

Report of the Joint Working Group on Quantitative In Vivo Functional Imaging in Oncology

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and
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Editors

Faina Shtern, M.D.
U.S. Public Health Service
Office on Women's Health

Daniel Winfield, M.S.
Research Triangle Institute

Contributors

Britton Chance, Ph.D., D.Sc.
University of Pennsylvania Medical Center

Steven E. Harms, M.D.
University of Arkansas for Medical Sciences

Jerry M. Collins, Ph.D.
U.S. Food and Drug Administration

Rakesh K. Jain, Ph.D.
Harvard University Medical School

William C. Eckelman, Ph.D.
National Institutes of Health Clinical Center

Stephen Lam, M.D., F.R.C.P.C.
University of British Columbia

Jeffrey L. Evelhoch, Ph.D.
Wayne State University

James L. Mulshine, M.D.
National Cancer Institute

Michael S. Feld, Ph.D.
Massachusetts Institute of Technology

Richard L. Wahl, M.D.
University of Michigan

John N. Weinstein, M.D., Ph.D.
National Cancer Institute

Acknowledgments

Daniel Sullivan, M.D.
National Cancer Institute

Wanda K. Jones, Dr.P.H.
U.S. Public Health Service
Office on Women's Health

Robert E. Wittes, M.D.
National Cancer Institute

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Planning Committee

The U.S. Public Health Service's Office on Women's Health and the National Cancer Institute gratefully acknowledge the members of the planning committee, who devoted time and effort to the development of the conference agenda.

Chair:	Faina Shtern, M.D.	U.S. PHS Office on Women's Health
Program Staff:	Susan M. Ascher, M.D. Daniel Sullivan, M.D.	U.S. PHS Office on Women's Health National Cancer Institute
Members:	Jeffrey Abrams, M.D. Joseph Aisner, M.D. David Benaron, M.D. Irving J. Bigio, Ph.D. Jerry M. Collins, Ph.D. William C. Eckelman, Ph.D. Jeffrey L. Evelhoch, Ph.D. Ellen G. Feigal, M.D. Michael S. Feld, M.D. S. Percy Ivy, M.D. Rakesh K. Jain, Ph.D. Kenneth A. Krohn, Ph.D. James L. Mulshine, M.D. Sarah J. Nelson, Dr.ReR.Nat. Donald B. Plewes, Ph.D. Rebecca Richard-Kortum, Ph.D. Bruce Rosen, M.D., Ph.D. Edward C. Trimble, M.D. Bruce J. Tromberg, Ph.D. Richard L. Wahl, M.D. John N. Weinstein, M.D., Ph.D. Michael Zalutsky, Ph.D.	National Cancer Institute Cancer Institute of New Jersey Stanford University Los Alamos National Laboratory U.S. Food and Drug Administration National Cancer Institute Wayne State University National Cancer Institute Massachusetts Institute of Technology National Cancer Institute Harvard University Medical School University of Michigan National Cancer Institute University of California at San Francisco University of Toronto University of Texas at Austin Massachusetts General Hospital National Cancer Institute University of California at Irvine University of Michigan Medical Center National Cancer Institute Duke University Medical Center

Speakers and Panelists

Session 1: Summary of Clinical Issues

Session Leaders: Stephen Lam, M.D., F.R.C.P.C.
John N. Weinstein, M.D., Ph.D.

Speakers: Carmen J. Allegra, Ph.D.
Molly Brewer, D.V.M., M.D.

Thomas W. Burke, M.D.
Anthony V. D'Amico, M.D., Ph.D.
Thomas Julian, M.D.
Karen Kelly, M.D.
David Kelsen, M.D.
Stephen Lam, M.D., F.R.C.P.C.
Michelle Follen Mitchell, M.D.

University of British Columbia
National Cancer Institute
National Cancer Institute
M.D. Anderson Cancer Center, University of Texas-
Houston Medical School
M.D. Anderson Cancer Center
Harvard University Medical School
Allegheny University
University of Colorado
Memorial Sloan-Kettering Cancer Center
University of British Columbia
M.D. Anderson Cancer Center

Session 2: State of the Art in Functional Imaging

Session Leaders: Steven E. Harms, M.D.
Richard L. Wahl, M.D.

Speakers: Britton Chance, Ph.D., D.Sc.
James G. Fujimoto, Ph.D.
Randall A. Hawkins, M.D., Ph.D.
Hedvig Hricak, M.D., Ph.D.
Steven M. Larson, M.D.
Thomas J. Miller
James B. Mitchell, Ph.D.
Mitchell D. Schnall, M.D., Ph.D.
Bruce J. Tromberg, Ph.D.

University of Arkansas for Medical Sciences
University of Michigan
University of Pennsylvania Medical Center
Massachusetts Institute of Technology
University of California at San Francisco
University of California at San Francisco
Memorial Sloan-Kettering Cancer Center
Carl Zeiss, Inc.
National Cancer Institute
University of Pennsylvania Medical Center
University of California at Irvine

Session 3: Tumor Biology/Pathology

Session Leaders: Jeffrey L. Evelhoch, Ph.D.
Rakesh K. Jain, Ph.D.

Speakers: Robert C. Bast, Jr., M.D.
R. Edward Coleman, M.D.
Rakesh K. Jain, Ph.D.
Stanley J. Korsmeyer, M.D.
Steven K. Libutti, M.D.
Sarah J. Nelson, Dr.Re.R.Nat.
Larry Norton, M.D.
Janet S. Rasey, Ph.D.
Rebecca Richards-Kortum, Ph.D.
Bruce Rosen, M.D., Ph.D.
Anthony F. Shields, M.D., Ph.D.

Wayne State University
Harvard University Medical School
M.D. Anderson Cancer Center
Duke University Medical Center
Harvard University Medical School
Dana-Farber Cancer Institute, Harvard University
National Cancer Institute
University of California at San Francisco
Memorial Sloan Kettering Cancer Center
University of Washington Medical Center
University of Texas at Austin
Massachusetts General Hospital
Wayne State University

Session 3: Tumor Biology/Pathology (continued)

MRI/MRS Panel

<i>Facilitator:</i>	Jeffrey L. Evelhoch, Ph.D.	Wayne State University
<i>Panelists:</i>	Stephen G. Bown, M.D., F.R.C.P.	University College London Medical School
	Robert Brasch, M.D.	University of California at San Francisco
	Truman R. Brown, Ph.D.	Fox Chase Cancer Center
	Steven E. Harms, M.D.	University of Arkansas for Medical Sciences
	Hans Hawighorst, M.D.	German Cancer Research Center
	Michael V. Knopp, M.D.	German Cancer Research Center
	John Kurhanewicz, Ph.D.	University of California at San Francisco
	Nina A. Mayr, M.D.	University of Iowa College of Medicine
	Michal Neeman, Ph.D.	Weizmann Institute of Science, Israel
	Sarah J. Nelson, Dr.Re.R.Nat.	University of California at San Francisco
	Bruce Rosen, M.D., Ph.D.	Massachusetts General Hospital
	Harold M. Swartz, M.D., Ph.D.	Dartmouth Medical School

Nuclear Medicine/PET Panel

<i>Facilitator:</i>	R. Edward Coleman, M.D.	Duke University Medical Center
<i>Panelists:</i>	Farrokh Dehdashti, M.D.	Washington University School of Medicine
	Janet F. Eary, M.D.	University of Washington
	William C. Eckelman, Ph.D.	National Institutes of Health Clinical Center
	Randall A. Hawkins, M.D., Ph.D.	University of California at San Francisco
	Steven M. Larson, M.D.	Memorial Sloan-Kettering Cancer Center
	Ronald D. Neumann, M.D.	National Institutes of Health
	Janet S. Rasey, Ph.D.	University of Washington Medical Center
	Anthony F. Shields, M.D., Ph.D.	Wayne State University

Optics Panel

<i>Facilitator:</i>	Rebecca Richards-Kortum, Ph.D.	University of Texas at Austin
<i>Panelists:</i>	David A. Benaron, M.D.	Stanford University Medical School
	James G. Fujimoto, Ph.D.	Massachusetts Institute of Technology
	Amir H. Gandjbakhche, Ph.D.	National Institute of Child Health and Human Development
	Calum E. MacAulay, Ph.D.	British Columbia Cancer Agency
	Gerwin J. Puppels, Ph.D.	Erasmus University Rotterdam
	Bruce J. Tromberg, Ph.D.	University of California at Irvine
	Tuan Vo-Dinh, Ph.D.	Oak Ridge National Laboratory

Session 4: Pharmacology

<i>Session Leaders:</i>	Jerry M. Collins, Ph.D.	U.S. Food and Drug Administration
	William C. Eckelman, Ph.D.	National Institutes of Health Clinical Center
<i>Speakers:</i>	Irving J. Bigio, Ph.D.	Los Alamos National Laboratory
	Christopher H. Contag, Ph.D.	Stanford University Medical Center
	Merrill J. Egorin, M.D.	University of Pittsburgh Cancer Institute
	Joanna S. Fowler, Ph.D.	Brookhaven National Laboratory
	Jerry D. Glickson, Ph.D.	University of Pennsylvania
	William N. Hait, M.D., Ph.D.	The Cancer Institute of New Jersey
	Walter N. Hittelman, Ph.D.	M.D. Anderson Cancer Center
	Kenneth A. Krohn, Ph.D.	University of Washington
	David R. Piwnica-Worms, M.D., Ph.D.	Washington University Medical School
	Hubert van den Bergh, Ph.D.	Swiss Federal Institute of Technology in Lausanne
	Robert M. Weisskoff, Ph.D.	EPIX Medical, Inc.

Session 4: Pharmacology (continued)

MRI/MRS Panel

<i>Facilitator:</i>	Robert Brasch, M.D.	University of California at San Francisco
<i>Panelists:</i>	Kathleen M. Donahue, Ph.D.	Medical College of Wisconsin
	Jerry D. Glickson, Ph.D.	University of Pennsylvania
	Orhan Nalcioğlu, Ph.D.	University of California at Irvine
	Paul S. Tofts, D.Phil.	University College London
	Robert M. Weisskoff, Ph.D.	EPIX Medical, Inc.

Nuclear Medicine/PET Panel

<i>Facilitator:</i>	William C. Eckelman, Ph.D.	National Institutes of Health Clinical Center
<i>Panelists:</i>	Ronald G. Blasberg, M.D.	Memorial Sloan-Kettering Cancer Center
	Joanna S. Fowler, Ph.D.	Brookhaven National Laboratory
	Kenneth A. Krohn, Ph.D.	University of Washington
	David R. Piwnica-Worms, M.D., Ph.D.	Washington University Medical School
	Michael Zalutsky, Ph.D.	Duke University Medical Center

Optics Panel

<i>Facilitator:</i>	David A. Benaron, M.D.	Stanford University Medical School
<i>Panelists:</i>	Irving J. Bigio, Ph.D.	Los Alamos National Laboratory
	Wai-fung Cheong, M.D.	Pharmacyclics, Inc.
	Christopher H. Contag, Ph.D.	Stanford University Medical Center
	Thomas H. Foster, Ph.D.	University of Rochester Medical Center
	Robert M. Hoffman, Ph.D.	AntiCancer, Inc.
	Allen R. Oseroff, M.D., Ph.D.	Roswell Park Cancer Institute
	Eva M. Servick-Muraca, Ph.D.	Purdue University
	Willem M. Star, Ph.D.	University Hospital Rotterdam
	Hubert van den Bergh, Ph.D.	Swiss Federal Institute of Technology in Lausanne

Session 5: Cellular and Molecular Targets

<i>Session Leaders:</i>	Michael S. Feld, Ph.D.	Massachusetts Institute of Technology
	James L. Mulshine, M.D.	National Cancer Institute
<i>Speakers:</i>	Ronald G. Blasberg, M.D.	Memorial Sloan-Kettering Cancer Center
	Janet F. Eary, M.D.	University of Washington
	Michael S. Feld, Ph.D.	Massachusetts Institute of Technology
	Rakesh K. Jain, Ph.D.	Harvard University Medical School
	Thomas J. Meade, Ph.D.	California Institute of Technology
	James L. Mulshine, M.D.	National Cancer Institute
	Ralph Weissleder, M.D., Ph.D.	Massachusetts General Hospital
	Michael Zalutsky, Ph.D.	Duke University Medical Center

MRI/MRS Panel

<i>Facilitator:</i>	Ralph Weissleder, M.D., Ph.D.	Massachusetts General Hospital
<i>Panelists:</i>	Robert J. Gillies, Ph.D.	University of Arizona Cancer Center
	Michael V. Knopp, M.D.	German Cancer Research Center
	King C.P. Li, M.D.	Stanford University
	Thomas J. Meade, Ph.D.	California Institute of Technology
	Brian D. Ross, Ph.D.	University of Michigan

Session 5: Cellular and Molecular Targets (continued)

Nuclear Medicine/PET Panel

<i>Facilitator:</i>	Kenneth A. Krohn, Ph.D.	University of Washington Medical Center
<i>Panelists:</i>	Ronald G. Blasberg, M.D.	Memorial Sloan-Kettering Cancer Center
	Janet F. Eary, M.D.	University of Washington
	Randall A. Hawkins, M.D., Ph.D.	University of California at San Francisco
	John A. Katzenellenbogen, Ph.D.	University of Illinois
	David R. Piwnica-Worms, M.D., Ph.D.	Washington University Medical School
	Michael Zalutsky, Ph.D.	Duke University Medical Center

Optics Panel

<i>Facilitator:</i>	Michael S. Feld, Ph.D.	Massachusetts Institute of Technology
<i>Panelists:</i>	Molly Brewer, D.V.M., M.D.	M.D. Anderson Cancer Center, University of Texas- Houston Medical School
	Daniel L. Farkas, Ph.D.	Carnegie Mellon University
	Maryann Fitzmaurice, M.D., Ph.D.	Case Western Reserve University
	Thomas Flotte, M.D.	Massachusetts General Hospital
	Joseph A. Izatt, Ph.D.	Case Western Reserve University
	E. Neil Lewis, Ph.D.	National Institutes of Health
	Michele Follen Mitchell, M.D.	M.D. Anderson Cancer Center

Introduction

In March 1996, the U.S. Public Health Service's Office on Women's Health (USPHS OWH) established a Federal Multi-Agency Consortium for Imaging and Other Technologies to Improve Women's Health. This consortium facilitates technology transfer from laboratories to patients. The membership of the consortium includes, but is not limited to, the National Cancer Institute, Food and Drug Administration, Health Care Financing Administration, Central Intelligence Agency, Department of Defense, Department of Energy, and National Aeronautics and Space Administration. The activities of this consortium have been critical for sharing expertise, resources, and technologies by multiple government agencies for the advancement of novel breast imaging for early diagnosis of cancer, such as digital mammography, magnetic resonance imaging (MRI) and spectroscopy (MRS), ultrasound, nuclear medicine, and positron emission tomography (PET), as well as related image display, analysis, transmission, storage, and minimally invasive biopsy and treatment.

The consortium sponsored a public conference entitled "Technology Transfer Workshop on Breast Cancer Detection, Diagnosis, and Treatment" convened on May 1-2, 1997.¹ During this meeting, consortium members developed recommendations for the scientific and technologic projects critical for advancement of novel breast imaging.

Subsequently, USPHS OWH and the National Cancer Institute (NCI) jointly sponsored the establishment of several working groups to define even further the research agenda in the areas of breast imaging examined by the May 1997 conference. These groups focused on specific recommendations for research priorities and technology development and transfer opportunities across multiple areas of breast imaging:

- Nonionizing imaging (e.g., ultrasound, MRI, optical imaging) for the development and testing of novel modalities free of ionizing radiation
- Functional imaging (e.g., PET, MR imaging and spectroscopy, and optical imaging and spectroscopy) for the achievement of comprehensive in vivo cellular and ultimately molecular biologic tissue characterization

- Image processing, computer-aided diagnosis, and three-dimensional digital display for enhanced lesion visualization and radiologic image interpretation
- Telemammography, teleradiology, and related information management for facilitated expert consultations
- Digital X-ray mammography, with an emphasis on digital display technologies and workstation design for image interpretation
- Image-guided diagnosis and treatment for potential replacement of open surgery with minimally invasive and/or noninvasive interventions
- Methodological issues for diagnostic and screening trials for imaging technologies, with specific focus on the development of computer models for analysis of patient outcomes and cost-effectiveness.

This report summarizes the results of the Conference of the Joint USPHS OWH/NCI Working Group on Quantitative In Vivo Functional Imaging in Oncology. Approximately 139 international scientific leaders, representing clinical practice, academic research, government agencies and laboratories, and medical imaging system manufacturers, attended the meeting held January 6-8, 1999, in Washington, D.C. This paper describes the group's findings and recommendations.

Goals of the Joint USPHS OWH/NCI Working Group

- 1) To review the clinical state of the art and challenges in cancer prevention, initiation, progression, and treatment.
- 2) To examine leading edge technologies in quantitative in vivo functional imaging in oncology, including current and future clinical applications and technical challenges.

- 3) To outline a research agenda, including short-, intermediate-, and long-term priorities in technology development, basic research, and clinical testing.
- 4) To translate oncology needs into technical requirements for the advancement of new and/or emerging technologies.

To achieve these goals, the meeting opened with two overview sessions, from both oncologic and technical perspectives:

Session 1: Oncology Overviews examined the clinical state-of-the-art, current, and future needs in cancer prevention, initiation, progression, and treatment in breast, genito-urinary, and aerodigestive tumors.

Session 2: State of the Art in Functional Imaging examined current and potential impact of MRI/MRS, nuclear medicine/PET, optical, and other technologies on patient care.

These opening overviews were followed by sessions that addressed three major areas of oncology where functional imaging may provide important information for tumor characterization and treatment planning, including prediction and early assessment of tumor response.

Session 3: Biology/Physiology examined tumor structure and pathophysiology, with specific focus on the following topics:

- Evaluation of tumor response
- Correlation of standard assessment of treatment response to functional imaging data
- Disease detection and staging at diagnosis-correlation of functional imaging to histopathology, molecular biology, and other clinical measures
- Investigation of hypoxia, necrosis/irreversibility of cell death, and apoptosis
- Elucidation of the alternations associated with changes in cell proliferation and angiogenesis.

Session 4: Pharmacology examined current oncologic needs and the potential role of functional imaging in providing noninvasive information on standard and intratumoral drug uptake, distribution, and metabolism. The following specific topics were addressed:

- Evaluation of standard/conventional pharmacokinetics and pharmacodynamics (PK/PD)
- Study of intratumoral PK/PD
- Comparison of classical pharmacology, which may be a better predictor of toxicity, versus tumor pharmacology, which may be a better predictor of response.

Session 5: Molecular Targets examined how molecular targets and pathways can be studied by noninvasive imaging, which may be uniquely linked to the elucidation of molecular mechanisms of cancer and guidance of treatment and drug development. The following specific topics were examined:

- Identification of the appropriate molecular targets, receptors, and binding sites
- Evaluation of novel treatments such as gene therapy.

Working Session: Working group members met to formulate consensus reports describing the current state of the art and recommendations for future priorities in technology development and related research.

Summary Session: The consensus reports were presented during the summary session. The reports addressed (1) the current state of the art and fundamental clinical/technical roadblocks, (2) technical parameters required to meet current and future clinical needs, and (3) future priorities in technology development and related basic and clinical research.

Subsequent to the working group meeting, its leaders developed written summary reports with input from session participants. These summary reports have been integrated into this article with editorial input from the working group chairs and sponsors.

References

1. Asher S, Shtern F, Winfield D, et al. Final report of the technology transfer workshop on breast cancer detection, diagnosis, and treatment. Washington, D.C., USA. May 1-2, 1997. *Acad Radiol* 1998; 5:S465-501.

Session 1: Summary of Clinical Issues

Among adults, cancer is one of the most common causes of death in the United States, second only to cardiovascular disease. The estimated deaths for 1998 and the 5-year survival rates of the major cancers affecting women and men are shown in Table 1. Participants in the Joint Working Group on Functional Imaging in Oncology examined current knowledge on the prevention, diagnosis, and treatment of these cancers and discussed how cutting-edge technology in quantitative in vivo functional imaging may address the clinical needs to improve the outcome for these patients.

