectiveness, which includes a denominator and numerator. The denominator measures health effects, such as years of life saved, quality-adjusted years of life saved, or additional people who quit smoking. The numerator consists of an equation that includes: 1) cost of applying the screening strategy; 2) expected cost of diagnosing positive cases; and 3) the expected savings attributable to the early detection. She recognized that evaluating these measures requires considerable work and suggested that this may be a barrier to investigating cost-effectiveness.

Dr. Urban pointed out that cost-effectiveness is a relative concept. Determining the efficiency of a modality requires that it be compared with other strategies. For example, to evaluate the relative cost-effectiveness of screening for breast cancer, six strategies might be evaluated, such as mammography at three different time intervals, every 3 years, 2 years, or annually, alone or in combination with clinical breast exam (CBE). Dr. Urban indicated that a strategy is "efficient" if no other strategy saves as many years of life at lower cost.

Dr. Urban explained that the cost per year of life saved can be expected to increase as the number of years added rises. The cost-effectiveness equation will determine which strategy will cost the least for the same amount of years added. Dr. Urban noted that evaluating cost-effectiveness is particularly useful for screening strategies, which are typically employed based on effective results in clinical trials. She emphasized that trials do not examine cost-effectiveness and, therefore, do not provide grounds for determining the relative efficiency of various modalities. She suggested that data from trials that have already been conducted, could be used in cost-effectiveness analyses to select the most efficient strategy. Dr. Urban also recommended that prior to initiating a clinical trial, an effort should be made to determine what the most efficient strategies might be.

Dr. Urban indicated that her motivation for choosing to study ovarian cancer screening efforts is that a trial for this cancer has not yet been conducted, although the Prostate, Lung, Colorectal and Ovarian Cancer (PLCO) trial is being planned. While ovarian cancer is rare, the potential for mortality reduction is considerable because in relative terms a higher percentage of women with ovarian cancer die from this disease than do women with breast cancer. In addition, the years of life lost attributed to death from both cancers are comparable.

Dr. Urban described the PLCO trial, in which 74,000 women will be randomized into four rounds of annual screening. One of the detection modalities this trial will explore is the biannual pelvic exam, which despite support for its use is not believed to be effective. Performing an annual screening using CA-125 will also be investigated. Researchers Skates

and Singer used a stochastic simulation model to evaluate this strategy, which revealed that its use may add approximately 3 years of life per case occurring in the screened population. Dr. Urban reported that the last strategy being examined is the transvaginal ultrasound, which is probably the most feasible screening method. This judgment was supported by the conclusions reached at an ovarian cancer consensus conference, which put forth that while there is no evidence that screening is an effective approach for decreasing mortality attributable to ovarian cancer, transvaginal ultrasound may ultimately prove to be a useful strategy.

Dr. Urban explained that she decided to research the cost-effectiveness of transvaginal ultrasound because she believed that even if the trial proved that it was an effective method for early detection, it might be too expensive to perform annually among the entire female population of the nation. She simulated the protocol's screening effort by replicating the work of Skates and Singer, except for a few modifications, including adding transvaginal ultrasound and altering some of the CA-125 strategies. Dr. Urban reported that the study required that she: 1) describe the disease among the target population; 2) examine the natural history of the disease in the absence of screening; 3) characterize the tumor in terms of sensitivity and specificity of the selected modalities; 4) detail the chosen screening strategies; and 5) analyze the data to determine the results. She added that the cost-effectiveness analysis that was utilized included discounting within the equation.

Dr. Urban stated that she explored the accuracy of the assumptions this study necessitated, including the sensitivity and specificity of the modalities, on which there was some peer-reviewed literature. In addition, she told members that she utilized Skates and Singers' estimates of the duration of the disease stages, which they based on the opinions of several oncologists. Dr. Urban noted that her team performed a sensitivity analysis to determine the possible effects of using these estimates and advised that more information was needed regarding length of disease stages. The final assumption involved the cost of performing the screening strategies, on which the literature was inconsistent. She remarked that the PLCO trial assumed much lower costs than some of literature indicated.