Table 1: U.S. Cancer Deaths and 5-Year Survival Rates (1998)

	Deaths	5-Year Survival
Lung cancer	160,100	13%
Ovarian cancer	14,500	42
Colorectal cancer	56,500	60
Cervical and endometrial cancer	11,200	70
Breast cancer	43,900	82
Prostate cancer	39,200	90

Source: American Cancer Society^{1,2}

Two types of developments in functional imaging are likely to have great impacts on cancer incidence and mortality: (1) improvements in existing methods (e.g., in X-ray mammography for breast cancer, in pap smear and coposcopy for cervical cancer) and (2) development of fundamentally new approaches to detection of early, preinvasive cancers (e.g., for lung and ovarian cancers, which have low survival rates and no proven early detection methods). For early detection programs to be cost-effective, they must be able to determine the biological behavior of preinvasive lesions to predict which lesions will likely progress to invasive cancer and require intervention.

For patients who are diagnosed as having invasive cancer, improvement in the following areas will likely have the greatest potential to improve the outcome:

- Staging methods to determine the extent of local invasion and regional or systemic metastasis
- Identifying treatment that is most likely to succeed

- Monitoring the short- and long-term response to treatment and characterizing the response to provide insight into response mechanisms
- Determining the reasons for treatment failure
- Studying the mechanisms of action and pharmacokinetics of chemopreventive and anti-neoplastic agents to allow rapid development of effective new agents.

The clinical needs and the extent to which quantitative in vivo functional imaging is being developed or applied vary for various tumor sites. For example, the challenges for early detection of superficial epithelial tumors in the respiratory and gastrointestinal tracts or cervix are not the same as those for more deep-seated tumors such as breast, ovarian, and prostate cancers. The clinical needs and the opportunities to apply new, cutting-edge imaging technologies to address these areas are outlined below for each tumor site.

Breast Cancer

Cancer of the breast is one of the most common malignancies in the United States, with about 180,000 new cases diagnosed each year. One out of every eight American women will develop breast cancer, and mortality is second only to that from lung cancer.

Early detection is critical for effective treatment. Thirty percent of all tumors identified by X-ray mammography are noninvasive, and 30% of invasive tumors identified are 1 cm in diameter or smaller. Patients with tumors 1 cm or less in size have a greater than 90% long-term survival. Although conventional, film-based X-ray mammography is of great importance to breast cancer screening, there are a number of limitations. Approximately 15% of breast cancers are undetectable by X-ray mammography. The utility of X-ray is particularly limited in younger premenopausal women (40-49 years and younger) and those on hormone-replacement therapy, who have a higher probability of radio-dense breast tissue. Residual disease following lumpectomy is difficult to assess. The overall positive predictive value of mammography is 45% to 55%. Sixty to eighty percent of breast biopsies following mammography turn out to be benign. Premalignant lesions cannot be differentiated from benign lesions, necessitating a

large number of biopsies. Failure to detect a premalignant lesion may deny a patient preventive therapy, and failure to diagnose breast cancer at an early stage increases overall treatment costs and litigation.

Another issue is the delivery of low doses of ionizing radiation in women carrying the ataxia-telangiectasia gene who may be more susceptible to the carcinogenic effects of radiation.³ This gene is carried by about 1% of the population. It has been shown that between 9% and 18% of all persons with breast cancer in the U.S. are heterozygote carriers of the ataxia-telangiectasia gene. Therefore, it is desirable to develop nonionizing radiation-based methods for early breast cancer screening.

The major potential for ultrasound is the ability to differentiate cyst formations from other types of lesions (e.g., solid abnormalities) in the breast parenchyma. Ultrasound, however, is limited by its resolution. Small tumors (less than 10 mm) are difficult to visualize using ultrasound. Further, it is difficult to differentiate a benign solid fibroadenoma from a malignant lesion.

Breast magnetic resonance imaging, which is described later in this paper, is a very promising method for screening and staging breast cancer. The examination is relatively expensive and, hence, unsuitable for routine screening, but it is widely available and could be appropriate for improving the characterization of problem cases (e.g., dense breasts). Lesions can be localized and biopsied under MRI guidance;⁴ however, the interventional MRI equipment required is expensive and usually limited to major university hospitals

Several optical technologies are also promising for breast cancer detection, including fluorescence spectroscopy^{5,6} and Raman scattering.⁷

Cytological or histological examination of tissues sampled by the core needle biopsy (CNB) procedure is critical to differentiate benign from malignant disease.⁸ CNB can be performed either stereotactically or under ultrasound. CNB has a high level of accuracy;⁹ however, because of lesion location, CNB may not be feasible in all patients.

Staging of breast cancer requires axillary dissection for a pathologic assessment of nodal status. This surgical process carries the morbidity of pain, lymph edema, and weakness. Sentinel node technology may

limit the number of unnecessary dissections by 70%; however, there are false-negatives and a significant learning curve.

In recent years, surgical procedures for breast carcinoma have become less invasive. The clinical challenge in connection with breast-conservation procedures is to identify tumor borders correctly and to determine if axillary lymph nodes are involved. Establishing disease-free margins can often be difficult with heterogeneous lesions. Often the procedure is placed “on hold” while the anesthetized patient and the surgeon await results from frozen sections. An instant, in situ diagnostic probe, in addition to speeding up the process, would permit the surgeon to check surrounding tissue areas and accessible axillary nodes.

Mastectomy and lumpectomy can leave cosmetic defects (although these can be partly ameliorated by reconstructive surgery). If the lesion(s) and their extent can be accurately determined, however, they can be treated locally (with microwaves, radio waves, or lasers), cryotherapy, brachytherapy (interstitial radiotherapy), photodynamic therapy, or local drug injection without resecting the adjacent normal tissue once these therapies are proven to be effective.

Response to neoadjuvant chemotherapy or hormone therapy is currently followed clinically. Although extremely effective in reducing primary tumor size and nodal status, several months or cycles of therapy are required to detect a response.¹⁰ During this time, alternative neoadjuvant therapy choices may be delayed. It is therefore important to have the means to determine the treatment response rapidly.

Advances in chemotherapy and hormone therapy have improved the survival of patients with breast cancer, but the reasons for treatment failure after long-term remission are poorly understood. Better characterization of the tumor response to treatment using functional imaging may provide an answer.

Lung Cancer

Lung cancer is the most common cause of cancer death worldwide, with a mortality rate exceeding that of colon, breast, and prostate cancers combined. Former heavy smokers retain an elevated risk for lung cancers even years after they stop smoking. With a large reservoir of current and former smokers and the increasing incidence of lung cancers among women, lung cancer will remain a major health issue for the

next several decades. Currently, the only patients who achieve long-term survival are those with stage 0 (5-year survival exceeding 90%) or stage I (5-year survival between 70% and 80%) disease. They constitute only a small minority of the lung cancer population, however. Furthermore, for those given curative treatment, second primary cancers occur at up to 4% per year. The 5-year survival of patients with second primary cancer is less than 50%.

Previous screening studies for early lung cancer had significant limitations.^{11,12} The studies, which were conducted only in men using 30-year-old technologies, assessed whether sputum cytology could be equivalent to a cervical pap test. Assessment of the efficacy of chest X ray was the secondary goal, and there was no unscreened control group. Sample size was calculated based on a 50% reduction in mortality with screening. The studies did not have enough statistical power to detect smaller benefits. Despite randomization, there were a higher number of lung cancer cases in the screened group even after screening was stopped. Therefore, these studies neither supported nor refuted the benefit of screening. Since then, sputum cytology examination has become more standardized,¹³ and more sensitive methods have been developed.^{14,15}

New methods for in vivo functional imaging of preinvasive cancer in the central airways can be useful, as demonstrated by the development of autofluorescence bronchoscopy that is several times more sensitive than conventional white-light bronchoscopy.¹⁶ Lesions as small as 1 mm can be localized. Autofluorescence bronchoscopy is the first optical imaging method approved by the U.S. Food and Drug Administration (FDA) for detection and localization of preinvasive lung cancer since the development of white-light endoscopy.

For peripheral lung cancer, spiral chest computed tomography (CT) has lowered the limit of detection resolution from about 7 mm for chest X ray to 1 mm. It also, in large part, has overcome the problem of “blind-spots” caused by overlapping structures.¹⁷ Recent case studies showed that the detection rate for early stage I lung cancer is about 1% to 2%. Although spiral chest CT is several times more sensitive than chest X ray, the prevalence of nonmalignant lesions such as those caused by previous tuberculosis, histoplasmosis, or coccidioidomycosis can be present in 20% to 40% of cases, depending on geographic location. Differentiation between benign and malignant nodules is problematic for nodules less

than 1 cm. Positron emission tomography is a promising tool to distinguish between malignant and inflammatory lesions; however, scanners of the highest resolution and sensitivity are not widely available, and tracers are not readily accessible at reasonable cost. MRI is relatively insensitive to calcified or fibrotic nonmalignant lesions, which can be an advantage. In addition, MRI also can differentiate between a blood vessel and a nodule. Its sensitivity for pulmonary nodules smaller than 5 mm, however, needs to be improved. The use of bronchoscopic optical methods to localize small peripheral lung nodules for precise biopsy without resorting to needle biopsy, thoracoscopic biopsy, or open lung biopsy has not been explored but is theoretically feasible as a minimally invasive method for the diagnosis of peripheral lung lesions.

For detection and localization of early preinvasive cancers in the central airways, quantitative fluorescence imaging methods have the potential to reduce interobserver variation and to improve diagnostic accuracy. In vivo morphological characterization of normal and abnormal bronchial tissues without biopsy (e.g., by optical coherence tomography [OCT] or confocal microscopy) has promise for studying the response of preneoplastic lesions to chemopreventive agents.

In patients with invasive lung cancer, the current staging methods using mediastinoscopy and chest CT are imprecise, as illustrated by the poor survival of patients with stage IB (T2N0M0) disease. Even for this supposedly localized stage, the 5-year survival is less than 60%. For patients with unresectable lung cancer, there is no curative treatment. The search for more effective antineoplastic agents has been hampered by the inability to study the mechanism of action of new and existing agents in vivo and to determine the reasons for treatment failure.

PET imaging has been approved for staging of lung cancer. It is currently the most sensitive in vivo method to detect regional or systemic metastases. Both PET and MRI imaging have the potential for studying the mechanism of action of new and existing antineoplastic agents at the cellular and even molecular levels. They may provide insight into the reasons for failure of current regimens.

PET imaging has been used to study the neurochemistry of tobacco addiction. Even with a combination of counseling, nicotine replacement, and antidepressants, the long-term success rate of

smoking cessation is only 20% to 40%. Further research into the mechanism of nicotine addiction using in vivo imaging would contribute to prevention of lung cancer and other tobacco-related diseases.

Prostate Cancer

The state of the art for the curative treatment of clinically localized adenocarcinoma of the prostate is radical prostatectomy (RP) or external beam radiation therapy (RT). The role of androgen suppression and other systemic therapies remain under investigation until current prospective randomized trials are completed and reported. The ability to identify patients with clinically significant localized disease who are curable using these approaches, however, is limited. Pretreatment prostate-specific antigen (PSA), biopsy Gleason score, and American Joint Commission on Cancer T-stage partially predict post-treatment PSA.¹⁸ To better select patients likely to be cured by RP or RT, however, more specific serum, tissue, and/or imaging parameters are needed.

Transrectal ultrasound, body coil MRI, and CT are no longer recommended for the staging of localized prostate cancer because of their low sensitivity and specificity.¹⁹ Although endorectal coil MRI has been shown in selected institutions to improve prediction of organ-confined disease and postoperative PSA, its routine use is not justified until it is standardized.²⁰ In addition, microscopic extraprostatic disease is not visualized; the limit of detection is on the order of 1 cm³.²¹ Magnetic resonance spectroscopy has promise when coupled with endorectal coil MRI for differentiation of malignant from benign prostatic epithelial cells; however, its use is currently limited because only a small region of the prostate (less than 1 cm³) can be sampled in a reasonable time (less than 1 hour).²² Moreover, the results need to be validated.

Other imaging tools under development include color Doppler imaging (CDI) and optical-based imaging. It has been suggested that CDI can predict the biologic aggressiveness of prostate cancer.²³ Specifically, CDI has been reported to identify areas of enhanced blood flow in regions of prostate cancer. Increased capsular flow was associated with a more advanced pathologic stage and a higher rate of PSA progression after RP in one study.²³

Molecular genetic studies have identified genetic mutations that may be of prognostic importance. For example, there is evidence that p27 underexpression may predict for a more biologically aggressive, and

therefore less curable, prostate cancer after RP.²⁴ In addition, a subgroup analysis of the Radiation Therapy Oncology Group 8,610 trial has suggested the presence of a mutated p53 gene predicts significantly lower survival after RT and androgen suppression when compared with RT alone.²⁵ The potential advantage of functional imaging at the molecular level is that the entire prostate gland can be imaged while with biopsy, the most aggressive area of the cancer may happen to be missed.

Recent data suggest that some p53 mutations in malignant prostatic epithelial cells are present in the primary tumor, lymph node, and bone metastasis, whereas other mutations of this gene are found only in the primary tumor.²⁶ Once molecular imaging and appropriate techniques become available, minimally invasive image-guided focal therapy could be used to ablate specific regions of metastasis-prone cells within the prostate gland. Focal ablative therapy could be followed by minimally invasive global therapy to the whole prostate gland to eliminate the less biologically aggressive disease. A more precise combination of less toxic therapies may lead to an improved therapeutic ratio for some patients.

Colorectal Carcinoma

Colon cancer is the second leading cause of cancer deaths in the United States, with approximately 150,000 new cases each year and approximately 56,000 deaths. Progress in treating this disease has been limited and major advances in the surgical or medical treatment of colorectal cancer are not anticipated in the immediate future. However, several reports have demonstrated that endoscopy and polypectomy can substantially reduce the incidence and mortality of the disease.

Gastroenterologists and surgeons perform colonoscopy for the purpose of removing polyps and/or obtaining mucosal biopsies to make a specific diagnosis determined primarily by a pathologist. The final histological diagnosis ultimately determines the medical or surgical therapy and thus is essential to the patient and treating physician. Biopsies may be obtained for several indications including determining the type of polyp observed at colonoscopy. Patients with invasive colorectal cancers, if surgical candidates, are most often referred for surgical resection. Pathological stage of the resected tumor often determines the requirements for adjuvant chemotherapy. With respect to surveillance for colorectal cancer, patients with only hyperplastic

polyps at colonoscopy and no history of colorectal adenomas may not require subsequent colonoscopy; whereas patient in whom adenomatous polyps are removed may be enrolled in an endoscopic surveillance program.

Adenomatous polyps are the most important precursors of colorectal cancer. The practice of removing polyps during colonoscopy is based on the assumption that removing them will prevent the progression to cancer. The concept, often referred to as the adenoma-carcinoma sequence, has never been directly proven. The most important evidence substantiating the theory came from the National Polyp Study, a cohort study in which the incidence of colorectal cancer in over 1,418 patients who had a complete colonoscopy and one or more adenomatous polyps removed was compared with the incidence of colorectal cancer in three reference groups.²⁷ Colonoscopic polypectomy resulted in a lower than expected incidence of colorectal cancer. The results of this landmark study not only substantiated the adenoma-carcinoma hypothesis but also served to justify the widely held practice of colonoscopic polypectomy.

Endoscopists are poor at predicting the histological basis of colorectal polyps detected at colonoscopy or sigmoidoscopy based solely on their endoscopic appearance. A prospective study of 3,281 patients examined over a six-month period detected 465 patients (14%) with one or more polyps.²⁸ The sensitivity of the endoscopic “diagnosis” for adenomas was 80% and the specificity 71%. Thus, without better ways to differentiate between hyperplastic versus adenomatous polyps, the endoscopist has to remove all polyps at endoscopy for complete histological analysis.

Optical techniques, particularly fluorescence, have been shown to be effective in diagnosing adenomatous polyps in real time during colonoscopy. Such techniques are also promising for detecting flat pre-cancerous lesions, which are difficult to detect on endoscopy. A fluorescence imaging colonoscope for wide-area screening and surveillance has also been developed.²⁹ Such techniques should be explored further to determine if polypectomies can be more specifically directed to polyps that are at high risk of progression to invasive cancer.

In colorectal cancer, thymidylate synthase is an important clinical target. Patients with lower levels of thymidylate synthase in their cancers have a far better

disease-free and overall survival rates than do patients with higher levels. Inhibition of thymidylate synthase improves clinical outcome. The overall response rate of current therapy for advanced colorectal carcinoma using “thymidylate synthase-directed” therapy (i.e., therapy with 5-fluorouracil plus leucovorin or one of the antifolate analogs that specifically inhibits thymidylate synthase) is approximately 25%.³⁰ Median survival of treated patients is approximately 11 to 12 months, but very few patients are cured of their disease with chemotherapy. More recently, topoisomerase I inhibitors and oxaliplatin-based therapies, as well as convenient oral regimens using the thymidylate synthase-directed reagents, have been under development. Combination drug therapy has resulted in consistent response rates around 50% in patients with advanced colorectal carcinoma, and it is possible that such therapies will also enhance the beneficial effects of 5-fluorouracil plus leucovorin in the adjuvant setting.

A host of new antifolate agents that specifically target thymidylate synthase are in clinical development. A number of investigators are also examining the use of DNA-damaging agents in combination with agents (e.g., Fas and TRAIL ligands) that specifically stimulate apoptosis. These agents stimulate cellular receptors, leading to activation of the caspase cascade and apoptosis. These agents have been reported to be synergistic with traditional cytotoxic agents. Functional imaging will allow more precise selection of these agents for individualized prognosis and treatment.

Esophageal Cancer

In the esophagus, the epithelium can pass from normal through various grades of dysplasia to carcinoma in situ. A classic example of this sequence is provided by Barrett’s esophagus. Patients with Barrett’s esophagus are usually surveyed with endoscopy and multiple random biopsies at intervals. A major drawback of this approach is the known difficulty of recognizing high-grade dysplasia or carcinoma in situ using conventional white-light endoscopy. What is needed is an objective method that can reliably detect these hidden abnormalities so that future cancers can be prevented or even directly treated at the earliest, most curable stage.

To overcome the limitation of conventional white-light endoscopy, several optical technologies are currently under investigation. Examples of these

include laser induced fluorescence spectroscopy/imaging,³¹⁻³⁴ elastic scattering spectroscopy,³⁵⁻³⁸ Raman spectroscopy,^{39,40} and OCT.⁴¹⁻⁴³

For the majority of patients with esophageal cancer who present with advanced disease, transesophageal fine needle aspiration biopsy of mediastinal lymph nodes guided by endoscopic ultrasound appears useful for preoperative staging. Preliminary studies suggest that functional imaging techniques such as PET may provide noninvasive alternatives.

Ovarian Cancer

Ovarian cancer is the second most common gynecologic malignancy, but it causes more deaths than any of the other gynecologic cancers.⁴⁴ Although breast cancer has a much higher incidence, the death rate from ovarian cancer is higher. Seventy percent of ovarian cancers are diagnosed in International Federation of Gynecology and Obstetrics (FIGO) stage III (intra-abdominal disease) or stage IV (disease above the diaphragm) because of lack of symptoms, inaccessibility of the ovaries, and poor screening techniques. These stages have 5-year survivals of 20% and less than 5%, respectively. Preinvasive ovarian cancer has never been documented, although it is hypothesized to exist because most epithelial cancers have preinvasive phases.⁴⁵ Genetic testing can identify high-risk women appropriate for screening,⁴⁶ but current techniques such as ultrasound lack adequate positive predictive value.⁴⁷⁻⁴⁹ Predictive value is the most important epidemiologic parameter for a screening test. Likewise, ultrasound has a poor positive predictive value. CA-125 is elevated in fewer than 50% of patients with stage I ovarian cancer.^{50,51}

New optical imaging techniques hold significant promise both for early diagnosis and for further understanding of the carcinogenic process in the ovary. Early work with reflectance spectroscopy, which is based on structural changes in tissue, shows excellent ability to distinguish malignant from benign areas on the ovarian surface and subsurface. Fluorescence spectroscopy, which is based on biochemical differences in tissue, also shows promise. Both techniques show significant differences in oxy and deoxy spectra from normal and neoplastic tissue. It would be useful to develop a probe with multiple optical modalities capable of scanning the ovaries to detect areas of early carcinogenesis.