Dr. Urban outlined the five strategies that she examined, of which three are single-modality strategies, including transvaginal and transabdominal ultrasound alone, use of elevated (above 35 u/ml) CA-125 (a replication of Skates and Singer as well as the PLCO trial modality), and use of serial measures to detect rising (doubling) CA-125, which has been shown by Zurawski to improve specificity. She presented the other two multimodal strategies, which both involve the use of CA-125 as a first step. If this step is found to be positive, ultrasound is performed. Dr. Urban asserted that this protocol allows the CA-125 to be used to identify high-risk individuals, which limits the participants who receive ultrasound to those who may benefit from it most. In addition, this strategy reduces the number of false-positive results; however, a drawback is that because the sensitivity of CA-125 is relatively low, some cancers may not be detected. The first multimodal strategy involves annual screening. The final multimodal strategy performs the above strategy every 6 months, which may be useful when assuming short disease stages, as in ovarian cancer.

Dr. Urban presented the results of her simulation-model-based study of screening women aged 50-80. She pointed out that the study assumed that ultrasound is 100 percent effective in detecting cancer but that among at least half of the participants there is some lag

time between development of preclinical cancer and its detectability by ultrasound. She also noted that annual screening by ultrasound is very expensive, at over $300,000 per year of life saved. Dr. Urban remarked that this figure is probably too high for this approach to be supported. She added that the approach does not offer a definitive point at which the cost to save a year of life is too high, it only allows comparison among various strategies.

Dr. Urban reported that detecting cancer using CA-125 at the levels Skates and Singer employed is less expensive, approximately $100,000 per year of life saved, than the ultrasound modality; however, only half as many lives are saved. The strategy which employed rising (doubling) CA-125 as a criterion for positivity revealed promising results. Dr. Urban concluded that the only efficient strategies are the multimodal ones. However, she remarked that the simulation model is not perfect, and will be refined to produce higher confidence in the results it achieves.

Because limited information was available for exploring the accuracy of the assumptions involved in the study, Dr. Urban said her team completed a variety of internal and external validity tests. Internal validation was used to check the model's estimates of the effects of screening. The model's estimates of gains on years of life saved were based on survival distributions for each stage of diagnosis within 10-year age categories. Dr. Urban explained that to ensure that the simulation model was generating accurate results, the team directly estimated years of life saved attributable to earlier diagnosis using the same SEER data outside the model, and examined whether this was consistent with the findings generated by the model. Dr. Urban stated that they similarly used the SEER Medicare file to determine savings in treatment costs attributable to earlier detection. She noted that for shifts from distant to regional stage, there was no savings in treatment costs. Modest savings were found for shifts from regional and distant to early stage.

Dr. Urban indicated that her team decided to closely examine the results for the strategy that employed doubling as a criterion for positivity of CA-125, which was performing better than expected. They concluded that this was a result of the lack of a large variance in CA-125 levels over time within each participant, despite the variance across the target population. Dr. Urban and her colleagues determined that this low variance was a result of relying upon the distributional assumptions that Skates and Singer had used, which worked for that team because they had not used doubling as a criterion for positivity of CA-125. Dr. Urban continued by informing members that her group then examined data from a screening trial program in which CA-125 was being performed at 6-month intervals. The data from this study revealed much higher variance within each participant. Dr. Urban indicated that her team performed sensitivity analyses using these new data in terms of the length of the disease stages. They also examined the results achieved when combining the factors of slow disease progression, which favored ultrasound based on the study's assumptions, and high variance within each woman, as well as reducing the price of ultrasound, to determine whether the strategy of ultrasound used alone could be made more cost-effective than the multimodal strategies. None of these modifications could achieve this goal; the multimodal strategies performed better than ultrasound alone.

Dr. Urban stated that one of the goals of the simulation had been to predict the results of the PLCO trial. She added that the trial will provide a great deal of useful information on the performance of both ultrasound and CA-125.

Dr. Urban described the results of a prevalence screening program, reported by Jacobs et al., in 1993, that her group replicated to prospectively validate the findings of their simulation model for the multimodal strategy. She noted that this screening program used a multimodal strategy that was very similar to the one her team employed, except that it included a cutoff of 30 u/ml for CA-125, not a doubling. To simulate the program using the model, a 5-year interval was assumed, which would allow prevalence to be observed, and a 1- and 2- year follow-up. She reported that the effectiveness results of the replication were very encouraging. While the program had screened 22,000 women and detected 19 cancers, the model had predicted that 17 cancers would be found. In addition, 11 of the cancers were screen-detected in the program and eight in the model, which, Dr. Urban noted, indicates that her team was working with conservative estimates regarding the performance of the CA-125 screening modality. The trial reported eight interval cancers, none of which were missed by ultrasound, but seven of which were missed by the CA-125 modality and one as a result of the participant's failure to return for ultrasound. Dr. Urban said that the model had predicted nine interval cancers. She pointed out that one of the advantages of a simulation program is that it allows researchers to examine the source of the interval cancer, which is not possible in a screening program or a clinical trial. Dr. Urban's group determined that four of the nine interval cancers were missed by the CA-125 and that five of them were new cancers. Dr. Urban commented that the team is continuing to compare the results of the simulations with other elements of this study. She added that the model must be refined, particularly in terms of the high number of CA-125 false-positive results, which was nearly three times that found in the screening program. This will improve the cost-effectiveness findings for the multimodal strategy even further.

Dr. Urban informed the Board that her team has received funding to develop a simulation model for breast cancer. She remarked that as a result of the extensive literature regarding breast cancer, there will be much more opportunity for replication and comparison. She highlighted one issue the model will explore—how promoting breast cancer screening will affect cost-effectiveness. It will examine how much money should be allocated to attracting more participants to screening efforts and, if funding is limited, identify the best way to use the available money. An attempt will be made to determine whether it is more cost-effective to expand screening efforts to target younger women or to achieve higher participation among the original target population.

Dr. Rimer commented that it was recommended during a trans-NIH meeting last year that cost measures be integrated into all prevention studies.

XXIII. UPDATE ON NCAB AD HOC WORKING GROUP ON NCI INTRAMURAL PROGRAMS—DR. J. MICHAEL BISHOP

Dr. Bishop informed the Board that the committee's name has been changed to reflect its focus on the performance of science, rather than the management structure of the intramural research program (IRP) at NCI. He thanked Dr. Kalt and NCI staff for assisting he and his cochair, Dr. Calabresi, and expressed concern at the continued growth in membership of the committee, which may encumber it from obtaining consistent participation by all members.

Dr. Bishop explained that the committee's origin lies in the Marks-Cassell Report, a Congressional mandate for evaluation of the IRP of the NIH that recommended similar evaluations for each individual Institute as well. The general goal is to assess which science programs are most successful and cost-effective.

Dr. Bishop said the committee will focus on funding strategies, scientific quality control, and leadership. The following major areas of inquiry have been identified thus far: *peer review*, i.e., quality, response, methods of conduct, appropriateness; *scientific leadership*, i.e., status, effectiveness; and *allocation of funds* at varying levels of the intramural and extramural research programs, i.e., methods, criteria, basis in scientific quality.

The committee will also look into program redundancy, but Dr. Bishop emphasized that he is not referring to downsizing. He said the committee has been asked to examine the possible effects on the IRP of downsizing the new clinical center, and how the NCI can respond proactively. Dr. Bishop proposed evaluating personnel resource issues, such as recruiting and proper levels of renewal of vital, young scientists.

Dr. Bishop informed the Board that the committee has refined its charge and scheduled its monthly meeting agenda into May. He noted that the meetings are closed to the public, but that all NCAB members are welcome to attend. Dr. Calabresi mentioned that the committee's next meeting would be held that night and the following morning. In response to a question by Dr. Salmon, Dr. Bishop explained that the committee's January meeting is not connected with the NCAB meeting.

Dr. Rimer noted that the next NCAB meeting following the January session will not be held until May, but offered Dr. Kalt's aid in planning travel and lodging for any Board members who wish to attend ad hoc committee meetings.