Standard treatment for advanced ovarian cancer includes an initial laparotomy for both staging and debulking^{52,53} and adjuvant chemotherapy. Survival is significantly improved when optimal surgical cytoreduction can be achieved prior to adjuvant chemotherapy.^{52,54,55} Other diagnostic methods, such as CA-125,⁵⁶⁻⁵⁹ ultrasound, cul-de-sac aspiration,⁶⁰ and CT^{52,53} are less effective. An accurate staging at the initial laparotomy is crucial for appropriate management. Many patients with advanced disease (stages III and IV) initially respond to standard therapy, as evidenced by negative second-look laparotomy or laparoscopy (SLL). For up to 50% of patients with negative second look, the disease will recur, and they will succumb to it.^{52,53,61} Survival is not significantly increased with SLL. In light of these false-negative SLLs, there is a need for improved diagnostic tests to detect occult manifestations of disease. This need is amplified by the increasing desire to replace SLLs with less invasive procedures.⁶²

Cervical Cancer

The combination of modern screening methods with increasingly effective treatment modalities has led to a dramatic reduction in cervical cancer mortality over the past five decades. Despite the significant decline in incidence and mortality of invasive cervical cancer, however, there has been an increase in the incidence of cervical intraepithelial neoplasia (CIN).⁶³ This observation reflects improved screening and detection methods, as well as a true increase in the incidence of precancerous cervical lesions. The CIN terminology divides cervical cancer precursors into three groups: CIN I, II, and III corresponding to mild, moderate and severe (carcinoma in situ) dysplasia, respectively.⁶³ Another commonly used terminology invokes the terms low-grade squamous intraepithelial lesion (L-SIL) for CIN I and high-grade SIL (H-SIL) for CIN II and III.⁶³ The mortality of cervical cancer is expected to rise by 20% in the next decade unless further improvements are made in current screening and detection techniques.⁶⁴

Current screening techniques for CIN and invasive cervical cancer include the single or combined use of exfoliative cytology (pap smear) and colposcopy⁶⁵ (i.e., the examination of reflected light from the uterine cervix and adjacent tissues by low-magnification microscopy⁶⁶). A false-negative pap smear error rate of 15% to 55% is associated with insufficient sampling and with reading errors.⁶⁷⁻⁶⁹ The diagnostic accuracy of colposcopic impression in

patients with abnormal cervical cytology ranges from 37.5% to 89.9%, and underdiagnosis of microinvasive disease was reported to be up to 100%.⁷⁰ Improvements in colposcopy could save patients from multiple biopsies, a standard-of-care practice that contributes to the estimated \$6 billion per year cost of CIN detection. Hence, there is a strong need for more powerful diagnostics suitable for a wide spectrum of physicians and nurse practitioners, allowing more sensitive and cost-effective screening and follow-up of premalignant cervical lesions. This is a particularly important concept in that CIN affects women of lower socioeconomic status, young women, and young mothers—people who may have less access to traditional laboratory diagnostics and surgical procedures.

In an attempt to fill this need, various techniques such as cervicography,^{71,72} speculoscopy,⁷³⁻⁷⁵ digital image colposcopy,^{76,77} and a polarprobe-based method⁷⁸ have been developed to discriminate among benign, premalignant, and malignant cervical tissues. Optical techniques are particularly promising because they can provide quantitative information on molecular composition and tissue structure. For example, recent advances in fluorescence spectroscopy,^{66,79-82} photon migration spectroscopy⁸³ and elastic scattering spectroscopy⁸⁴ provide encouraging evidence that optical methods can noninvasively probe the metabolic state of cervical tissues *in vivo*. The sensitivity and specificity of fluorescence spectroscopy matches that of colposcopy.

Optical spectroscopy and imaging methods also can be performed with a variety of exogenous probes. For example, topically administered molecular probes (e.g., the photosensitizer ALA, ligand-specific fluorophores, gene-expression probes) that are compatible with optical spectroscopy tools (e.g., fluorescence, photon migration, elastic scattering, Raman) can be developed to enhance the specificity and sensitivity of the technique to molecular events in the transformation process. With automation of assay readout and data analysis, this approach could reduce the need for clinical expertise, minimize the number of biopsies, and improve precision. In the case of light-activatable exogenous probes, diagnosis and therapy could easily be combined in a cost-saving single-step procedure.

With respect to staging, for patients with invasive cervical cancer, the current FIGO methodology underestimates surgical stage in 25% to 67% of the

patients and overestimates it in about 2%. MRI is more sensitive than CT for preoperative staging, and there is room for innovative imaging techniques.

Endometrial Cancer

Most women with endometrial cancer have early symptoms and diagnosis. Approximately two-thirds of patients have low-grade tumors confined to the uterus. The remaining one-third have lesions with a propensity for extrauterine spread and recurrence. Most women with extrauterine disease have small metastases that are not routinely detected preoperatively. An elevated preoperative CA-125 level may be useful in predicting which patients have extrauterine disease.⁸⁵⁻⁸⁷ Current imaging techniques such as MRI can reliably identify myometrial invasion with an accuracy of about 80%.^{88,89} The accuracy is less than 20% in detecting extrauterine disease, however.

The functional anatomy of regional lymph node spread in endometrial cancer is still incompletely understood. Gaps in current imaging methods exist in the detection of regional lymph node metastases. The utility of lymphangiograms for detection of nodal involvement is limited and is problematic, especially for elderly patients or those with diabetes. Involvement of the peritoneal cavity is difficult to assess because of the large surface area, small disease volume, and frequent occurrence of benign abnormalities. Distant metastases to other organs, such as liver, lung, bone or brain, also are difficult to detect because small volume disease often escapes detection by current imaging methods. Recurrence after treatment is mostly diagnosed by symptoms and physical examination. Although some cures are still possible after a single-site failure, better means of surveillance for recurrent disease would be useful.

Research Priorities

Key research priorities for *in vivo* imaging technologies to improve the diagnosis and treatment of cancer lie in the following areas:

For Early Detection

- Develop imaging modalities that do not require ionizing radiation. For example:
 - Optical technologies (e.g., fluorescence or elastic scattering spectroscopy or imaging, OCT, confocal microscopy, Raman spectroscopy) for endometrial and cervical cancer

- Low-cost MRI methods and equipment for breast cancer.
- Test novel approaches, such as in vivo optical probes, for detection of early preinvasive cancer in high-risk groups (e.g., heavy smokers with abnormal biomarkers in sputum cells or abnormal spiral chest CT; patients with Barrett's esophagus; individuals with two first-degree relatives with ovarian cancer and BRCA gene mutation).
- Develop novel technologies for cheaper and more accurate detection of high-grade preinvasive lesions (e.g., optical probes for bronchial and cervical lesions).

For Diagnosis

- Develop functional imaging techniques for real-time tissue characterization, both for primary diagnosis and for perioperative enhancement of surgical precision (e.g., detecting multicentric, multifocal disease and assessing the extent of primary tumor or axillary lymph node involvement during breast conservation procedures).
- Differentiate between benign and malignant disease (e.g., assessing small [<7 mm] pulmonary nodules identified by spiral chest CT).
- Perform studies to evaluate whether functional measurements can be more predictive than conventional cytology or histopathology for diagnosis and for monitoring field carcinogenesis.

For Monitoring Chemoprevention

- Adapt functional methods for detection and characterization of preinvasive lesions as noninvasive, nonperturbing alternatives to biopsy. For example, chemoprevention trials provide the opportunity to correlate in vivo optical signatures with histopathology, molecular biomarkers and quantitative pathology using computer-assisted image analysis of exfoliated cells and tissue sections.

For Treatment

- Aid in selection of therapy by evaluation of in vivo molecular markers. This development could dramatically improve the statistics of clinical trials (i.e., reducing the number of patients

required) in addition to the obvious potential benefits for standard practice.

- Detect cellular and tissue changes at an early stage to direct therapy.
- Monitor response to treatment, permitting a quicker switch to other therapy for nonresponders.
- Direct the application of nonexcision modalities, including but not limited to interstitial laser photocoagulation, photodynamic therapy, and regional delivery of antineoplastic drugs.
- Aid in characterizing mechanisms of response, especially long-term response, after treatment.
- Provide means for rapidly evaluating new potential antineoplastic agents during preclinical studies and clinical trials.

References

1. Landis SH, Murray T, Bolden S, Wingo PA. Cancer Statistics, 1998. CA: A Cancer Journal for Clinicians 1998; 48:10.
2. American Cancer Society: Cancer Facts and Figures-1996. American Cancer Society Publication, 1996.
3. Swift M, Morrell D, Massey RB, Chase CL. Incidence of cancer in 161 families affected by ataxia-telangiectasia. New England Journal of Medicine 1991; 325:1831-1836.
4. Esserman L, Hylton N, Yassa L, et al. Utility of magnetic resonance imaging in the management of breast cancer: Evidence for improved preoperative staging. J Clin Oncol 1999; 17:110-119.
5. Gupta PK, Majumder SK, Uppal A. Breast cancer diagnosis using N_2 laser excited autofluorescence spectroscopy. Lasers in Surgery and Medicine 1997; 21:417-422.
6. Yang Y, Celmer EJ, Zurawska-Szczepaniak M, Alfano RR. Excitation spectrum of malignant and benign breast tissues: a potential optical biopsy approach. Lasers in the Life Sciences 1997; 7:249-265.
7. Manoharan R, Shafer K, Perelman L, et al. Raman spectroscopy and fluorescence photon migration for breast cancer diagnosis and imaging. Special issue Symposium in Print of Photochemistry and Photobiology, "Innovations in Optical Techniques for Biodiagnostics" 1998; 67:15-22.

8. Willis SL, Ramzy I. 1995. Analysis of false results in a series of 835 fine-needle aspirates of breast lesions. *Acta Cytologica* 39:858-864.
9. Parker SH, Burbank F, Jackman RJ, et al. Percutaneous large-core breast biopsy: A multi-institutional study. *Radiology* 1994; 193:359-364.
10. Fisher B, Brown A, Mamounas E, et al. Effect of preoperative chemotherapy on local regional disease in women with operable breast cancer: Findings from National Surgical Adjuvant Breast and Bowel Project B-18. *J Clin Oncol* 1997; 15:2485-2493.
11. National Cancer Institute Cooperative Early Lung Cancer Detection Program: Summary and Conclusions. *Am Rev Resp Dis* 1984; 130:565-567.
12. Kubik A, Polak J. Lung cancer detection: results of a randomized prospective study in Czechoslovakia. *Cancer* 1986; 57:2427.
13. Kennedy TC, Proudfoot SP, Franklin WA, et al. Cytopathological analysis of sputum in patients with airflow obstruction and significant smoking histories. *Cancer Res* 1996; 56:4673.
14. Tockman MS, Mulshine JL, Piantadosi S, et al. Prospective detection of preclinical lung cancer: Results from two studies of hnRNP overexpression. *Clinical Cancer Research* 1997; 3:2237.
15. Payne P, Sebo TJ, Doudkine A, et al. Sputum screening by quantitative microscopy: a re-examination of a portion of the National Cancer Institute Cooperative Early Lung Cancer Study. *Mayo Clin Proc* 1997; 72:697-704.
16. Lam S, Kennedy T, Unger M, Miller Y, et al. Localization of bronchial intraepithelial neoplastic lesions by fluorescence bronchoscopy. *Chest* 1998; 113:696-702.
17. Kaneko M, Eguchi K, Ohmatsu H, et al. Peripheral lung cancer: screening and detection with low-dose spiral CT versus radiography. *Radiology* 1996; 201:798.
18. D'Amico AV, Whittington R, Broderick GA, et al. Biochemical Outcome after Radical Prostatectomy, External Beam Radiation Therapy, or Interstitial Radiation Therapy For Clinically Localized Prostate Cancer. *J Am Med Assoc* 1998; 280:969-974.
19. Rifkin MD, Zerhouni EA, Gatsonis CA, et al. Comparison of Magnetic Resonance Imaging and Ultrasonography in Staging Early Prostate Cancer. *NEJM* 1990; 323:621-626.
20. D'Amico AV, Whittington R, Tomaszewski JE, et al. A critical appraisal of the role of endorectal coil magnetic resonance imaging in the prediction of pathologic stage and postoperative prostate specific antigen failure in patients with clinically organ confined prostate cancer. *Journal of Clinical Oncology* 1996; 14:1770-1777.
21. Jager GJ, Ruijter ETG, vd Kaa CA, et al. Dynamic turboFLASH subtraction techniques for contrast-enhanced MR imaging of the prostate: correlation with histopathologic results. *Radiology* 1997; 203:645-652.
22. Kaji Y, Kurhanewicz J, Hricak H, et al. Localizing Prostate Cancer in the presence of postbiopsy changes on MR images: Role of proton MR spectroscopic imaging. *Radiology* 1998; 206:785-781.
23. Ismail M, Petersen RO, Alexander AA, Newschaffer C, Gomella LG. Color Doppler imaging in Predicting the Biologic Behavior of Prostate Cancer: Correlation with Disease Free Survival. *Urology* 1997; 50:906-912.
24. Cordon-Cardo C, Koff A, Drobnjak M, et al. Distinct Altered Patterns of p27^{KIP1} Gene expression in Benign Prostatic Hyperplasia and Prostatic Cancer. *JNCI* 1998; 90:1284-1291.
25. Grignon DJ, Caplan R, Sarkar FH, et al. p53 status and Prognosis of Locally Advanced Prostatic Adenocarcinoma: a Study Based on RTOG 8610. *J Natl Cancer Inst* 1997; 89:158-165.
26. Navone NM, Labate ME, Troncoso P, et al. P53 Mutations in prostate cancer bone metastases suggest that selected p53 mutants in the primary site define foci with metastatic potential. *J Urol* 1999; 161:304-308.
27. Winawer SJ, Zauber AG, Ho MN, et al. Prevention of colorectal cancer by colonoscopic polypectomy. *N Engl J Med* 1993; 329:1977-1981.
28. Norfleet RG, Ryan ME, Wyman JB. Adenomatous and hyperplastic polyps cannot be reliably distinguished by their appearance through the fiberoptic sigmoidoscope. *Dig Dis Sci* 1988; 33:1175-1177.
29. Wang TD, Crawford JM, Feld MS, Wang Y, Itzkan I, Van Dam J. In vivo identification of colonic dysplasia using fluorescence endoscopic imaging. *Gastrointestinal Endoscopy* 1999; 49:447-455.
30. The Advanced Colorectal Cancer Meta-analysis Project. Modulation of fluorouracil by leucovorin in patients with advanced CRC: evidence in terms of response rate. *J Clin Oncol* 1992; 10:896-903.

31. Romer TJ, Fitzmaurice M, Cothren RM, et al. Laser-induced fluorescence microscopy of normal colon and dysplasia in colonic adenomas: implications for spectroscopic diagnosis. *Am J Gastro* 1995; 90:8107.
32. Cothren RM, Richards-Kortum R, Sivak MV, et al. Gastrointestinal tissue diagnosis by laser induced fluorescence spectroscopy at endoscopy. *Gastrointest Endosc* 1990; 36:105-111.
33. Vo-Dinh, T, Panjehpour M, Overholt BF, et al. In vivo cancer diagnosis of the esophagus using differential normalized fluorescence (DNF) indices. *Lasers Surg Med* 1995; 16:41-47.
34. Nilsson AMK, von Holstein CS, Andersson-Engels S, Willén R, Walter B, Svanberg K. Clinical detection studies of Barrett's metaplasia and oesophageal adenocarcinoma by means of laser-induced fluorescence. *Gut* 1996; 39:711-716.
35. Mourant JR, Boyer JD, Johnson TM, et al. Detection of gastrointestinal cancer by elastic scattering and absorption spectroscopies with the Los Alamos Optical Biopsy System. *Proc SPIE* 1995; 2387:210-217.
36. Bohorfoush AG, Bigio IJ, Ross AB, et al. White light optical biopsy for detection of dysplasia or malignancy. *Gastroenterology* 1995; 108:A449.
37. Perelman LT, Backman V, Wallace M, et al. Observation of periodic fine structure in reflectance from biological tissue: a new technique for measuring nuclear size distribution. *Phys. Rev. Letters* 1998; 80:627-630.
38. Wallace MB, Perelman LT, Backman V, et al. Endoscopic detection of dysplasia in patients with Barrett's Esophagus using light-scattering spectroscopy. *Gastroenterology*, in press.
39. Mahadevan-Jensen A, Richards-Kortum R. Raman spectroscopy for the detection of cancers and precancers. *J Biomedical Optics* 1996; 1:31-70.
40. Feld MS, Manoharan R, Salenius J, et al. Detection and characterization of human tissue lesions with near infrared Raman spectroscopy. *Proc SPIE* 1995; 2388:99-104.
41. Tearney GJ, Brezinski ME, Southern JF, Bouma BE, Boppart SA, Fujimoto JG. Optical biopsy in human gastrointestinal tissue using optical coherence tomography. *Am J Gastro* 1997; 92:1800-1804.
42. Brezinski ME, Tearney GJ, Bouma B, et al. Optical biopsy with optical coherence tomography. *Annals of the New York Academy of Sciences* 1998; 838:68-74.
43. Brezinski ME, Tearney GJ, Boppart SA, Swanson EA, Southern JF, Fujimoto JG. Optical biopsy with optical coherence tomography: feasibility for surgical diagnostics. *Journal of Surgical Research* 1997; 71:32-40.
44. American Cancer Society, <http://www.cancer.org>
45. Puls L, Powell D, DePriest P, et al. Transition from benign to malignant epithelium in mucinous and serous ovarian cystadenocarcinoma. *Gynecol Oncology* 1992; 47:53-57.
46. Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 1994; 266:66-71.
47. Karlan BY. The status of ultrasound and color Doppler imaging for the early detection of ovarian carcinoma. *Cancer Investigation* 1997; 15:265-269.
48. Van Nagell JR Jr, DePriest PD, Puls LE, et al. Ovarian cancer screening in asymptomatic postmenopausal women by transvaginal sonography. *Cancer* 1991; 68:458-62.
49. NIH Consensus Development Panel on Ovarian Cancer. Ovarian cancer screening, treatment, and follow-up. *JAMA* 1995; 273:491-497.
50. deBruijn HW, van der Zee AG, Aalders JG. The value of cancer antigen 125 (CA-125) during treatment and follow-up of patients with ovarian cancer. *Current Opinion in Obstetrics & Gynecology* 1997; 9:8-13.
51. Berek JS, Bast RC Jr. Ovarian cancer screening: the use of serial complementary tumor markers to improve sensitivity and specificity for early detection. *Cancer* 1995; 76(10 Suppl):2092-2096.
52. Schwartz PE. Cytoreductive surgery for the management of stage IV ovarian cancer [editorial; comment]. *Gynecol Oncol* 1997; 64:1-3.
53. Curtin JP, Malik R, Venkatraman ES, Barakat RR, Hoskins WJ. Stage IV ovarian cancer: impact of surgical debulking [see comments]. *Gynecol Oncol* 1997; 64:9-12.
54. Kriegmair M, Baumgartner R, Knuechel R, et al. Fluorescence photodetection of neoplastic urothelial lesions following intravesical instillation of 5-aminolevulinic acid. *Urology* 1994; 44:836-841.
55. Munkarah AR, Hallum AVR, Morris M, et al. Prognostic significance of residual disease in patients with stage IV epithelial ovarian cancer [see comments]. *Gynecol Oncol* 1997; 64:13-17.
56. Niloff JM, Bast RC Jr, Schaetzl EM, Knapp RC. Predictive value of CA 125 antigen levels in