XXIV. CONTINUING AND NEW BUSINESS AND FUTURE AGENDA ITEMS—DR. BARBARA RIMER

Dr. Rimer reminded the Board that future agenda items were suggested as part of the Activities and Agenda Subcommittee report. She updated the Board on the meeting with Dr. Calabresi, Dr. Broder, and Dr. Varmus, relaying Dr. Varmus' intent to communicate with the Board with respect to establishing future committees, and to attend the May meeting to discuss the ad hoc committee's report and other issues. Dr. Calabresi remarked that Dr. Varmus had expressed interest in the SENCAP report, particularly in the increase in research funding, and seemed eager to help the NCAB within the constraints of his budget. Dr. Calabresi said he was pleased with the overall tenor of the meeting.

Dr. Rimer mentioned that she had recently represented the NCAB on an advisory committee to the NIH Director. Among the topics discussed were funding of certain kinds of embryo research; innovative funding methods, including a biomedical research service to pay for certain types of physician investigators; and a new track for clinical research to help physician investigators understand epidemiologic research.

Dr. Rimer suggested that the Board discuss the Towns Report at the January meeting, after members have an opportunity to review the Report. She cited two issues of concern to the Board—process issues about decision making (to be addressed by Dr. Broder in January) and the complex issue of future mammography policy. Dr. Rimer asked whether NCI should be involved in a consensus conference with the Agency for Health Care Policy Research and other agencies and how to effectively communicate the decision to the public.

Dr. Rimer then opened the topic of the Towns Report and NCI's mammography screening policy for brief discussion by the Board. Board members were given copies of responses to the Towns Report from NCI and the investigators, Drs. Cornelia Baines and Tony Miller.

Dr. Salmon asked about funding for the AHCPR and a screening study in Great Britain. Dr. Grever answered that those programs will be funded with 1995 dollars. The AHCPR funds were marked for a 2-year consensus process to create clinical practice guidelines; the British study was a contracted effort to increase the speed of collecting mortality data to allow an early decision about whether to conduct a larger study. Dr. Grever described it as a small step to allow early assessment of the value of a study, not a commitment to a long-term study. Dr. Salmon noted that the Board would like to be kept updated about such projects, regardless of whether they are contracts or grants.

Dr. Rimer read a paragraph from a letter by Ms. Visco regarding the Towns Report, "Please note that the report is not exactly correct when it states that the National Breast Cancer Coalition opposes NCI revised guidelines. Our position was that we understand the results of the studies, and we demand that all premenopausal women be part of critical trials testing different screening mechanisms, including mammography, MRI, ultrasound, clinical breast exam, self-exam, and whatever else is available." Dr. Rimer said she has received substantial feedback from the Board on this issue, and plans to discuss it further in January, along with the possibility of the Board's involvement in a consensus meeting process.

Dr. Rimer drew attention to an important article in last week's *New England Journal of Medicine* about variability in mammography reading with a rejoinder by Dr. Daniel Kopans. Dr. Day, who read the article, pointed out that there has always been variability in interpretation of diagnostic tests by different observers with respect to TB slides, MRIs, x-rays, etc., so the fact that interobserver variation occurs in mammography is not a surprising finding. Drs. Salmon and Day noted that such variability can be reduced with training and choice of film type.

Dr. Sondik asked the Board, when reading the Towns Report and corresponding responses, to be as balanced as possible about the issue of mammography screening in 40- to 49-year-olds and leave the judgment in the individual's hands. He pointed out that current data are conclusive for women over age 50, but not for women ages 40 to 49, so it would be inappropriate for NCI to make a recommendation one way or the other. He stressed the importance, as a science agency, of being straightforward with the public to maintain credibility and trust.

Dr. Sigal pointed out that the practical effect of leaving the decision to the individual is that the affluent would have a choice, while women without means would not. Dr. Sondik

explained that this is the reason AHCPR is investigating guidelines rather than making a statement of science. Dr. Day queried whether the analyses are valid in light of the small sample size, film quality, length of observation, and other factors. He expressed his feeling that many technical and scientific issues remained unresolved, and asked, for the January meeting, whether SEER data can be obtained about change in stage at diagnosis, age at diagnosis, and the relative use of mammography before and after the public controversy.