- second-look procedures for ovarian cancer. *Am J Obstet Gynecol* 1985; 151:981-986.
57. Berek JS, Knapp RC, Malkasian GD, et al. CA 125 serum levels correlated with second-look operations among ovarian cancer patients. *Obstet Gynecol* 1986; 67:685-689.
 58. Rome RM, Koh H, Fortune D, Cauchi M. CA125 serum levels and secondary laparotomy in epithelial ovarian tumours. *Aust N Z J Obstet Gynaecol* 1987; 27:142-145.
 59. Rubin SC, Hoskins WJ, Hakes TB, et al. Serum CA 125 levels and surgical findings in patients undergoing secondary operations for epithelial ovarian cancer. *Am J Obstet Gynecol* 1989; 160:667-671.
 60. Engblom PR, Grenman SE, Klemi PJ, et al. The role of cul-de-sac aspiration cytology in the follow-up of ovarian cancer. *Acta Oncol* 1995; 34:783-785.
 61. DiSaia PJ, Creasman WT. Epithelial ovarian cancer. St. Louis, MO: Mosby-Year Book, Inc., 1993.
 62. Muderspach L, Muggia FM, Conti PS. Second-look laparotomy for stage III epithelial ovarian cancer: rationale and current issues. *Cancer Treat Rev* 1996; 21:499-511.
 63. Wright T, Kurman R, Ferenczy A. Precancerous lesions of the cervix. In Kurman R, ed. Blaustein's pathology of the female genital tract. New York, NY: Springer-Verlag, 1995.
 64. Beral V, Booth M. Predictions of cervical cancer incidence and mortality in England and Wales [letter]. *Lancet* 1986; 1:495.
 65. Ferenczy A, Hilgarth M, Jenny J, et al. The place of colposcopy and related systems in gynecologic practice and research [editorial] [see comments]. *J Reprod Med* 1988; 33:737-738.
 66. Ramanujam N, Mitchell MF, Mahadevan A, et al. In vivo diagnosis of cervical intraepithelial neoplasia using 337-nm-excited laser-induced fluorescence. *Proc Natl Acad Sci* 1994; 91:10193-10197.
 67. Gay JD, Donaldson LD, Goellner JR. False-negative results in cervical cytologic studies. *Acta Cytol* 1985; 29:1043-1046.
 68. Kierkegaard O, Byrjalsen C, Frandsen KH, Hansen KC, Frydenberg M. Diagnostic accuracy of cytology and colposcopy in cervical squamous intraepithelial lesions. *Acta Obstet Gynecol Scand* 1994; 73:648-651.
 69. Van Le L, Broekhuizen FF, Janzer-Steele R, Behar M, Samter T. Acetic acid visualization of the cervix to detect cervical dysplasia. *Obstet Gynecol* 1993; 81:293-295.
 70. Hopman EH, Voorhorst FJ, Kenemans P, Meyer CJ, Helmerhorst TJ. Observer agreement on interpreting colposcopic images of CIN. *Gynecol Oncol* 1995; 58:206-209.
 71. Allen DG, Ashton P, Wintle M, Conway C, Planner RS. The use of cervicography in the follow-up of cervical intraepithelial neoplasia treated by CO2 laser. *Aust N Z J Obstet Gynaecol* 1995; 35:349-350.
 72. Coibion M, Autier P, Vandam P, et al. Is there a role for cervicography in the detection of premalignant lesions of the cervix uteri? *Br J Cancer* 1994; 70:125-128.
 73. Lonky NM, Mann WJ, Massad LS, et al. Ability of visual tests to predict underlying cervical neoplasia. *Colposcopy and speculscopy. J Reprod Med* 1995; 40:530-536.
 74. Massad LS, Lonky NM, Mutch DG, et al. Use of speculscopy in the evaluation of women with atypical Papanicolaou smears. improved cost effectiveness by selective colposcopy. *J Reprod Med* 1993; 38:163-169.
 75. Mann W, Lonky N, Massad S, Scotti R, Blanco J, Vasilev S. Papanicolaou smear screening augmented by a magnified chemiluminescent exam. *Int J Gynaecol Obstet* 1993; 43:289-296.
 76. Shafi MI, Dunn JA, Chenoy R, Buxton EJ, Williams C, Luesley DM. Digital imaging colposcopy, image analysis and quantification of the colposcopic image. *Br J Obstet Gynaecol* 1994; 101:234-238.
 77. Chenoy R, Billingham L, Irani S, Rollason TP, Luesley DM, Jordan JA. The effect of directed biopsy on the atypical cervical transformation zone: assessed by digital imaging colposcopy. *Br J Obstet Gynaecol* 1996; 103:457-462.
 78. Schneider A, Zahm DM. New adjunctive methods for cervical cancer screening. *Obstet Gynecol Clin North Am* 1996; 23:657-673.
 79. Mahadevan A, Mitchell MF, Silva E, Thomsen S, Richards-Kortum RR. Study of the fluorescence properties of normal and neoplastic human cervical tissue. *Lasers Surg Med* 1993; 13:647-655.
 80. Ramanujam N, Mitchell MF, Mahadevan A, Thomsen S, Silva E, Richards-Kortum R. Fluorescence spectroscopy: a diagnostic tool for cervical intraepithelial neoplasia (CIN). *Gynecol Oncol* 1994; 52:31-38.
 81. Ramanujam N, Mitchell MF, Mahadevan A, et al. Spectroscopic diagnosis of cervical intraepithelial neoplasia (CIN) in vivo using laser-induced fluorescence spectra at multiple

- excitation wavelengths. *Lasers Surg Med* 1996; 19:63-74.
82. af Klinteberg C, Wang I, Lindquist C, Vaitkuviene A, Svanberg K. Laser-induced fluorescence studies of premalignant and benign lesions in the female genital tract. *SPIE* 1997; 3197:34-39.
 83. Patterson M, Pogue B, Wilson B. Part 4. In Müller BC, Alfano R, Arridge S, et al., eds. *Medical optical tomography: functional imaging and monitoring*. Washington: SPIE Opt. Eng. Press, 1993.
 84. Bigio IJ, Johnson T, Mourant J, et al. Determination of the cervical transformation zone using elastic-scattering spectroscopy. *Proc. Soc. Photo-Opt. Instrum. Eng.* 1996; 2679:85-91.
 85. Soper JT, Berchuck A, Olt GJ, et al. Preoperative evaluation of serum CA125, TAG 72, and CA 15-3 in patients with endometrial carcinoma. *Am J Obstet Gynecol* 1990; 163:1204-1209.
 86. Koper NP, Massuger LFAG, Thomas CMG, Kiemeny LALM, Verbeek ALM. Serum CA125 measurements to identify patients with endometrial cancer who require lymphadenectomy. *Anticancer research* 1998; 18:1897-1902.
 87. Sood AK, Buller RE, Burger RA, et al. Value of preoperative CA125 level in management of uterine cancer and prediction of clinical outcome. *Obstet Gynecol* 1997; 90:441-447.
 88. Hricak H, Rubinstein LV, Gherman GM, Karstaedt N. MRI imaging evaluation of endometrial carcinoma: results of an NCI cooperative study. *Radiology* 1991; 179:829-832.
 89. Sironi S, Colombo E, Villa G, et al. Myometrial invasion by endometrial carcinoma: assessment with plain and gadolinium-enhanced MR imaging. *Radiology* 1992; 185:207-212.

Session 2: State of the Art in Functional Imaging

This session examined the potential for magnetic resonance imaging and magnetic resonance spectroscopy, nuclear medicine and positron emission tomography, optical imaging, and other technologies to impact patient care. The session also addressed three major areas of oncology where functional imaging may provide important information for tumor characterization and treatment planning, including prediction and early assessment of tumor response. The technologies ranged in maturity from reasonably well-established (MRI/MRS and nuclear medicine/PET) to less well developed, such as optical imaging, and to nascent technologies, such as electron paramagnetic resonance imaging (EPRI). While the technologies vary considerably in their characteristics, common themes emerged for implementing and optimally using all of these technologies in oncologic imaging, as detailed in the research recommendation section. The overviews are presented by modality and disease-specific applications, with research recommendations listed in general and specific terms.

MRI/MRS

Magnetic resonance imaging and, to a lesser extent, magnetic resonance spectroscopy are reasonably established technologies, with a fairly large base of installed imaging devices in the U.S. capable of performing either exam. MRI is in routine use and has excellent resolution, contrast sensitivity for water, good temporal resolution, and the ability to assess vascular permeability, among other parameters. MRS can provide considerable biochemical information regarding cancers, but at present it takes longer to acquire, is less available, and has considerably lower resolution than MRI. MRS is, however, being applied to a greater extent in the research and, to some extent, clinical setting than ever before.

MRI for Breast Cancer

MRI of the breast is now a routine part of clinical care in many centers.¹⁻³ The following is a summary of current indications for breast MRI.

Lesion Characterization

MRI is indicated in patients with inconclusive clinical and/or mammographic examinations. Patients in particular that may benefit include women with radiographically dense breasts, silicone augmentation

(both implants and injections), and postoperative or post-traumatic scars.

Local Staging

Breast MRI is indicated in patients who present with a positive node biopsy (axillary or supraclavicular) with a likely breast primary and negative clinical and mammographic examinations of the breast. MRI should be used routinely to demonstrate the extent of disease in breast conservation candidates to improve cosmesis and to avoid redundant therapy. MRI should be used in patients undergoing induction chemotherapy to determine the extent of residual disease burden and chemotherapeutic response. The response of the MRI image to tamoxifen may be beneficial in developing surrogate markers for chemoprevention trials. Breast MRI is probably the best method for coordinating the delivery of minimally invasive therapy.

High-Risk Surveillance

The high negative predictive value of breast MRI may be used in patients at high genetic or histologic risk for breast cancer. Routine examinations can effectively be used to exclude breast cancer and reduce the need for prophylactic mastectomy.

A number of tools need to be developed for optimal uses in breast MRI. Of critical importance is the development of a practical and efficient method for using MRI to localize and biopsy lesions that are only seen on MRI. Along with an MRI stereotaxic system, MRI-compatible wires, needles, and core guns are needed. The pulse sequences for breast MRI remain unavailable for many centers. Many currently used breast MRI coils were satisfactory for implant evaluation but are not capable of producing high-resolution images for cancer definition. Image processing tools, such as automated volume calculations, need to be developed for clinical use.

Contrast Agents

New contrast agents under development have promise for improved breast MRI imaging. Some agents provide better relaxivity for better lesion conspicuity. Other agents prolong the intravascular half-life for longer imaging times and higher resolution. There is potential for improving the specificity contrast enhanced MRI by exploiting the tumor angiogenesis thought to be linked to lesion malignancy. New

agents under development can be applied in the important role of lymph node imaging and may reduce the need for lymph node dissections. New contrast agents are needed to improve the delivery of minimally invasive therapy.

Integrating MRI with Treatment Trials

Of major importance in the development of breast MRI is the integration with treatment trials.⁴ Breast MRI may be used to achieve surrogate endpoints in trials that evaluate new chemotherapeutic or chemoprevention regimens. A trial is needed that will evaluate the significance of MRI-detected lesions that are clinically and mammographically occult. MRI may be used to select patients that might be treated with lumpectomy alone. Education is important in the ultimate clinical use of this new technology. Quality assurance standards must be developed. Technology guidelines are needed to aid in establishing appropriate usage. Standards for interpretation and a lexicon of terms that can be employed consistently from center to center are needed.

MRI for Gynecological and Genitourinary Cancers

MRI is indicated in the staging of cervical, uterine, and prostate cancers.⁵⁻⁹ This information is used to select appropriate treatment and plan therapy. Follow-up examinations are used to determine disease stability, recurrence, or progression.

Better MRI tools needed for imaging these applications include faster scanners and better pulse sequences for more inherent contrast. Image processing tools include image fusion and automated volumetric calculations. New contrast agents include those with better cancer or tissue specificity. Cancer-specific agents would take advantage of angiogenesis and/or stromal proliferation. Tissue-specific agents are designed to image lymph nodes or cancer-specific monoclonal antibodies.

More clinical trials are needed to validate the use of MRI in gynecological and genitourinary applications. A multicenter database should have facilities for survival, recurrence, cost-benefit, cost-effectiveness, and outcome analysis. Tests should be employed for the validation of new techniques and methods. Interpretation guidelines should include tests for interobserver variability. The overall goal should be to demonstrate a positive effect on management of patients with gynecological and genitourinary malignancies. These trials may be organized with

joint sponsorship with the American College of Radiology Imaging Network.

Education is critical in the effective use of this new technology. Disease-tailored, standardized protocols are needed. Interpretation and practice guidelines should be employed to obtain consistent results over a variety of practice locations and conditions. A lexicon for standardized terms can help in the coordination of care across the nation.

Nuclear Medicine and PET Imaging

Nuclear medicine and PET imaging are increasingly maturing as imaging methods.¹⁰⁻¹¹ Nuclear medicine methods using single photon imaging are in place in most hospitals and many outpatient centers throughout the U.S. Several nuclear medicine procedures are routinely used in oncology. Single photon imaging is performed with a standard gamma camera and allows for the detection of radiotracers such as TC-99m, I-131, In-111 and Ga-67, which are used in many clinics and which decay by the emission of a single photon with each nuclear decay. A three-dimensional display of the distribution of single photon emitter biodistribution in vivo is possible by using a rotating gamma camera and computer system with single photon emission computed tomography (SPECT) scans. By contrast, PET imaging requires specialized instrumentation—a PET scanner to image positron emitting radioisotopes. PET is a more sensitive technique than SPECT and able to quantify more precisely the radioactivity levels in tissues. The most commonly used positron emitter in clinical practice is fluorine-18, with a 109-minute half-life. It is most typically used attached to 2-deoxyglucose as fluorine-18-fluoro-2-deoxy-D-glucose (FDG), which localizes rapidly to many human cancers following intravenous injection. Neither PET nor SPECT have the anatomic resolution that MRI can obtain; however, both have remarkable sensitivity for detecting low levels of radioactivity in vivo. Thus, in some studies, they are more sensitive than MRI for lesion detection.

Nuclear medicine imaging using single photon methods has been applied for detecting several diseases. In prostate cancer, the radionuclide bone scan plays a key and routine role in staging patients with elevated prostate-specific antigen levels and in follow-up of patients after therapy. More recently, a monoclonal antibody labeled with In-111 has become available for imaging this type of tumor. Soft tissue

disease can be detected using In-111 capromomab pentetide (ProstaScint), although its precise role in managing prostate cancer is not yet fully established. At present, it seems able to detect recurrent prostate cancer in patients with a low, but rising, PSA level in the blood with greater sensitivity than other methods. The clinical database is small, however, and much more study is needed in larger patient populations. PET imaging with FDG has not shown the same level of success with prostate cancer as has been seen with FDG in other tumors. As an example, up to 50% of lesions have been “missed” using FDG PET in some series. Lesion detection near the bladder may be impaired because (1) FDG is excreted from the kidneys into the bladder; (2) background tracer activity levels are high; and (3) in studies to date, the often blastic prostate cancer bone metastases were more often seen on bone scan than on PET with FDG. More study of PET with FDG in prostate cancer is needed to determine the role of the method and whether technical improvements may help in the detection process. New PET tracers are being evaluated in prostate cancer and show some promise. Both carbon-11-choline and carbon-11-acetate have recently been shown to have promise for detecting disease, but carbon-11 is challenging to use due to its short (20-minute) half-life—studies can only be done if a dedicated cyclotron is nearby.

Gynecological neoplasms, such as ovarian and cervical cancer, can be imaged using nuclear medicine methods. Bone metastases can occur from cervical cancer (and less commonly from ovarian cancer), and these can be detected by radionuclide bone scan. More specific imaging of ovarian cancer has been feasible by using a monoclonal antibody to a high molecular weight mucin (i.e., TAG72) expressed on many ovarian cancers. A murine monoclonal antibody labeled with In-111 (In-111 satumomab pentetide [Oncoscint]) has been approved by the FDA for detecting ovarian cancers. While the performance of this antibody is superior to computed tomography for detecting recurrent ovarian cancer in the abdomen, it is not widely used because the target/background signal is only modest and there is considerable challenge in interpreting the images. More promising are recent reports of studies using FDG that have shown that this tracer can detect small foci of ovarian cancer well and that PET with FDG is more accurate than CT in patients who have undergone therapy for ovarian cancer. Very recent data have shown that cervical cancer can be well-imaged using FDG and PET, with initial studies

showing better detection than with cross-sectional methods such as CT or MRI. While excreted activity in the ureters and in the bladder can be challenges, in general the method appears valuable. Additional clinical trials, possibly multicenter in nature are needed to define more precisely the role of PET with FDG in cervical cancer imaging. This is of particular relevance as cervical cancer therapy with radiation alone has been shown to be less effective in patients with advanced stage disease than if chemotherapy is included.

Breast cancer is another disease in which nuclear medicine imaging has had an important role for many years. The radionuclide bone scan and the gated cardiac blood pool imaging study, both single photon methods, are used quite routinely in the management of patients with breast cancer. More recently, a single photon imaging technique, TC-99m Sestamibi (MIBI) imaging, has been approved by the FDA for evaluating the breast itself. This is based on the fact that most breast cancers accumulate the tracer MIBI, which is a cationic isonitrile that presumably is accumulated in the mitochondria due to the negative charge gradient in that organelle. The use of this agent is controversial, however, as it is not approved by the FDA as a substitute for biopsy. The performance characteristics of MIBI are such that it may fail to detect 10% to 20% of palpable cancers and more than 50% of nonpalpable lesions. The sensitivity of the method is least good for small lesions (<1 cm in size). Thus, while the technique is more robust than mammography in independently evaluating an indeterminate breast mass, it is not as sensitive nor as specific as biopsy. Consequently, the clinical use of this agent has not become extremely widespread. More focused uses in breast imaging, such as in postbiopsy, to assess chemotherapy response, to assess for multidrug resistance (MIBI is a substrate for the multidrug resistance transporter), and in women with radiodense breasts (due to implants or increased glandular density) need more study.

PET imaging also has been explored in imaging breast cancer.¹² Preclinical studies have shown PET with FDG to be superior to PET with MIBI in terms of localization to breast cancers.¹³ Clinical studies have shown that PET with FDG can detect about 90% of primary breast cancers that are smaller than 1 cm in size and that accuracies in the 80% to more than 90% range have been seen in noninvasively characterizing such masses as malignant or benign.

PET can detect cancers in breasts after silicone augmentation and can effectively image cancers in some women with radiodense breasts. Detection of breast cancers in lesions smaller than 1 cm is less effective, however, due to limits in scanner resolution.

PET can also image beyond the breast, due to the reasonably high tumor to background tracer uptake ratios achieved using the FDG tracer. Several studies have shown the ability of PET to detect noninvasively both axillary and internal mammary lymph node metastases from breast cancer. While the precise accuracy of PET is not yet fully determined, it is dependent on reader performance (i.e., whether biased to sensitivity or specificity) and machine performance. Sensitivities of 80% to 100% have been reported, with specificities of 60% to nearly 100%. At present, a multicenter prospective NIH-sponsored trial is evaluating the accuracy of PET for staging breast cancer metastases to the axilla; therefore, more refined data should be forthcoming. The current generation of PET scanners cannot detect micrometastases. There is a strong interest in research to develop higher resolution scanning devices that could detect small nodal metastases as well as in dedicated devices that could image smaller primary breast cancers.

PET also can detect disease in the soft tissues very well. At present, small studies from several groups indicate that PET is a very accurate technique for detection of metastases to the brachial plexus and in helping separate brachial plexopathy due to radiation damage from tumor involvement with breast cancer. PET with FDG also can detect visceral metastases and bone metastases. The sensitivity of FDG for lytic bone metastases is excellent; however, it is lower for blastic lesions. Fluorine-18 alone has shown promise as a bone metastasis detection agent in several studies, with localization due to the remodeling of bone due to tumor involvement.