Dr. Greenwald promised to bring any available data, but cautioned that there is not likely to be much on point at this time. Dr. Rimer offered to attempt to obtain data from a cohort survey by Dr. Ellen Sieber of UNC alumni regarding their reaction to the controversy. Dr. Sondik suggested asking for the critiques of the Canadian study and responses by the Canadian investigators. He highlighted the fact that mammography did find cancers in women aged 40 to 49 and changed survival, but did not affect mortality, suggesting that survival is an imperfect measure for a screening study. Dr. Sondik said he considers the key question to be whether screening has a beneficial effect in reducing mortality, but he acknowledged a point made by Dr. Day that one can measure factors other than mortality change, such as morbidity.

Dr. Day noted a polarization of viewpoints in the medical community and the general public on the issue of mammography screening. He emphasized that communication is critical because of the impact it has on people's behavior. Dr. Rimer said she has conducted focus groups with women in their 40s to survey their reactions, so she is acutely aware of the impact communications have on women and the loss of credibility of the scientific community.

Dr. Goldson expressed his view that the NCAB's wavering position on this issue is damaging to the NCI. He expressed his belief that the Board should not make conclusive statements that have such strong effect on the public when it does not believe the data are conclusive.

Dr. Salmon echoed Dr. Day's point about morbidity in relation to the advantages of early detection. He reminded the Board of the B06 trial, indicating that lumpectomy (which requires early detection) with radiation was as effective and less mutilating than mastectomy. Dr. Day said he feels it is inconsistent for the Board to represent that early mammography is important in one way and not another. Dr. Rimer pointed out that using morbidity as the defining variable, instead of mortality, would affect the way NCI assesses all screening tests.

Dr. Broder described the issues as complicated, and emphasized the need for NCI, as a scholarly organization, to set standards and make the best decisions possible with the facts available, even when those facts may lead to unpopular conclusions.

Dr. Broder referred to an editorial in the *New England Journal of Medicine* by Dr. Kopans that suggested implementation of a two radiology rule, i.e., at least two radiologists should read a mammogram and pool their efforts. He said the Board should consider the possibility that mammography in younger women may demand a higher level of technical expertise than realized, requiring a consultative or specialty approach. Dr. Broder opined that the Board must look at the available data to make such recommendations and must consider the potential differences in results at a given institution versus in the general population.

Dr. Broder described the Canadian study as well done by knowledgeable people in good faith. He said it is not constructive to degrade or dismiss the study and suggested reexamining the structure of how screening mammography is performed. Dr. Broder emphasized the larger principle of accepting feedback from clinical trials, and that a respectable basic laboratory scientist cannot ignore experiments. He observed that special effort should be made to learn from scientific results that are opposite of expectations.

Dr. Broder denounced speculation that NCI's analysis of the mammography issue was linked with reimbursement issues. He stated his personal view that any informed woman who wants mammography should have it and should be entitled to reimbursement.

Dr. Rimer thanked Dr. Broder for his comments, remarking that there appears to be no easy solution to this difficult policy question. She announced plans to discuss the mammography issue and consensus conference further in January with the additional information from SEER and the meta-analysis, looking at women in their 40s.

Dr. Kalt reminded the Board that Dr. Jerome Green, Director of the Division of Research Grants, will retire in the early spring of 1995, but will hopefully attend a future NCAB meeting to discuss review of clinical research.

Dr. Kalt also informed Board members that his own Division is being downsized, forcing a careful look at processes, particularly program review. He outlined a plan to streamline the P01 grant review process by forming a group of reviewers, applicants, NCI staff from review and programs, and NCAB members to examine the current process and decide how it can be made less labor-intensive. Dr. Kalt said this approach will be discussed further in January, and will probably be applied to other major areas of work effort that pertain to peer review.

XXV. ADJOURNMENT

Dr. Rimer requested that all Board members remain for a brief closed session. She thanked all those in attendance and the presenters. There being no further open business, Dr. Rimer adjourned the 92nd National Cancer Advisory Board Meeting at 4:54 p.m.

Date

Dr. Barbara Rimer, Chairperson