Other PET tracers have been used in evaluating breast cancer, and more are being studied. Fluorinated estrogen analogs can detect estrogen receptor positive tumors and have shown proof of principle as agents to characterize lesions' estrogen receptor status, as well as to monitor the response of such agents to estrogen receptor antagonist therapies. FDG, which quantitatively assesses glucose metabolism in tumors, also can be used to assess treatment response in breast cancer. Several clinical studies have shown

that declines in FDG uptake in tumors occur rapidly (i.e., before changes in tumor size) in response to effective chemotherapy and that these changes can, relatively soon after treatment is started, predict longer term outcomes of the therapy. These studies, however, are small in size, and confirmation is needed in larger studies with a broad spectrum of treatment agents.

Another promising potential role of PET in assessing/predicting responses of breast cancers to therapy involves the use of FDG. It has been shown in preclinical models that estrogen stimulation of estrogen receptor positive tissues can raise glucose metabolic rates quite rapidly. Thus, an increase in FDG uptake might be expected if estrogen were given. Recently, investigators have shown in patients that, in women with metastatic breast cancer treated with tamoxifen, there is an initial increase in FDG uptake in the group of women who ultimately respond. The fact that tamoxifen is a partial estrogen analog may explain this "metabolic flare." Thus, for treatment monitoring, FDG appears to have considerable potential in breast and other cancers.

Areas of promise also include instrumentation beyond that for imaging alone. As an example, intraoperative probes, which can be used by the surgeon to locate radioactive tissues, have been used to identify sentinel lymph nodes. More recently, however, these probes have been used in animal tumor models to locate the margins of tumors, based on their avidity for FDG. The possibility of using positron-sensitive probes to guide surgical resections is in development but could lead to more precise lumpectomies and a reduction in the need for reoperations to secure tumor margins clear of tumor cells. Another area of interest is developing biopsy techniques based on localization of radiotracers to the breast, so that radionuclide-guided biopsies, rather than simply anatomic-based biopsies, can be performed. An additional area of considerable interest regarding nuclear medicine methods is the development of imaging devices that can perform both PET and single photon imaging. These devices are rapidly penetrating the market, although their PET performance is not optimal in the earliest versions due to limited counts. As another approach, hybrid CT and PET devices are evolving rapidly, with the ability to produce high-quality anatomic images for registration with PET (or even SPECT).

The dissemination of nuclear medicine techniques, especially PET, is currently limited by a shortage of dedicated PET imaging equipment. Many NIH comprehensive cancer centers do not have such instrumentation due to its cost. Further, the technical envelope for PET has expanded only slowly, as industry might have been concerned that higher quality (i.e., higher sensitivity and resolution) PET scanners might be too expensive to make or sell. Such very high resolution devices should prove very useful in cancer imaging. The growth of clinical PET imaging for diseases such as lung cancer (where Medicare pays for evaluation of solitary pulmonary nodules as well as for tumor staging) and the more recent broadening of Medicare indications to include clinical issues in melanoma, lymphoma, and colorectal cancer mean that there is less research time available on PET scanners for studies in cancer (due to the competing clinical volume).

Optical Imaging Methods

Optical imaging includes a family of potentially very high spatial resolution and real-time imaging methods. Optical diagnostic technologies have the potential capability to perform diagnosis on tissue in situ (i.e., without the need for excision and processing). These methods can probe superficial structures accessible to light with micron-level spatial resolution with a centimeter depth of interrogation. Such high-resolution imaging can be sensitive to cytologic and morphologic changes, extracellular matrix structure and composition, tissue dysplastic transformation, blood flow, and other parameters. Optical technologies can provide a powerful means for detecting preneoplastic and early neoplastic changes, which is important from an outcome viewpoint since treatment is difficult once invasive carcinoma and metastases develop.

A second major application theme for optical diagnostic technologies is the guidance of surgical intervention and treatment. This technology might provide more precise guidance of surgical intervention by aiding in the determination of tumor margins or by facilitating surgery on or near important normal structures, such as nerves or blood vessels. Additionally, optical techniques may provide real-time assessment of tissue response to treatment.

There are several generic technologies for optical imaging, including near infrared (NIR) imaging, optical microscopy, optical coherence tomography,

and light scattering and absorption. These technologies are discussed below.

Near Infrared Imaging

Optical imaging uses a “diagnostic window” in the NIR region and has now advanced to the stage where deep cancers of the breast can be imaged. They are characterized by a wide variety of intrinsic and extrinsic contrast agents. The method is economical, very fast, sensitive, and highly portable. While of lower resolution than MRI, NIR imaging is “near field” technology that is not limited by the Rayleigh criterion and is readily coregisterable with MRI where selected voxels can be characterized with optical data to afford added sensitivity or specificity. Another strength of NIR imaging is its very high sensitivity for very low contrast agent concentrations. A wide variety of NIR-targeted probes can be delivered in a “cryptic” form and decoded by tumor-specific enzymes to an optically detectable form (i.e., molecular beacons). These probes also may signal gene expression by tagging the conventional markers (e.g., B galactosidase).

NIR imaging is being used in at least half a dozen laboratories in the U.S. and abroad to detect breast cancer. Several large companies and a number of laboratories have devised suitable NIR imagers that are engaged in preliminary clinical trials. Available contrast currently is based upon intrinsic signals of blood concentration or blood pooling related to angiogenesis and of blood deoxygenation due to hypermetabolism. Extrinsic contrast is imaged with tricarboyanine probes related to tumor hypermetabolism and extrinsic blood pooling agents, similar to MRI’s gadolinium chelates.

Optical Microscopy

Optical microscopy can entail the use of wide field and scanning microscopies and spectroscopic microprobes (e.g., Raman, elastic scattering, fluorescence). Confocal and multiphoton confocal microscopy use the spatial focusing properties of light to obtain depth information or to enhance imaging in optically scattering tissues. These imaging technologies feature very high image resolution. Recent advances in multiphoton confocal microscopy use short-pulse illumination and multiphoton excitation of chromophores. These multiphoton techniques yield enhanced imaging depth and enable living cell imaging with reduced photobleaching effects. These technologies represent one of the major recent advances in optical microscopy and have been

used extensively in research. Clinical application requires development of endoscopic mediation.

Optical Coherence Tomography

OCT is a recently developed technique that provides cross-sectional images of tissue in situ.¹⁴ OCT imaging relies on detecting scattered light, and the depth of imaging is limited to within 2 to 3 mm of the surface in most tissues. However, the “histology-like” images with resolution as high as 5 to 10 μm are rendered in real time with no need for excision; thus, this technique has the potential for diagnosis of early neoplasia and surgical guidance. It is largely still a research technique.

Light Scattering and Absorption

Two types of optical imaging systems readily deconvolute absorption and scattering of tissues: time and frequency systems (interchangeable by Fourier transformation) and continuous light system where scattering and absorption are merged.¹⁵⁻¹⁷ Extensive studies on in vitro and in vivo optical properties of human tumors show increased absorption due to angiogenesis with variable changes of light scattering that may be related to hormonal perturbation of the population of cell organelles, mitochondria, etc.

Light scattering is capable of quantitative characterization of tissue optical properties (absorption and scattering), quantitative measurements of endogenous biochemical constituents (e.g., tissue hemoglobin concentration [oxy-, deoxy-, and total], oxygen saturation, water, fat), and quantitative measurements of exogenous probes and drugs. Photon migration techniques seek to perform measurement or imaging in deep (i.e., several centimeters) tissues by using multiply scattered light. These techniques take advantage of the fact that light at NIR wavelengths is not highly absorbed by tissue and thus can penetrate several centimeters. Multiple scattering of the light degrades image information; therefore, most of these techniques focus on either low-resolution imaging or functional assessment of tissue at low resolution. These techniques are still experimental.

Light absorption methods include fluorescence imaging and spectroscopy. With this approach and with photoacoustic imaging, endogenous tissue fluorescence and exogenous contrast probes can be used to localize tumors, characterize tissue biochemical environment, and quantify flow and extravasation kinetics. Spectral imaging, which

represents a hybrid optical diagnostic, obtains spectroscopic information and renders it in image form. In principle, almost any spectroscopic method also can be combined with imaging. Some of these techniques use computer-based image processing in combination with microscopy. These techniques have had a major impact on research and are being implemented in cytological diagnostics. One also can combine spectroscopy with photon migration in order to perform functional assessment of deep tissue structures. One example is the spectroscopic detection of oxy- and deoxy-hemoglobin for noninvasive assessment of tissue oxygenation.

Summary of Optical Imaging

Optical imaging is a promising group of methods making a transition from laboratory studies to early phase clinical investigations. The modalities are developing rapidly, are attractive because of the potential for high spatial and temporal resolution, and free of ionizing radiation. Current limitations include lack of commercial instruments, lack of suitable methods for tissue characterization in vivo, and the limited access to deeper parts of the body due to the limited path length of most optical wavelengths in tissues. Given the extensive tissue volumes that can be probed at high resolution, methods for analyzing complex data sets need to be developed and improved.

Future studies may exploit not only targetable probes and molecular beacons for deep tumors in vivo but also a variety of signals other than NIR, such as infrared, Raman, and other methods, thereby combining many of the advantages of radioactive labels or specific biochemical markers. Optical imaging is unique, however, in having the great advantage of a very high (i.e., pmole) sensitivity and speed for cryptic and noncryptic molecular beacons, thus affording a unique complement to MRI, PET, and SPECT. The future of the optical method lies in its simplicity, affordability, and portability.

Electron Paramagnetic Resonance Imaging

A magnetic resonance technique similar to MRI except operating a very low magnetic fields (e.g., 9-40 mT), EPRI probes the magnetic properties of species containing unpaired electrons (e.g., free radicals, transition metals). This imaging method is in the very early development phase and is less advanced than MRI, nuclear medicine/PET or optical

imaging. At present, the depth of interrogation of EPRI is only a few centimeters, but it may become possible to interrogate up to 20 cm deep in living tissues in the future. Excellent resolution (i.e., submillimeter) appears to be possible using water-soluble spin probes.

The development of clinical EPRI has been hampered by the lack of (1) adequate levels of endogenous paramagnetic species in vivo and (2) biologically compatible exogenous paramagnetic spin probe tracers. Recently, with the availability of exogenous, nontoxic, biologically compatible paramagnetic spin probes such as trityl and nitroxides, EPRI has acquired the potential to provide valuable three-dimensional physiologic and metabolic information in the tissue of interest. Such functional information obtained from the tissue of interest by EPRI can complement the anatomical information obtained from other high-resolution diagnostic techniques such as MRI and guide treatment efforts in clinical oncology. For example, nitroxides are redox-sensitive paramagnetic tracers, which are useful to delineate noninvasively tissue heterogeneity such as that occurring between normal and malignant tissue with respect to distribution, redox status, and oxygen concentration. Determining tissue oxygen levels can be important to cancer treatment (e.g., to determine the oxygenation status of tumors) as well as in determining oxygen status in cardiac and brain tissue. Studies using spin label oximetry or EPRI have shown significant hypoxia in experimental animal tumors compared to normal tissue. These results suggest that tumor hypoxia results in more rapid reduction of nitroxides than in normal tissue, which in turn may explain the selective radioprotection of normal tissue by nitroxides.

Based on these proof-of-principle experiments and with the vast experience of MRI (which is similar to EPRI) in diagnostic radiology, EPRI is evolving into a promising technique for functional/physiological imaging and will augment the anatomic imaging methods by coregistering valuable physiological information. Proton-electron double resonance imaging (PEDRI), or Overhauser-enhanced MRI, may fuse EPRI and MRI and yield high spatial resolution images with qualitative oxygen sensitivity. The ability of EPRI to provide quantitative and noninvasive pO_2 information in the entire physiological range with high sensitivity in the hypoxia regions, as may be expected in pathophysiological states, makes it a unique

technique among the emerging functional imaging modalities to image tissue hypoxia with the desired resolution and to guide efficacious therapeutic efforts.

Further development will be required to bring EPRI into clinical use. The technology to accomplish this goal is available; however, support will be required as systems are developed to accommodate larger volumes and enhance resolution. The unique functional/physiologic dimension that EPRI will contribute to the diagnostic repertoire will enable clinicians to enhance their ability to provide detailed information regarding disease states and treatment options. A great body of work will be needed to move EPRI into clinical evaluation.

Research Priorities and Overall Recommendations

Across all modalities, the following general themes were raised repeatedly.

Needs for Established Technologies

- Clinical integration into cancer imaging trials.
- Infrastructure support to allow access.
- Careful correlation with cancer biological and clinical “truths.”
- Fundamental developments to improve.

Needs for Emerging/Nascent Technologies

- Instrument development and proof of concept in small-scale preclinical and clinical studies.
- Close interactions with clinicians to determine what problems can be solved.
- Transfer of promising technology to commercial vendors.
- Careful correlation with molecular biology/physiology.

Common Needs

- Multimodality data integration for comprehensive tissue characterization.
- Cellular, molecular, and tissue correlates of imaging signals.

- Data archival, rapid analysis, and clinical outcome data.

Specific recommendations by modality follow.

MRI/MRS

- Develop faster scanners with better pulse sequences for more inherent contrast, image fusion, image processing, automated volumetric measurements.
- Develop contrast media with greater specificity than currently available reagents (e.g., cancer-specific imaging of angiogenesis/stromal proliferation, tissue-specific imaging of small tumors within lymph nodes via monoclonal antibodies or other receptor binding contrast delivery reagents).
- Validate and standardize current techniques across centers and establish a multicenter database.
- Conduct studies to assess interobserver variability.
- Conduct clinical trials to evaluate changes in patient management, cost-effectiveness, cost-benefit, and outcome. These studies should be conducted across patients of varying ages to be certain results are not affected by age (especially for breast cancer studies). These studies could potentially be developed in joint sponsorship with the American College of Radiology Imaging Network.
- Establish closer professional relationships with the major clinical trial groups.
- Educate patients, radiologists, referring physicians, and third-party payors on MRI/MRS and their clinical roles.
- Develop disease-tailored, standardized protocols; interpretation guidelines; practice guidelines; and a lexicon for standardized terms.

Nuclear Medicine and PET

- Develop human and animal PET scanners of higher resolution and sensitivity, and optimize hybrid CT-PET and PET-SPECT devices.

- Further develop organ-specific imaging and biopsy devices and probes.
- Improve methods for image fusion, reconstruction, rapid transmission correction, and quantitation.
- Develop and validate (in vitro and in vivo) tracers for specific and biologically relevant processes, including tracers that can diagnose, and at higher doses treat, cancers.
- Develop suitable methods to apply tracers to clinical studies of flow, proliferation, apoptosis, signal transduction, gene transfer, drug resistance, drug delivery, and hypoxia.
- Develop data-transfer methods (e.g., “kits” for production) regarding specific tracers, especially fluorine-18 compounds.
- Structure regulatory issues regarding PET tracers and other nuclear tracers to reflect the general safety of the trace amounts of radioactivity used.
- Develop better national access to PET and PET tracers and revitalize the cyclotron infrastructure.
- Undertake genome-based initiative for diagnostic and therapeutic radiopharmaceuticals.
- Develop radiopharmaceuticals to monitor gene therapy.
- Integrate nuclear medicine/PET into early-, mid-, and possibly late-phase multicenter trials. Evaluate nuclear medicine/PET in pilot trials of new dedicated organ scanners, probes, and biopsy devices.
- Use nuclear medicine/PET to develop more rapid and specific human cancer drug screening programs, comparing these imaging techniques with traditional and new methods.
- Interact with drug industry to develop new nuclear medicine/PET drug development paradigms. Assess costs of nuclear medicine/PET drug development paradigms versus more traditional drug development approaches.

- Use nuclear medicine/PET to assess the toxicity of cancer therapies.
- Examine nuclear medicine/PET-based therapy planning (e.g., radiation, chemotherapy, new treatments).
- Use multiparameter nuclear medicine/PET to characterize tumor phenotypes.
- Conduct prospective clinical trials of accuracy and outcomes, consider changes in management, and examine costs and benefits.
- Train new scientists with focus on cancer biology, and educate imaging/referring physicians and the general public about nuclear medicine/PET.

Optical Imaging Methods

- Develop in vivo, endoscopic, and catheter-based delivery systems for high-resolution imaging of internal tissue locations.
- Develop scalable integrated systems combining high-resolution imaging with spectroscopy for quantitative functional tissue characterization.
- Develop analytical methods (e.g., software) to allow extraction of tumor-related optical signatures from normal tissues in large and complex high-resolution data sets.
- Develop specific molecular probes that are compatible with optical technologies and that have unique spectral signatures that are sensitive to physiology.
- Enhance our understanding of molecular and cellular determinants of optical signals via basic preclinical and ex vivo model research.
- Use high-resolution and quantitative optical techniques to characterize variations in normal and tumor tissue structure and physiology to assess risk of neoplastic transformation, to assess malignant versus benign status, to follow response to therapies, to understand disease progression, and to optimize drug/radiation dosimetry.

- Apply knowledge of optical signatures to develop practical and cost-effective methods to assist in diagnosis, screening, and intraoperative guidance.
- Combine and coregister multiple imaging modalities with optical methods to enhance complementarity of optical information with other functional imaging methods.
- Disseminate technologies and methods.
- Support device development.
- Stimulate multicenter clinical trials, and support interdisciplinary collaborations.
- Ensure sufficient population of imaging scientists and physicians are trained to develop optical technologies.

Electron Paramagnetic Resonance Imaging

- Explore the capabilities of high-resolution EPRI to show normal and tumor physiology in animals.
- Explore the capabilities of particulate-localized spectroscopy to reflect normal and tumor physiology.
- Explore the capabilities of PEDRI.
- Develop second-generation imagers with larger bore (i.e., 20-60 cm) and electronics for faster image acquisition.
- Explore even lower frequency
- Use suppression strategies to image tumor antigens and evolution of biomolecules relevant to signaling and apoptosis.
- Develop spin probes that distribute selectively in intravascular, intracellular, and nuclear water compartments.
- Develop new spin probes with an order-of-magnitude reduction in line width and an order-of-magnitude increase in spatial resolution, oxygen concentration resolution, and signal-to-noise ratio.

- Develop new resonators for particulate spectroscopy for placement and removal from tissue and for EPRI.
- Work toward FDA approval for infusible spin probes and for particulates.
- Establish strategies to suppress signal except in the presence of specific biomolecules (e.g., tumor surface antigens, intracellular cytochrome-c [liposomes]).
- Explore heterogeneity of tumor/normal tissue oxygenation with EPRI or PEDRI in animal models.
- Conduct human trials with particulate-localized spectroscopy to explore the relationship between tumor/normal tissue oxygenation and radiation/chemotherapy therapy scheduling.
- Conduct human trials with EPRI or PEDRI to explore human tumor oxygenation heterogeneity and the response of tumor heterogeneity to therapy.
- Train imaging scientists and other physicians to have familiarity with this emerging technology.

References

1. Harms SE. Breast magnetic resonance imaging. *Seminars in Ultrasound, CT & MR* 1998; 19:104-120.
2. Harms SE, Flamig DP, Hesley KL, et al. MR imaging of the breast with rotating delivery of excitation off resonance: clinical experience with pathologic correlation. *Radiology* 1993; 187:493-501.
3. Williams MB, Pisano ED, Schnall MD, Fajardo LL. Future directions in imaging of breast diseases [see comments]. *Radiology* 1998; 206:297-300.
4. Harms SE. Integration of breast magnetic resonance imaging with breast cancer treatment. *Topics in Magnetic Resonance Imaging* 1998; 9:79-91.
5. Yu KK, Hricak H, Subak LL, Zaloudek CJ, Powell CB. Preoperative staging of cervical carcinoma: phased array coil fast spin-echo versus body coil spin-echo T2-weighted MR imaging. *AJR* 1998; 171:707-711.
6. Kaji Y, Kurhanewicz J, Hricak H, et al. Localizing prostate cancer in the presence of postbiopsy changes on MR images: role of proton MR spectroscopic imaging. *Radiology* 1998; 206:785-790.
7. Hricak H. Widespread use of MRI in gynecology: a myth or reality? *Abdominal Imaging* 1997; 22:579-588.
8. Kurhanewicz J, Vigneron DB, Hricak H, et al. Prostate cancer: metabolic response to cryosurgery as detected with 3D H-1 MR spectroscopic imaging. *Radiology* 1996; 200:489-496.
9. Kurhanewicz J, Vigneron DB, Hricak H, Narayan P, Carroll P, Nelson SJ. Three-dimensional H-1 MR spectroscopic imaging of the in situ human prostate with high (0.24-0.7-cm³) spatial resolution. *Radiology* 1996; 198:795-805.
10. Eckelman WC. Sensitivity of new radiopharmaceuticals. *Nuclear Medicine & Biology* 1998; 25:169-173.
11. Lang L, Aloj L, Kiesewetter DO, et al. A review of new oncotropic tracers for PET imaging. *Nuclear Medicine & Biology* 1996; 23:669-672.
12. Wahl RL. Overview of the current status of PET in breast cancer imaging. *Quarterly Journal of Nuclear Medicine* 1998; 42:1-7.
13. Torizuka T, Zasadny KR, Recker B, Wahl RL. Untreated primary lung and breast cancers: correlation between F-18 FDG kinetic rate constants and findings of in vitro studies. *Radiology* 1998; 207:767-774.
14. Tearney GJ, Brezinski ME, Bouma BE, et al. In vivo endoscopic optical biopsy with optical coherence tomography. *Science* 1997; 276:2037-2039.
15. Chance B. Near-infrared images using continuous, phase-modulated, and pulsed light with quantitation of blood and blood oxygenation. *Annals of the New York Academy of Sciences* 1998; 838:29-45.
16. Villringer A, Chance B. Non-invasive optical spectroscopy and imaging of human brain function. *Trends in Neurosciences* 1997; 20:435-442.
17. Haglund MM, Berger MS, Hochman DW. Enhanced optical imaging of human gliomas and tumor margins. *Neurosurgery* 1996; 38:308-317.

Session 3: Tumor Biology/Pathology

This session of the workshop examined the current role and potential for future developments in the application of functional imaging to assess tumor structure and pathophysiology. The session included overviews on related aspects of clinical and basic oncology; technical overviews of optical spectroscopy and imaging, MRI, MRS, and nuclear medicine/PET; and consideration of their application to provide clinically relevant information related to tumor structure and pathophysiology. Among the specific topics addressed were the following:

- Disease detection and staging at diagnosis and the correlation of functional imaging to histopathology, molecular biology and other clinical measures
- The use of functional imaging to assess changes in cell proliferation, hypoxia, necrosis, apoptosis, and angiogenesis
- Prediction and/or evaluation of tumor response to treatment by functional imaging
- Correlation of standard assessment of treatment response to functional imaging data.

Oncology, Tumor Biology, and Pathophysiology

Most cancers are clonal diseases that develop from the progeny of single cells. Several genetic alterations must occur in a single clone for a normal cell to undergo transformation to a malignant cell capable of uncontrolled proliferation, invasion and metastasis. Mutations often involve signaling molecules and produce activation of oncogenes and the loss of function in tumor suppressor genes. Distinctive profiles of genetic change have been correlated statistically with different stages of histopathologic tumor progression. Current molecular targets for functional imaging include MUC-1, epidermal growth factor receptors (EGFR), HER-2/*neu*, and folate binding proteins on the tumor cell surface. Specific receptors (e.g., somatostatin receptor, estrogen receptor) and cell surface integrins can be targeted by ligands or analogs.

Programmed cell death is a distinct genetic pathway required for the successful development of

multicellular lineages and maintaining homeostasis in such tissues in the adult. This pathway is also heavily used by chemotherapy and radiotherapy to induce the death of cancer cells. Evolving biochemical and biophysical evidence about this pathway indicates the presence of conformational changes in a variety of molecules upon activation of the death pathway. The post-translational modifications of Caspases and BCL-2 family members, coupled with mitochondrial dysfunction and altered plasma membrane lipid polarity offer attractive molecular tags on cells committed to die. Real-time measurements in vivo with functional imaging could provide critical information concerning which, where, and when tumor cells are committed to die following therapy. Such methodology would provide a critical tool to assess rapidly the effectiveness of therapies, especially for the solid tumors.

The physiology of tumors plays a central role in the growth, progression, metastasis, detection, and treatment of solid tumors. For example, angiogenesis and the resulting vascular network are essential for supporting nutrients and for removing products of metabolism during tumor growth. Similarly, blood and lymph vessels provide pathways for cancer cells to metastasize to distant organs. The efficacy of all nonsurgical methods of cancer treatment depends substantially on tumor blood flow, metabolic microenvironment of cells, and molecular transport, in addition to the intrinsic cellular parameters. For example, hypoxia, which is governed by local blood flow and oxygen consumption rates, can be a primary determinant of response to radiation and several chemotherapeutic agents and induces several cytokines that regulate vascular growth. Therefore, better understanding of tumor pathophysiology is urgently needed (1) to improve methods of cancer detection and treatment in current use and (2) to develop new methods that overcome or exploit the vascular and interstitial barriers and the unique microenvironment in a solid tumor.¹

The evaluation of tumor response to therapy has generally relied on changes in tumor size. Based on size, the success or failure of therapy generally takes 2 to 4 months to become apparent. During this time the patient may be receiving toxic therapy, despite the lack of benefit. Functional imaging offers the opportunity to measure response soon after the start

of therapy. Functional imaging also may be useful in evaluating patients undergoing treatment in phase I trials. Such trials usually employ a limited number of patients with advanced cancer and varying tumor types. While phase I trials are primarily used to measure toxicity, they are carefully evaluated for any evidence of efficacy as well. When choosing tumors to treat in phase II trials, investigators analyze the results of *in vitro* and animal testing in addition to the phase I trials. Since functional imaging may provide an early indicator of alterations in tumor metabolism, its use in phase I trials may help in suggesting tumors for further exploration in phase II trials. Thus, functional imaging techniques may assist in evaluating new drugs by speeding the measurement of response. In addition, functional imaging may be particularly important in developing new classes of drugs that are cytostatic or target tumor vasculature since information regarding the putative mechanism of action can be provided noninvasively.

Optical Imaging and Spectroscopy

Optical methods have important advantages, including high molecular specificity (sensitive to concentrations at the nanomolar level), high spatial resolution (sensitive to sub-cellular level morphology, can probe volumes as small as 1 femtoliter), and high temporal resolution (1 femtosecond). Portable, inexpensive optical devices with small fiber optic probes for minimally invasive access can target small lesions in epithelial tissues. Thus, optical imaging and spectroscopy can be used to probe tissue morphology and biochemistry *in vivo* in near real time, providing two important opportunities. First, inexpensive optical surveillance methods can be developed for clinical application that have the potential to enable prevention and/or early intervention through early detection, before advanced pathophysiologic changes take place.²⁻⁴ Second, at the more basic level, *in vivo* optical microscopies provide an opportunity to study gene expression and physiologic function in tumor and host *in vivo*.⁵

Several groups have shown that fluorescence,^{2,3} reflectance,⁶⁻⁸ and Raman^{4,9} spectroscopies have the potential to identify dysplastic and early neoplastic areas *in vivo* in a variety of epithelial tissues. Several biologic and physiologic targets exist for fluorescence spectroscopy. Autofluorescence, which is produced by aromatic amino acids (e.g., tyrosine, tryptophan, phenylalanine), hydrogen carriers (e.g., NADH and FAD), porphyrins, and components of the extracellular matrix (e.g., cross-linked collagen and

elastin), gives information about both the metabolic rate and structural composition of tissue. Fluorescence can be partially reabsorbed by hemoglobin, and vascularity and pO_2 influence reabsorption. Reflectance spectroscopy has a different set of biologic and physiologic targets. The intensity of reflected light depends upon scattering and absorption. Scattering is affected by subcellular organelles, including mitochondria, endoplasmic reticulum, and nuclei. Absorption is produced in part by hemoglobin and depends upon vascularity and pO_2 . Proteins, lipids, and nucleic acids produce Raman scattering, and their spectra depend on the microscopic environment in tissue.

An alternative optical approach to the detection and diagnosis of intraepithelial neoplasia is to image the cells within the epithelium noninvasively using the light reflected from the tissue. In concept, this is similar to histological analysis of biopsy specimens, except that three-dimensional resolution is achieved without removing tissue and contrast is provided without histochemical stains. Imaging systems based on confocal microscopy provide *in vivo* tissue images with histologic resolution that illustrate morphologic and biochemical changes; images can be obtained from the superficial and deep layers of the epithelium.¹⁰ Optical coherence tomography is a novel biomedical imaging technique in which tomographic images of subsurface biological microstructure are obtained with $\sim 10\ \mu\text{m}$ spatial resolution to a depth of 1 to 3 mm.¹¹

Optical imaging techniques also can be exploited to develop microscopies to study gene expression and (patho)physiologic function *in vivo*. Confocal microscopy, multiphoton microscopy, interferometric imaging techniques (e.g., OCT), multispectral imaging (e.g., fluorescence, reflectance), and near-field scanning optical microscopy all have the potential to study *in vivo* physiology. These microscopies can be applied to study physiological barriers in solid tumors and to develop novel strategies to exploit and/or to overcome these barriers for improved cancer detection and treatment.⁵ Some of these technologies can provide real time, *in vivo* monitoring of angiogenesis, inflammation/immune response, hemodynamics (e.g., blood flow rate), pO_2 , pH, calcium concentration, drug localization, vascular permeability, interstitial and lymphatic transport, cellular events, and gene expression at 1–10 μm resolution.

Magnetic Resonance Imaging and Spectroscopy

Due to the intrinsic characteristics of the MR experiment, the MR signal observed from a system under investigation carries a wealth of information that can be extracted with a judicious choice of pulse sequence, contrast or molecular agent, and stimulus (e.g., light, pO_2 , temperature). For example, theoretical calculations on physiologic models of tissue perfusion show that magnetic susceptibility-based MRI techniques are sensitive to the geometry of tissue microvasculature (e.g., capillary size).¹² Thus, MRI opens a window of opportunity to study in vivo critical disease processes, such as tumor angiogenesis, and the effect of treatments associated with them. Work performed in a number of laboratories has demonstrated the capability of these MRI techniques to study in vivo tissue physiology, and, in particular, relative cerebral blood volume and flow, pharmacokinetics of contrast/molecular agents in the brain, breast and other tissues/organs, blood and tissue oxygenation, pH, gene expression, tissue structure (e.g., compartments size and orientation) and compliance. Studies in animal tumor models have helped to elucidate the regulation of angiogenesis and the potential role hormones may play in stimulating angiogenesis.¹³ Several groups have reported that analysis of the pattern of contrast agent uptake kinetics from dynamic contrast-enhanced MRI can identify patients responsive to treatment either before treatment or as a result of the effect of the first course of treatment.^{14,15} Dynamic contrast-enhanced MRI also can assess temperature changes induced by interstitial laser photocoagulation or ultrasound in real time that may delineate the ultimate extent of heat-induced necrosis.

MRS imaging complements MRI techniques by mapping the distribution of tissue metabolites. Thus far, proton and phosphorus-31 have been the most studied in humans, but fluorine-19 has been observed in some human studies, and animal studies have included carbon-13. MRS has also been used for ex vivo analysis of tissue biopsies or surgical samples. In addition to helping to interpret the findings of in vivo MRS, the information provided by ex vivo MRS may complement conventional histologic analysis. MRS imaging techniques can detect pH, altered phospholipid metabolism, and metabolic markers of hypoxia and viability. Tumor pH assessed by phosphorus-31 MRS prior to treatment with hyperthermia and radiation helps to identify sarcoma patients who will benefit from treatment. In work

from several groups in both prostate cancer and brain lesions, proton MRS has been shown to detect regions of abnormal cellular metabolism that correspond to pathology, distinguish benign from malignant lesions, delineate the extent of disease better than contrast-enhanced or T2-weighted MRI, assist in targeting biopsies or focal therapy, assess response to treatment, and distinguish residual disease from necrosis.^{16,17} Preliminary studies from several groups have indicated that evaluating the levels of choline-containing compounds in breast cancer may both improve the specificity of MR characterization of breast lesions and provide a marker for treatment response and predict metastatic potential.

The application of MRI and MRS to early detection, staging, and characterization of breast cancer and gynecologic and genitourinary malignancies is discussed earlier in this paper.

Another use of MR, which detects a signal from (unpaired) electrons rather than nuclei, as mentioned earlier, is electron paramagnetic resonance imaging. The most advanced and actively implemented capability of in vivo EPRI is for the repeated and accurate measurement of pO_2 from a well-defined site.¹⁸ This has been fully implemented in experimental animals, and applications in human subjects are about to begin. Other EPRI measurements relevant to tumor biology have been made in animals using naturally occurring or exogenous probes have been demonstrated (e.g., free radical intermediates of drugs and biochemical intermediates).

Further refinements of MRI, MRS, and EPRI techniques, coupled with future advances in technology and a better understanding of tumor biology, will help expand the opportunities to use MR in clinical oncology for the diagnosis of cancer and evaluation of emerging cancer therapies.

Nuclear Medicine and PET

Nuclear medicine uses radiopharmaceuticals and instrumentation to perform in vivo functional imaging in oncology. The instrumentation includes the use of intraoperative probes, planar gamma camera imaging, single photon emission computed tomography imaging, and positron emission tomography imaging. The instrument used depends on the radionuclide, the radiopharmaceutical, and the desire for qualitative and quantitative data. Intraoperative probes are increasingly used in surgery because of their ability to

identify sentinel lymph nodes. This technique is widely used in patients who have melanoma or breast cancer, and the procedure greatly facilitates the surgery.

Planar gamma camera imaging provides important information concerning the distribution of single photon emitting radiopharmaceuticals in the body. In addition to providing important clinical imaging procedures like bone and gallium scans, it is used in determining the kinetics of radiolabeled antibodies that are used to treat malignancies such as lymphoma and brain tumors. SPECT imaging provides the ability to quantify volumes and radiopharmaceutical accumulation in organs and tumor masses. With the new techniques for correcting for attenuation of the emitted photons, the accuracy of the quantification has improved.

PET imaging provides functional information through its imaging of the positron emitting radionuclides carbon-11, nitrogen-13, oxygen-15, and fluorine-18. Fluorine-18-labeled fluorodeoxyglucose is the most widely used radiopharmaceutical. It is a marker of glucose metabolism, and multiple studies have shown its clinical utility in the evaluation of malignancies such as lung cancer, colorectal cancer, lymphoma, melanoma, head and neck cancer, breast cancer, and others. The most common use of PET at present is in the detection and staging of cancer. Studies have found that (1) PET is sufficiently sensitive and specific in evaluating patients with solitary pulmonary nodules and (2) those found to have a low probability of cancer can be observed rather than being subjected to a thoracotomy.¹⁹ In those discovered to have lung cancer, PET has gained acceptance and Medicare approval to assist in staging of the mediastinum, often sparing patients other procedures, such as mediastinoscopy.²⁰ The detection of recurrent cancer in patients with rising serum markers has also been demonstrated, for example in patients with recurrent colorectal cancer and rising CEA.²¹ Functional imaging with PET also offers the opportunity to measure response soon after the start of therapy. Studies involving small numbers of patients have used a number of markers of tumor metabolism to document response to therapy. In addition to using FDG, studies have employed markers of protein and DNA synthesis, using carbon-11-methionine and carbon-11-thymidine, for example.^{22,23}

There are a wide variety of tumor-specific receptors that have the potential to be targeted for PET imaging. Tumor imaging targeted to a specific

receptor offers a greater specificity than can be achieved by imaging with markers of metabolic processes. The use of receptor-based imaging compounds offers a noninvasive method for in vivo evaluation of the concentration and the functional status of the receptor and the degree of receptor blockade after therapy. For example in vivo assessment of ER status of breast cancer and changes after therapy have been evaluated using 16 α [fluorine-18]fluoro-17 β -estradiol, an estrogen analog with PET.²⁴

Tumor hypoxia produces resistance to the lethal effects of ionizing radiation. Imaging of human tumors using [fluorine-18]fluoromisonidazole has shown decreases in hypoxia in some but not all tumors during a course of fractionated radiotherapy, and no correlation between tumor size and extent of hypoxia.²⁵ In addition to the above metabolic markers, flow, permeability, and blood volume can be measured with PET but have not found routine clinical application to date.

Key Findings and Recommendations

The following principal roles related to tumor biology and pathophysiology were identified for functional imaging in oncology:

- Disease detection at an early stage in order to minimize the risk of disease spread and maximize the likelihood of successful treatment.
- Molecular/pathophysiological characterization and assessment of disease extent in order to better stage patients and tailor treatment to individual patients.
- Early assessment of response to treatment to identify patients in need of altered treatment strategy and help to evaluate new drugs by speeding the measurement of response.
- Identification of residual disease at the completion of treatment and/or early identification/characterization of recurrent disease to identify patients in need of further therapy and help guide the selection of treatment.
- Determination of the basis for treatment success or failure to guide the development of improved treatment.

While promising results addressing each of these roles have been obtained with the functional imaging methods considered at this workshop, much work remains, particularly in their integration.

The current understanding of the molecular genetics, biology, and immunology of cancer is a result of significant investment by the National Institutes of Health (NIH) and other federal agencies. The advances in molecular and cellular biology of cancer have been spectacular and will have immediate benefits for the molecular diagnosis of cancer. Commensurate investment in tumor biology and pathophysiology research is vital to realize the fruits of molecular biology in cancer prevention and treatment. The imaging technologies outlined in this report are critical components of this effort and have the potential of noninvasively obtaining quantitative data on the biology/physiology of tumors during growth, regression and relapse.

Research Priorities

Short Term

- Encourage research by and continue to organize and support research conferences for investigators with expertise in various functional imaging modalities.
- Standardize methods for coregistration of data from various modalities.
- Evaluate and standardize methods for data acquisition, analysis, interpretation, and integration with current clinical end points.
- Develop and test improved methods/agents to characterize tumor vascularity.
- Integrate functional imaging into development of new antiangiogenic or cytostatic drugs.
- Conduct pilot studies for development and testing of new tracers/contrast agents.
- Develop methods for MR-guided therapy and monitoring response with feedback control.
- Evaluate PET versus coincidence detection.
- Add specificity to existing optical technologies.
- Facilitate the development of advanced MR techniques and access to clinical MR scanners for trials.
- Conduct further studies in the use of PET for staging.

- Implement real-time targeting/monitoring for optical methods.
- Improve depth of in vivo optical imaging by improved probe and technology development.

Intermediate Term

- Foster relationships among research institutions and industry.
- Conduct multicenter prospective clinical trials to compare functional imaging response with clinical results.
- Evaluate functional imaging techniques in terms of their effect on patient outcomes and cost-effectiveness.
- Improve MR sensitivity and spatial resolution (e.g., higher field, better radiofrequency coils, new contrast agents).
- Develop/improve MRI/EPRI methods for measuring hypoxia.
- Develop innovative, portable instrumentation for clinical optical studies.

Long Term

- Study mechanisms of success or failure of various therapies.
- Improve the understanding of the connections between biology and information obtained from functional imaging.
- Develop predictive criteria based on functional imaging results for response to specific histologic and clinical treatments.
- Conduct multidisciplinary clinical trials that assess the relative merits of functional imaging techniques in terms of effect on patient outcome and cost-effectiveness.
- Develop and apply methods that enable prevention/early intervention through risk assessment and early detection, before advanced pathophysiologic changes occur.
- Identify and develop molecular markers for gene expression and surface receptor expression that can be detected noninvasively by functional imaging.
- Develop methods for assessing apoptosis, cell viability, and tumor aggressiveness.
- Establish an open imaging database for educational and research purposes, and for

providing data to test and compare methods of data processing.

References

1. Jain RK. The next frontier of molecular medicine: delivery of therapeutics. *Nature Med* 1998; 4:655-657.
2. Richards-Kortum R, Sevick-Muraca E. Quantitative optical spectroscopy for tissue diagnosis. *Ann Rev Phys Chem* 1996; 47:555-606.
3. Wagnieres GA, Star WM, Wilson BC. In vivo fluorescence spectroscopy and imaging for oncologic applications. *Photochem Photobiol* 1998; 68:603-632.
4. Mahadevan-Jansen A, Follen Mitchell M, Ramanujam N, Malpica A, Thomsen S, Richards-Kortum R. Near-infrared Raman spectroscopy for detection of cervical precancers. *Photochem Photobiol* 1998; 68:123-132.
5. Jain RK. Delivery of molecular and cellular medicine to solid tumors. *J Controlled Release* 1998; 53:49-67.
6. Perelman LT, Backman V, Wallace M, et al. Observation of periodic fine structure in reflectance from biological tissue: a new technique for measuring nuclear size distribution. *Phys Rev Lett* 1998; 80:627-30.
7. Mourant JR, Boyer JD, Johnson TM, et al. Detection of gastrointestinal cancer by elastic scattering and absorption spectroscopies with the Los Alamos Optical Biopsy System. *Proc SPIE* 1995; 2387:210-217.
8. Zonios G, Perelman LT, Backman V, et al. Diffuse reflectance spectroscopy of human adenomatous colon polyps in vivo. *Applied Optics*, in press.
9. Hanlon EB, Manoharan R, Koo T-W, et al. Prospects for in vivo Raman spectroscopy. In: Leach MO, ed. *Physics in medicine and biology*. Philadelphia, PA: Institute of Physics, 1999.
10. Rajadhyaksha M, Grossman M, Esterowitz D, Webb RH, Anderson R. In vivo confocal scanning laser microscopy of human skin: melanin provides strong contrast. *J Invest Dermatol* 1995; 104:946-952.
11. Brezinski ME, Tearney GJ, Bouma B, et al. Optical biopsy with optical coherence tomography. *Ann New York Acad Sci* 1998; 838:68-74.
12. Boxerman JL, Hamberg LM, Rosen BR, Weisskoff RM. MR contrast due to intravascular magnetic susceptibility perturbations. *Magn Reson Med* 1995; 34:555-566.
13. Schiffenbauer YS, Abramovitch R, Meir G. Loss of ovarian function promotes angiogenesis in human ovarian carcinoma. *Proc Natl Acad Sci, USA* 1997; 94:3203-8.
14. Hawighorst H, Weikel W, Knapstein PG, et al. Angiogenic activity of cervical carcinoma: assessment by functional magnetic resonance imaging-based parameters and a histomorphological approach in correlation with disease outcome. *Clin Cancer Res* 1998; 4:2305-2312.
15. Mayr NA, Yuh WT, Zheng J, et al. Prediction of tumor control in patients with cervical cancer: analysis of combined volume and dynamic enhancement pattern by MR imaging. *AJR Am J Roentgenol* 1998; 170:177-182.
16. Wald LL, Nelson SJ, Day MR, et al. Serial proton magnetic resonance spectroscopy imaging of glioblastoma multiforme after brachytherapy. *J Neurosurg* 1997; 87:525-534.
17. Parivar F, Hricak H, Shinohara K, et al. Detection of locally recurrent prostate cancer after cryosurgery: evaluation by transrectal ultrasound, magnetic resonance imaging, and three-dimensional proton magnetic resonance spectroscopy. *Urology* 1996; 48:594-599.
18. O'Hara JA, Goda F, Demidenko E, Swartz HM. Effect on regrowth delay in a murine tumor of scheduling split-dose irradiation based on direct pO_2 measurements by electron paramagnetic resonance oximetry. *Radiat Res* 1998; 150:549-556.
19. Lowe V, Fletcher J, Gobar L, et al. Prospective investigation of positron emission tomography in lung nodules. *J Clin Oncol* 1998; 16:1074-1108.
20. Graeber GM, Gupta NC, Murray GFJ. Positron emission tomographic imaging with fluorodeoxyglucose is efficacious in evaluating malignant pulmonary disease. *J Thorac Cardiovasc Surg* 1999; 117:719-727.
21. Flamen P, Stroobants S, Van Cutsem E, et al. Additional value of whole-body positron emission tomography with fluorine-18-2-fluoro-2-deoxy-D-glucose in recurrent colorectal cancer. *Clin Oncol* 1999; 17:894-901.
22. Lindholm P, Leskinen-Kallio S, Grenman R, et al. Evaluation of response to radiotherapy in head and neck cancer by positron emission tomography and [^{11}C]methionine. *Int J Radiat Oncol Biol Phys* 1995; 32:787-794.
23. Shields AF, Mankoff DA, Link JM, et al. [^{11}C]Thymidine and FDG to measure therapy response. *J Nucl Med* 1998; 39:1757-1762.

24. Dehdashti F, Flanagan FL, Mortimer JE, Katzenellenbogen JA, Welch MJ, Siegel BA. PET assessment of “metabolic flare” to predict response of metastatic breast cancer to antiestrogen therapy. *Euro J Nucl Med* 1999; 26:51-56.
25. Rasey JS, Koh W-J, Evans ML, et al. Quantifying regional hypoxia in human tumors with positron emission tomography of [^{18}F]fluoromisonidazole: A pretherapy study of 37 patients. *Int J Radiat Oncol Biol Phys* 1996; 36:417-428.

Session 4: Pharmacology

The goal of this session was to examine current oncologic needs and the potential role of functional imaging in providing noninvasive information on drug uptake, distribution, and metabolism. Other goals include better defining the role of imaging in drug selection and clinical outcome.

Positron Emission Tomography

A number of areas related to this topic were discussed in formal presentations. The role of PET imaging in the measurement of metabolic response to cytotoxic therapy was discussed. For most cancers, more than one treatment option has been established, through prospective randomized clinical trials, as safe and effective for some patients. Oncologists have long sought tests that could select the most appropriate therapy for the individual patient without relying on probability considerations. PET provides this opportunity to serially and noninvasively measure several chemical characteristics of a tumor. The most well known of the PET procedures uses FDG as an indicator of cellular energetics; however, a broad range of radiopharmaceuticals are available and are being validated for their role in characterizing cancers. Among this list are tracers for measuring blood flow and capillary permeability, several aspects of energy metabolism, cellular growth and growth factors, and other biochemical characteristics that influence the response of a tumor to therapy.

The FDG procedure is widely used because it is simple, it can be interpreted with several well-established data analysis procedures, and it provides much useful clinical information. In addition to FDG, cellular energetics can be evaluated using carbon-11-glucose labeled at C₁ or C₆ or randomly labeled. The University of Washington PET group has demonstrated that the ratio of metabolic rates for glucose (MRGlc) and FDG (MRFDG) varies between tumors and that there is no legitimate “lumped constant” to allow MRGlc to be inferred from MRFDG.¹ While this was suspected because of the heterogeneity of enzymes in cancer, it has now been established experimentally. FDG results should be clearly labeled as the MRFDG and should not be disguised as the MRGlc calculated from FDG results, even if the lumped constant is assigned a value of unity. This is an important consideration in the evaluation of this biochemical marker.

The role of PET for imaging cellular growth is becoming increasingly appreciated. Many of the initial methods for imaging growth used amino acids, with emphasis on carbon-11-methionine. However, amino acid kinetics have never been validated as reflective of protein synthesis. In this regard, methionine is particularly problematic because the methyl-carbon-11 also is involved in transmethyations. Also, protein and enzyme biosynthesis, as well as energy metabolism, continues after an individual cancer cell has been doomed to death—that is, prevented from reproducing. Carbon-11-thymidine, a nucleoside specifically incorporated into DNA, provides a more specific measure of cellular reproduction. This procedure has been validated, and its value in imaging cancer was discussed.

Other radiopharmaceuticals are being developed for imaging important cellular growth factors. The most fully developed of these is fluorine-18-fluoroestradiol for imaging hormonally sensitive breast cancer. New developments can be anticipated in imaging with labeled cytokines and receptor ligands, such as the sigma receptors that are overexpressed in a number of tumors. Yet additional PET methods can be used to characterize biochemical factors that limit response to cancer therapy, including hypoxia imaging with fluorine-18-fluoromisonidazole and imaging of multidrug resistance.

The session also considered approaches to understanding and treating addiction instead of treating cancer in smoking-related cancers. PET has been used to understand better the addiction process, especially the relative pharmacokinetics of addictive drugs and other drugs of the same class. Thirty percent of all cancer deaths are related to cigarette smoking and therefore are preventable, either by preventing the initiation of smoking or by early smoking cessation. This places a sense of urgency on understanding, preventing and treating smoking addictions. Molecular imaging, by virtue of its ability to measure the pharmacokinetics and pharmacodynamic effects of addictive drugs directly in the human brain, holds promise in understanding mechanisms underlying addictive behavior, and this knowledge can be used to develop new treatment strategies. Similarly, imaging can be used to investigate new strategies to interfere with the

biochemical effects of addictive drugs. Because studies can be carried out in living addicted subjects, relationships between brain chemistry and behavior can be investigated. Optimally, transdisciplinary approaches to addiction treatment will integrate knowledge on the interplay between the drug, the environment and genetics.

Magnetic Resonance Imaging

In addition to discussions on the use of PET in pharmacology, several investigators outlined the use of MRI in PK/PD. Using new imaging techniques to directly measure drug levels and drug effects both over time and over space within the same animal has the potential to revolutionize the way drugs are studied in vivo. As an emerging discipline, MRI of PK/PD is a prime example of biomedical engineering: the successful execution and optimization of these studies requires an understanding of both the underlying biology/physiology and the engineering/physics of the imaging techniques. For cancer in particular, where heterogeneity both within the tumor and across time is of critical importance for therapy, imaging PK/PD may be the most accurate way both to judge the effectiveness of new treatments, and to help plan or modify the treatment of individual subjects. Examples demonstrate both the power and subtlety of quantitative image analysis using MRI. Heterogeneity in the underlying tissue properties and behavior of water molecules can affect how any given amount of contrast material produces measurable signal changes in MRI. These have been demonstrated in both a breast cancer study, in which quantified rate constants were better predictors of disease presence than simple signal enhancement, and a brain tumor study, in which quantifying increases in blood volume was a more specific predictor of cancer than simple signal changes due to blood-brain barrier disruption. Finally, the nature of the probe itself is critical in that small and large contrast agents show large differences in permeability in animal cancer models.

Ligands labeled with nuclear magnetic resonance (NMR)-active isotopes can be used to follow drug action, especially the action of 5-fluorouracil. Since the classic fluorine-19 NMR studies of 5-fluorouracil in 1984, this drug has served as the paradigm for development of NMR spectroscopy of cancer drugs.² Pharmacokinetics of 5-fluorouracil can be monitored in mouse liver by fluorine-19 NMR spectroscopy while drug activation is monitored in a subcutaneous

tumor.² After the large resonance originating from the parent drug declines, a smaller resonance originating from dihydrofluorouride, together with a very small α -fluoro- β -alanine peak, remains. A small peak composed of a mixture of the three cytotoxic nucleotides provides an index for monitoring drug activation. Dr. Hull's group at Heidelberg later showed that some tumors exhibit both activation and catabolism of 5-fluorouracil in the tumor, whereas others exhibit only catabolism; the relative extent of activation and catabolism is reflected in the overall response of the tumor to chemotherapy.³ The potential power of NMR pharmacokinetics as a predictor of therapeutic efficacy is demonstrated by the studies by McSheehy et al. from Griffiths' group.^{4,5} Coadministration of 5-fluorouracil with methotrexate increases the rate of nucleoside/nucleotide production by about a factor of three. Interferon- α decreases the rate of 5-fluorouracil elimination from the tumor by about a factor of two, whereas thymidine increases both drug retention and activation.

These studies have been extended to human subjects with liver metastases of colorectal tumors.⁶ Over 30 patients have been examined, but currently used methods have not detected metabolites of the drug; rather, only the half-life of the drug in the tumor has been measured. Patients with retention times longer than 20 minutes are termed "trappers" and those with shorter drug half lives are referred to as "nontrappers." Among the trappers, there is an approximate 60-40 split between responders and nonresponders; however, virtually all the nontrappers do not respond. Consequently, if a patient exhibits a half-life shorter than 20 minutes, administration of the drug can be discontinued. This information will spare a large proportion of the patients from the harmful side effects of the drug during ineffective treatment with this agent. A higher predictive capability might be achieved by NMR if metabolites of the drug could be detected, as they are in animal models. This will require the development of more effective methods of localization of the fluorine-19 signal in the tumor and improvement of the signal-to-noise ratio by decoupling, polarization transfer, and/or increasing the magnetic field.

The possibility of labeling other antineoplastic agents with fluorine to facilitate monitoring of their pharmacokinetics by MRS has been explored. Placement of the fluorine atom at the active sites of drugs like cyclophosphamide resulted in a decrease in

pharmacological activity. To overcome this problem, antineoplastic agents have been labeled with carbon-13 and detected by polarization-transfer carbon-13 MRS. Alternatively, one also could detect them by the inverse detection, monitoring proton while decoupling carbon-13. This technique was demonstrated in mice with subcutaneous RIF-1 tumors using the newly developed antineoplastic agent temozolomide, which is active against human brain tumors and melanoma.

It is not always necessary to chemically or isotopically label a drug that can be detected by MRS, as illustrated by the cisplatin analog iproplatin. This drug contains 12 equivalent protons that resonate at the same frequency as lactate and lipids; however, by monitoring multiquantum coherence transfer from the methine to the methyl protons, the iproplatin methyl resonance can be exclusively detected. This approach needs to be extended to more commonly used cisplatin derivatives such as carboplatin.

Finally, a new class of agent—Gd-Texophrin, which can be detected by MRI—has been prepared. This porphyrin, which is now in phase III clinical trial as a radiation sensitizer, contains a Gd atom at the center of the porphyrin ring. The Gd atom is ligated to water and serves as a very potent MRI contrast agent. In humans, image enhancement by Gd-Texophrin was limited to the tumor site and was distinct from the pattern of enhancement induced by administration of the nonspecific relaxation enhancer Gd-DTPA.

Optical Methods in Site-Specific Pharmacokinetics

Optical methods also offer potential for site-specific pharmacokinetics. Site-specific pharmacokinetics are especially important in the development of new chemotherapy and photodynamic therapy agents, for which relative concentrations in various tissues and time histories of concentrations are key factors for efficacy of treatment. Optical methods to measure noninvasively drug concentrations in tissue are based on detection of the fluorescence or determination of the absorption coefficient. Fluorescence measurements have the advantage of being sensitive to small concentrations of fluorescent drugs, but are difficult to calibrate for the purposes of quantitative results. The absorption coefficient can be determined by photon migration methods, which are suitable for drugs that have an absorption band in part of the near

infrared and provide average values over relatively large tissue volumes. Small source-detector separations can be used to determine drug concentrations in small tissue volumes if careful attention is paid to the optical geometry of the measurement. For this method, the drug can have an absorption band anywhere in the visible-NIR range, but the absorption band must be strong to measure small concentrations. All three methods have been demonstrated successfully in vivo for therapeutic dosages of chemotherapy or photodynamic therapy agents.

In Vivo Optical Applications

Internal biological light sources that report externally the inner workings of mammalian biology can be built into animal models of human physiology and disease. Although mammalian tissues are relatively opaque, low-level light transmitted through tissues can be externally monitored with sensitive detection systems. The genes that encode biological sources of light, most notably that of the firefly, can be moved fairly easily into bacteria or mammalian cells. The cells tagged by expressing these genes, or alternatively the genes themselves, can be transferred into animal models. The entire living animal then can be screened so that the light reveals the relative number of labeled cells at various tissue sites. Evaluation of novel antineoplastic therapies would be enhanced by noninvasive detection of tumor cells in living animal models. Since light is transmitted through mammalian tissues, it was possible to externally and quantitatively monitor growth and regression of human tumor cells tagged with bioluminescence, engrafted into immunodeficient mice. The efficacy of both immunotherapeutic treatment with ex vivo expanded human T-cell–derived effector cells and chemotherapy was evaluated. In the absence of therapy, animals showed progressive increases in signal intensity over time. In contrast, reductions were observed in treatment groups. Immunotherapy dramatically reduced signals at high effector to target cell ratios, and significant decreases were observed with lower ratios. This model system allowed sensitive, real-time spatiotemporal analyses of the dynamics of neoplastic cell growth and facilitated rapid optimization of effective treatment regimens. The natural occurrence of several colors of biological light, and the ability to change the color of emission through mutation, presents the possibility of simultaneously monitoring multiple processes such as tumor progression and immune response in living

mammals. Noninvasively monitoring light emitted from within a living mammal provides an opportunity for obtaining more specific information about interactive physiological processes and whole biological systems in less time, using fewer animals. This is especially relevant to the costly processes of drug discovery where significant advances have been made in generating large numbers of potentially useful compounds, and screening in animals has remained a significant bottleneck. External monitoring of biological light sources accelerates in vivo analyses with broad applications in the fields of infectious disease, oncology, gene therapy, mammalian development, and other areas in which animal models are used as predictors of human biological processes. PK/PD monitoring is important to optimize the further clinical development of agents intended to photosensitive tumors.

Pharmacology without Imaging Tools

As a bridge between scientists involved in classic therapeutic development and those involved in the implementation of imaging technologies, this session also addressed pharmacologic development without imaging tools. Data were presented on the interplay between microtubules (i.e., a very specific target) and genetic susceptibility, as mediated via the p53 oncogene. Understanding the combination of these factors was shown to be essential in the interpretation of cytotoxicity of taxanes and vinca alkaloids. The payoff for careful attention to targets in the genetic context was that cells resistant to one class of molecules could be collaterally sensitive to another class. The emerging uses of biomarkers in therapeutic development demonstrate the framework into which imaging could be introduced. For PK/PD studies in mice, as part of the preclinical development of new anticancer drugs, imaging techniques could be applied to more direct exploration of targets—for example, the impact of 17-allylaminogeldanamycin on heat shock protein and intracellular signaling.

Research Priorities

While there are some aspects of development that are highly specific for a single mode of imaging (e.g., optical, MR, PET), there are many areas of common ground for which there are general recommendations:

- Imaging researchers and basic scientists should work in close proximity to achieve the primary goal of relevant clinical studies.

- Developing imaging probes to study the functional interaction with contemporary molecular targets should be a priority
- Imaging should be used to study the biodistribution of new drugs, which can be facilitated by labeling with radioisotopes, stable isotopes, or chromophores.
- Animal studies, including the use of gene knockouts as models for target-probe interactions, are useful tools for translational research, particularly with the use of small animal imaging.
- The time course of the imaging signal is usually very complex, and further development of mathematical algorithms and modeling is necessary to extract the maximum amount of quantitative pharmacologic information from each imaging procedure.
- More emphasis is needed on the design of probes with respect to their ligand specificity and metabolic stability in order to minimize nonspecific signals during imaging.

References

1. Spence AM, Muzi M, Graham MM, et al. Glucose metabolism in human malignant gliomas measured quantitatively with PET, ^{11}C glucose and FDG: analysis. *J Nucl Med* 1998; 39:440-448.
2. Stevens AN, Morris PG, Iles RA, Sheldon PW, Griffiths JR. 5-fluorouracil metabolism monitored in vivo by ^{19}F NMR. *Br J Cancer* 1984; 50:113-7.
3. Schlag P, Yi J, Friedl P, Hull W, Berger M. Animal experiments on the toxicity of FUDR and 5-FU within the framework of regional infusion chemotherapy of the liver. *Langenbecks Archiv für Chirurgie* 1990; 375:81-86.
4. McSheehy PMJ, Seymour MT, Ojugo ASE, et al. A pharmacokinetic and pharmacodynamic study in vivo of human HT29 tumours using F-19 and P-31 magnetic resonance spectroscopy. *Eur J Cancer* 1997; 33:2418-2427.
5. McSheehy PMJ, Prior MJW, Griffiths JR. Enhanced 5-fluorouracil cytotoxicity and elevated 5-fluoronucleotides in the rat Walker carcinosarcoma following methotrexate

- pretreatment: a F-19-MRS study in vivo. *Brit J Cancer* 1992; 65:369-375.
6. Wolf W, Waluch V, Presant CA. Non-invasive F-19-NMRS of 5-fluorouracil in pharmacokinetics and pharmacodynamic studies. *NMR in Biomedicine* 1998; 11:380-387.

Session 5: Cellular and Molecular Targets

The purpose of this session was to review the range of potential targets for functional imaging within particular contexts. Since the various imaging modalities relate to the physical properties of the imaging target, it is necessary to organize the discussion along the lines of the particular imaging tools. As these techniques are rapidly evolving—several of them might have important impacts on human health problems—this session examined several promising research directions. Although the discussion attempted to highlight situations that involve general challenges for the field, much of the information discussed is relevant only in specific imaging contexts. More experience and clinical correlation will be necessary before a systematic development approach for the related imaging techniques can be defined.

Biological Targets for Functional Imaging

There are several potentially important *in vivo* analytical tools for functional imaging. Identifying compelling proof-of-concept applications helps ensure that sufficient resources are available to develop this new area quickly. One promising area for application is in monitoring the epithelial response to locally delivered chemoprevention agents. In the early stages of many epithelial cancers, the area of genetic injury is localized to the organ's epithelial lining cells. Dedicated epithelial imaging devices may be particularly useful in detecting characteristics of a tissue involved in the clonal expansion stage of early cancer.

One example of this situation is the precancerous lesion leukoplakia. In about 10% of the cases, this white mucosal lesion is associated with progression to invasive oral cancer. In an ongoing chemoprevention trial, a cyclooxygenase inhibitor delivered as a rinse is being evaluated for its benefit in arresting the progression of oral leukoplakia. From previous work with this oral rinse, it is expected that a favorable response to this drug would be associated with the reversion of oral inflammation. This would potentially result in a hyperplastic oral epithelium reverting back to a thinner, more orderly state of the epithelium. Using optical devices, it may be possible to monitor the thickness of the epithelium to correlate to the ambient level of the proinflammatory cytokines. These cytokines have been shown to

sustain the environment that favors cancer progression. Although, there are challenges such as accounting for the heterogeneity of the oral precancer process, clinicians would be able to accelerate the development of cancer prevention drugs if validated monitoring tools, such as optical imaging devices, were available. Selecting applications for functional imaging in areas of clinical management where existing technologies are not answering clinicians needs may represent a useful strategy for allowing rapid maturation of this exciting new field.

Monitoring In Vivo Gene Expression with MRI

MRI offers a noninvasive means to map organ structure and function by sampling the amount, flow, or environment of water protons *in vivo*. Such intrinsic contrast can be augmented by the use of paramagnetic contrast agents in both clinical and experimental settings; however, these agents are little more than anatomical reporters that at best can label individual fluid compartments or distinguish tissues that are magnetically similar but histologically distinct. To permit a more direct imaging of the physiological state of cells or organs, several new classes of “smart” MRI contrast agents change their influence on nearby water protons in a conditional fashion. The agents modulate fast water exchange with the paramagnetic center, yielding distinct “strong” and “weak” relaxivity states. Two types of biological events trigger the modulation: (1) enzymatic processing of the agent and (2) binding of an intracellular messenger. These agents represent the first examples of direct, three-dimensional MRI visualization of gene expression and intracellular second messenger concentration.

Tumor Biology and Physiology

The success of tumor detection using, for example, contrast agents and monoclonal antibodies is governed by their selective uptake in tumors. In radiation therapy, as mentioned above, the response to treatment depends in part on local oxygen tension. In chemotherapy, immunotherapy, gene therapy, and photodynamic therapy, the effectiveness of treatment depends on the localization within tumors of an appropriate therapeutic agent (e.g., cytotoxic drugs,

biological response modifiers, effector cells, gene targeting vectors, porphyrins), in therapeutically adequate quantities, with its tolerable accumulation levels in sensitive normal tissues. Again, whether or not a therapeutic agent reaches its target cells depends on blood perfusion and molecular transport within the tumor. Even then, the target cell may be resistant to that agent. The sensitivity to drugs and radiation is a function not only of tumor cells' inherent sensitivity characteristics but also their proliferation kinetics and phase within the cell cycle. These kinetics are in turn modified by the nutritional and metabolic microenvironment of the tissue. Finally, in hyperthermia, the temperature distribution and interstitial microenvironment, which are both governed by the local blood flow rate, determine the outcome of treatment. Therefore, better understanding of tumor pathophysiology is urgently needed to improve methods of cancer detection and treatment in current use and to develop new methods that overcome or exploit the vascular and interstitial barriers and the unique microenvironment in a solid tumor.

Using Tumor Biology to Map Gene Expression

Tumor biology with specific imaging approaches can be applied to in vivo mapping of gene expression. For example, gene therapy involves the introduction of a therapeutic gene into the body. Imaging techniques are being developed to map the topography and level of such gene expression, primarily through the use of imaging marker genes (IMG), which are coexpressed with a given therapeutic gene. To date, two fundamentally different IMG strategies have been investigated: (1) marker genes encoding intracellular enzymes and (2) marker genes encoding cell surface-bound receptors or other ligands. The first approach harnesses the ability of certain enzymes to modify imaging prodrugs so that tissue accumulation of such drugs correlates with expression of the gene. The second approach uses cell surface expression of a receptor or a ligand-binding protein, which can then be probed with novel imaging tracers. Various nuclear medicine, PET, MR, and optical techniques have been developed for imaging of gene expression at the whole body level.

Targets for Optical Spectroscopy and Imaging

Using optical technologies in tumor functional imaging is undergoing enormous growth since they

offer a potentially powerful diagnostic tool. The information is carried in light signals, and biopsy may not be required. Processing can occur in real time, and the ability to create spectroscopic images of functional indicators of disease has been demonstrated.

A functional imaging technique must have two characteristics: it must provide an appropriate signal in response to a cellular/molecular target and it must be capable of supplying quantitative information. Optical spectroscopy has these properties. It can provide three types of information: classification, chemical, and morphological. The third is perhaps the most interesting as it directly relates to diagnostic parameters studied by pathologists. Three optical techniques are of particular interest to molecular imaging: fluorescence, the Raman effect, and reflectance. All have been tested in vivo.

At present, fluorescence is the most widely used spectral diagnostic technique. The short penetration depth of visible light makes fluorescence (and reflectance) best suited to tissue surfaces such as the epithelial linings of the body. It has mainly been used for disease classification. Its targets are the naturally occurring fluorophores in the body. These fluorophores include structural proteins, porphyrins, NADH, flavins, and (in the near ultraviolet) aromatic amino acids.

As an example, consider the detection of transitional cell carcinoma (TCC) in bladder epithelium. Fluorescence spectra of TCC and normal tissue excited with light at 400 nm show characteristic features corresponding to collagen and porphyrins. Researchers used principal component analysis to characterize clinical data from 47 sites in 10 patients and develop a diagnostic algorithm.¹ A cross-validation analysis classified 44 out of 47 sites correctly.

The Raman effect provides high information content, has narrow spectral features, and is molecule-specific; however, it is more difficult to implement than fluorescence. Raman signals from tissue are 1,000 times weaker than those of fluorescence, and they can be masked by broad-band fluorescence background. In addition, specially designed optical fiber probes are required to avoid probe-induced background.

The Raman effect is an inelastic scattering process. Many molecules, when subjected to optical radiation, can be set into vibrational motion. This energy comes from the incident photon, whose energy is

proportional to its frequency, and is thereby reduced. This molecular information, encoded in the Raman scattered light, can be extracted by means of a spectral analyzer. Since every molecule possesses a unique Raman spectrum, the molecular composition of a tissue sample can be determined. Thus, considering that the onset of cancer is accompanied by changes in chemical composition, Raman scattering is a potentially powerful diagnostic technique.

The targets of Raman scattering include a wide range of specific tissue molecules. Proteins, lipids, and nucleic acids all exhibit distinct Raman signatures. Near infrared light is most suitable for Raman spectroscopy of tissue. These wavelengths penetrate deeply into tissue, making this technique well-suited to probing solid lesions.

As an example, consider the detection of breast lesions. As cancer develops, biochemical changes occur in the tissue—an increase in structural proteins, accumulation of calcium salts, increases in metabolites, and changes in DNA content and concentration. These are reflected in corresponding structural changes—alterations in extracellular matrix (fibrosis), microcalcification and necrosis, increased proliferation, and cellular alterations. Thus, chemical and morphological changes are interrelated. The Raman spectra of normal breast tissue and an adenocarcinoma show distinct differences. Normal breast tissue exhibits many features characteristic of lipids, whereas carcinoma exhibits protein features. In 24 samples of normal and carcinoma breast tissue, these distinctions were used to completely separate the tissue types. Note that, as opposed to the fluorescence example discussed above, the Raman classification is based on directly observed specific molecular differences.²

Optical reflectance is the simplest spectroscopic technique as it is based on the analysis of the pattern of reflected light, as based on the elastic scattering of white light. The targets for reflectance are tissue absorbers and scatterers. Hemoglobin is the most important tissue absorber. Mitochondria and cell nuclei are examples of important tissue scatterers. Reflectance thus can provide morphological information about the tissue.

As an example, consider dysplasia, the precursor to cancer, in Barrett's Esophagus. Because dysplasia in Barrett's Esophagus is flat and cannot be seen with an endoscope, it is difficult to detect, is difficult to diagnose, and precludes interobserver agreement.

Reflectance can be used to measure the size distribution of cell nuclei at the epithelial surface of the esophagus. Enlarged cell nuclei are the key feature used by pathologists to identify dysplasia.

The reflectance spectrum from Barrett's tissue can be obtained during esophagoscopy by means of an optical fiber probe inserted into the biopsy channel of the endoscope. It consists of a large background from submucosal tissue on which is superimposed a small (2% to 3%) component that is oscillatory in wavelength due to scattering by cell nuclei in the mucosal layer. The amplitude of this component is related to the density of epithelial nuclei, which is a measure of nuclear crowding. The number of oscillations over the visible wavelength range is related to nuclear size. The larger the nuclei, the larger number of oscillations. Tissue is classified as dysplastic when an increased density of enlarged nuclei is detected.

In a clinical study of reflectance in 79 samples from 49 patients, dysplasia (high-grade and low-grade) could be distinguished from non-dysplastic Barrett's tissue with a sensitivity of 92% and a specificity of 97%, as referenced to the averaged independent diagnoses from four gastroenterological pathologists.^{3,4}

In conclusion, optical spectroscopy and imaging provide physical and molecular information about human tissue. Disease classification, chemical content and morphological composition can be determined. Optical data can supply the same information provided by histochemistry and histopathology. Thus, optical techniques have high potential as functional imaging tools in oncology.

Targets for Nuclear Medicine/PET

There are significant factors that must be considered in searching for better molecular targets that can improve the sensitivity and specificity of oncologic imaging: (1) whether the receptor is overexpressed on tumor or truly reflects malignant transformation; (2) the number of receptors per cancer cell required for external imaging; (3) the effects of receptor processing within the cell on retention of the label within the target cell; (4) designing ligands such that the intact molecule or its labeled catabolites clear rapidly from normal tissues; and (5) the nature of the image application (i.e., tumor detection, monitoring therapy, or receptor quantification) in vivo.

Growth factor receptors, particularly epidermal growth factor receptors, represent a reasonable paradigm of the development of more molecularly specific targets. There is a long history of attempting to exploit the overexpression of EGFR and *cerbB-2* on a variety of cancers for imaging several types of malignancies, primarily through the use of radiolabeled monoclonal antibodies (mAbs). The presence of receptor on liver and other tissues, however, has confounded this approach. To circumvent these problems, one can target EGFR variant III (EGFRvIII). This receptor represents a gene mutation that has been found only on oncogenically transformed cells, including glioma, breast carcinoma and non-small cell lung carcinoma. The receptor has a deletion of 801 base pairs of the coding sequence, resulting in the deletion of amino acids 6–273 from the EGFR extracellular domain. Progress towards using EGFRvIII as a molecular target for imaging includes (1) the development of mAbs specifically reactive with EGFRvIII but not wild-type EGFR; (2) the development of labeling methods that residualize the radionuclide within the tumor cell after internalization and processing; (3) demonstration of selective uptake in EGFRvIII xenografts; and (4) determination of at least 100,000 EGFRvIII receptors per tumor cell, a level compatible with nuclear medicine imaging approaches. Smaller molecular weight scFv fragments and combinatorial-derived anti-EGFRvIII peptides will be pharmacologically more appealing carriers for imaging EGFRvIII expressing gliomas, non-small cell lung carcinomas and breast carcinomas.

Evaluation of Gene Therapy and Signal Transduction Pathways

Pilot studies have established that imaging gene expression with radiolabeled tracers is feasible and is being extended to patient studies. Quantitative in vitro and in vivo imaging data have demonstrated a consistent relationship between radiotracer accumulation, reflecting the level of expression of the marker/reporter gene—for example, herpes simplex thymidine kinase (HSV1-tk) and independent measures of HSV1-tk expression (e.g., mRNA levels and a functional sensitivity assay to the antiviral drug Ganciclovir). The potential for clinical imaging also has been demonstrated using quantitative autoradiographic,⁵ gamma camera,⁵ and positron emission tomographic imaging techniques.⁷

New approaches to indirect imaging of gene expression using a marker/reporter gene coupled to a

therapeutic gene of interest and radiotracer techniques were presented. This indirect imaging strategy is based on proportional coexpression of therapeutic and marker gene activity, which occurs using specific gene coexpression cassettes. This strategy provides the opportunity for a wider application of imaging therapeutic gene expression in patients undergoing gene therapy trials. Three new imaging paradigms using radiotracer-imaging methods should be able to image transcriptional activation/repression, post-transcriptional modulation, and specific steps of specific signal transduction pathways. Preliminary data supporting these three new imaging paradigms are currently being obtained.

Although there are several clinical issues related to imaging gene expression, post-transcriptional modulation, and specific signal transduction pathways, imaging gene expression in patients can provide very important quantitative, spatial, and temporal information. Currently, many clinical gene therapy protocols cannot adequately address the following issues:

- Has gene transfection or transduction been successful?
- Is the distribution of gene expression optimal?
- Is the level of gene expression sufficient?
- How long does gene expression persist?
- Is there potential for organ-specific toxicity?
- In combined pro-drug-gene therapy protocols, when is gene expression maximum and when is the optimal time to begin and to end pro-drug treatment?

These fundamental issues are important issues to address as the field matures.

Using Carbon-11-Thymidine in Evaluating Renal Cancer

The current state of the art requires large studies to reliably discriminate study endpoints due to the imprecise measurement of current clinical parameters. PET imaging with highly sensitive agents can accomplish the goals of quantitating response to therapy with great precision using much smaller studies. The data also can be used to guide the evaluation of a new, mechanistically defined clinical

strategy. Imaging cellular proliferation in the setting of a clinical trial evaluating the use of interleukin-2 (IL-2) with carbon-11-thymidine for renal cancer is an example of a quantitative clinical trial endpoint. Carbon-11-thymidine is not limited by blood flow, has no endogenous synthesis, and has negligible local reuse, making it an attractive marker. After injection, normal metabolism produces known degradation products. Carbon-11-thymidine shows rapid uptake in all tissues; however, it is only retained in tumor. Also, tumor and inflammation can be delineated and this marker does cross the blood-brain barrier.

A clinical example for the use of carbon-11-thymidine is in the evaluation of experimental therapy of renal carcinoma. Renal cell carcinoma is a disease of middle-aged populations that often presents with intravascular spread. There is a high rate of metastatic disease, and systemic chemotherapy is ineffective. Since the tumor often undergoes coagulative and liquefactive necrosis, it is hard to characterize antitumor response radiologically. The administration of IL-2 is associated with a significant response rate of between 13% and 35% and a complete response rate of between 3% and 11%. It is unclear as to what characteristics predispose to a favorable antitumor response to IL-2.⁸ While the therapeutic index with IL-2 therapy is narrow, it would be useful to determine if particular patients would benefit from the addition of other lymphokines. The use of FDG might help define some of the distinct clinical cohorts best addressed with specific biological agents. The goal for the oncologic community is to be able to characterize a tumor's biological profile so that mechanistically appropriate therapy can be administered. These goals can make a large contribution and impact of evaluation and testing of experimental therapies for patients with the refractory cancers.

Research Priorities

Develop High-Resolution Imaging Technology

Imaging of cellular and subcellular targets requires the use of high-resolution techniques to reveal tumoral heterogeneities (i.e., spatial imaging) and rapid events (i.e., temporal imaging). Research avenues should include several imaging technologies with varying resolutions, including NMR, nuclear (scintigraphy, PET, SPECT), and optical imaging. Optical imaging technologies may include intravital microscopy, confocal or multiphoton intravital microscopy, optical coherence tomography, diffuse optical tomography, and others.

Create Novel Technologies for Imaging

Molecular probes represent a key element in visualizing specific targets *in vivo*. The major priority for molecular imaging in the near and mid-term should be to facilitate the development of new, molecular-specific probes to be applied *in vivo*. A variety of chemistries are available to generate probes that are specifically active. These can be based on combinatorial chemistry, nucleic acids, peptide libraries, and antibody technology, among others. Probes can either bind specifically to ligands or be enzymatically or transcriptionally activated. Despite extensive technology research, development of novel biocompatible molecular probes has been slow, primarily because of lack of funding. Developing imaging marker genes detectable by NMR, nuclear, or optical imaging should be a priority.

Transfer Novel Technologies to the Translational Research Community

Rapid translation of developed probes and technologies to a clinical setting is highly desirable. Although a number of probes and imaging techniques have been developed by single investigators, barriers to translational research have hampered the introduction into the clinical practice.

Develop Organelle-Specific Targeting Strategies

Subcellular targeting is of considerable interest in delivering molecular probes to their intended target. For example nuclear localization signals, membrane translocation signals, or cellular sorting domains are expected to become useful in drug development and imaging at the subcellular level.

Elucidate the Biological/Physical Basis of Imaging Signals

Investigating the cellular and molecular basis of signal generation will be important in understanding and developing novel imaging strategies. Once novel probes are developed, every effort should be made to define mechanistically the origin of signal and its subcellular fate and metabolism. For example, an endogenous contrast mechanism can be used as a source of image contrast for NMR and optical imaging, yet the molecular basis is not well-understood.

Facilitate Interdisciplinary and Multimodality Research Interactions

Frequently the best ideas come from interdisciplinary and multidisciplinary research. For example, chemists and biochemists need to enter this field. A mechanism to fund exploratory grants by (bio)chemistry/imaging consortia would be helpful. Additionally, a program

to supplement existing imaging grants with funds to recruit (bio)chemists and students also would be important. Funding mechanisms to foster such programs should be developed. Interdisciplinary programs ensure that ideas, concepts, and development plans are sound and better conceived than is often the case.

References

1. Arendt JT, Levin HS, Klein EA, Cothren RM, Feld MS. Detection of transitional cell carcinoma of the urinary bladder at cystoscopy using fluorescence spectroscopy. *Journal of Cancer Research*, in press.
2. Manoharan R, Shafer K, Perelman L, et al. Raman spectroscopy and fluorescence photon migration for breast cancer diagnosis and imaging. Symposium in Print of Photochemistry and Photobiology, "Innovations in Optical Techniques for Biodiagnostics" 1998; 67:15-22.
3. Perelman LT, Backman V, Wallace M, et al. Observation of periodic fine structure in reflectance from biological tissue: a new technique for measuring nuclear size distribution. *Phys. Rev. Letters* 1998; 80:627-630.
4. Wallace MB, Perelman LT, Backman V, et al. Endoscopic detection of dysplasia in patients with Barrett's Esophagus using light-scattering spectroscopy. *Gastroenterology*, in press.
5. Tjuvajev JG, Stockhammer G, Desai R, et al. Imaging the expression of transfected genes in vivo. *Cancer Res* 1995; 55:6126-6132.
6. Tjuvajev JG, Finn R, Watanabe K, et al. Noninvasive imaging of herpes virus thymidine kinase gene transfer and expression: a potential method for monitoring clinical gene therapy. *Cancer Res* 1996; 56:4087-4095.
7. Tjuvajev JG, Avril N, Oku T, et al. Imaging herpes virus thymidine kinase gene transfer and expression by positron emission tomography. *Cancer Res* 1998; 58:4333-4341.
8. Rosenberg SA. Keynote address: perspectives on the use of interleukin-2 in cancer treatment. *Cancer J Sci Am* 1997; 3(Suppl 1):S2-6.