Meeting Report

Tumor Receptor Imaging: Proceedings of the National Cancer Institute Workshop, Review of Current Work, and Prospective for Further Investigations


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Abstract

In February 1994, the National Cancer Institute held a workshop to evaluate the current and future role of emission tomographic imaging methods, positron emission tomography and single-photon emission computed tomography, in improving the accuracy of cancer diagnosis and the effectiveness of treatment and in elucidating basic aspects of human cancer biology. Reviews covered many of the receptor and transport systems for hormones and growth factors, as well as metabolic changes important in human cancer, and topical presentations reviewed the current status of receptor-based imaging in the most well-characterized systems: somatostatin receptor imaging of neuroendocrine tumors, estrogen receptor imaging of breast cancer, and epidermal growth factor receptor and tumor metabolic imaging. A critical analysis was made of the current research and of new directions for the future development and use of receptor-imaging methods in oncology. In each area, recommendations were made for further investigation, where emerging understanding of tumor cell biology and defined molecular targets might be combined with the methods of radiopharmaceutical design and evaluation, to develop new approaches to critical issues in the diagnosis, staging, and treatment of cancer through tumor receptor imaging.

Introduction

Cancer is now the second leading cause of death in the United States and, by the next century, is predicted to emerge as the leading cause of death (1). Although significant progress has been made in cancer diagnosis and treatment, the annual mortality statistics attest to both the magnitude of the problem and the challenge it poses to medical science.

Major advances in understanding the molecular biology of cancer are now making possible new diagnostic and treatment strategies linked to the fundamental molecular characteristics of cancer (2). Imaging methods based on radiopharmaceuticals targeted to receptor systems and metabolic characteristics that are specific to cancer offer intriguing opportunities for the development of a new generation of diagnostic methods. Some of these are already beginning to be realized in areas of neuroendocrine tumor imaging with somatostatin analogues and breast tumor imaging with steroids and metabolic probes, and promising developments are under way in the area of tumor growth factor receptors and transport systems. Although still at an early stage of development, these imaging methods may facilitate both improved cancer diagnosis and staging, and early assessment of the effectiveness of treatment, by providing a more accurate in vivo characterization of tumor receptor status and metabolic activity.

In February 1994, the Diagnostic Imaging Research Branch of the Division of Cancer Treatment of the National Cancer Institute (Rockville, MD) convened a workshop on 'Tumor Receptor Imaging' in Bethesda, MD. Organized as a series of formal presentations that covered the major areas of ongoing research, followed by topical panels and open discussion in which each area was subject to critical analysis, this workshop covered many of the new advances in cancer biology involving receptors for hormones and growth factors and certain biochemical changes in the malignant state that may serve as targets for radiopharmaceutical design for tumor imaging.

This report presents a summary of the workshop presentations and discussions covering ongoing work in three areas: somatostatin receptor imaging of neuroendocrine tumors, steroid receptor imaging of hormone-responsive cancers, and imaging based on tumor growth factor receptors and transport systems. It also provides an analysis of current research and new possibilities for using tumor receptor imaging to address important issues in cancer diagnosis and therapy, and it con-
cludes each area with a series of recommendations for further investigation.

Overview: Nuclear Medicine and Cancer—New Opportunities from Tumor-Receptor Imaging

In a comprehensive review entitled, ‘‘Tumor Imaging: Current Status, Advances and Limitation of Nuclear Medicine’, Steven M. Larson (Memorial Sloan-Kettering Cancer Center, New York, NY) noted that general nuclear medicine procedures contribute significantly to the practice of oncology. For example, common procedures such as bone scans and gallium scans are used to detect and stage a variety of musculoskeletal and soft tissue tumors, while nuclear medicine cardiac wall motion studies are helpful in monitoring potential cardiotoxic effects of some forms of chemotherapy (3).

In the last decade, nuclear medicine has benefited greatly from advances in technology of imaging; in particular, improved instrumentation such as modern PET and SPECT, and computerized image processing, especially methods for ‘‘fusion’’ of emission tomographic images with CT or MRI images. The preparation of new radiopharmaceuticals and the development of quantitative modeling methods enable the study of hitherto inaccessible chemical processes occurring at nanomolar concentrations in living tissues in vivo. These methodological tools are now available for fundamental studies on tumors and practical applications in clinical oncology. The ‘‘new cancer cell biology,” i.e., the explosion of basic knowledge about cancer causation and prevention, can be used as a road map for radiopharmaceutical development (2). These radiopharmaceuticals may provide in return, through images based on interactions with specific molecular targets, detailed information on receptor levels and metabolism in vivo. This information about individual patients can be used to address critical issues in cancer diagnosis and effectiveness of therapy.

Metabolic imaging of tumors by PET is already a clinical reality, and some aspects of this method have been reviewed recently (4). This method depends on accelerated metabolism in tumor versus normal tissue: increased glucose utilization, DNA synthesis, and protein synthesis are hallmarks of the transformed cell, with the rate of increase roughly corresponding to the rate of growth of these tumors, and positron-labeled sugars, amino acids, and nucleotides have been successfully used for tumor imaging based on their increased uptake by tumors. Metabolic imaging with the glucose analogue FDG (Fig. 1) appears to have considerable potential in lung, breast, and colon carcinoma, lymphoma, thyroid carcinoma, and primary brain tumors. Anticipated benefits include improvements in distinguishing benign from malignant tumors, staging of cancers, detecting recurrence, and predicting and monitoring the response of tumors to antineoplastic treatment.

The proliferation of cancer cells is regulated by a number of hormones and growth factors that bind to membrane and intracellular receptors. These hormone growth factor receptor systems activate signal transduction pathways that are important in controlling transcription and cell growth, and are potential targets for imaging agents that could be used to identify specific tumors and that might provide an assessment of tumor receptor levels. Examples of several hormone growth factor receptors and transporter proteins of potential use in imaging specific tumors are shown in Table 1, along with ligands or antibodies that bind to these receptors. In addition to the specific tumor receptor and transporter systems shown in Table 1, there are many tumor-associated antigens which are being investigated for tumor-specific imaging with antibodies (16–19).

Agents used to image these specific tumor receptor systems may be ligands for these receptors (e.g., somatostatin derivatives for the somatostatin receptor and estrogens for the ER), substrates (e.g., sestamibi for the MDR transporter P-glycoprotein), or antibodies for several of the growth factor receptors.

mAbs (20) have played an important role in detecting tumor-associated antigens that eventually are shown to have important biological functions, and they can serve as archetypal ligands for these antigen receptors. They are often highly specific and have been used to map the receptor distribution in

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3 The abbreviations used are: PET, positron emission tomography; AR, androgen receptor; CT, computed tomography; EGF, epidermal growth factor; ER, estrogen receptor; 99mTc, technetium-99m; FDG, 2-[18F]fluoro-2-deoxy-D-glucose; FES, 16α-[18F]fluoroestradiol-17β; GR, glucocorticoid receptor; MDR, multidrug resistance (expression of mdr gene); PR, progesterone receptor; SPECT, single-photon emission computed tomography; TGF-α, transforming growth factor α.
Table 1  Examples of transmembrane growth factor receptors, intracellular steroid receptors, and transport proteins of potential use in specific tumor imaging

<table>
<thead>
<tr>
<th>System</th>
<th>Ligand</th>
<th>Tumor</th>
<th>Function</th>
<th>Imaging agent/antibody</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptors</td>
<td>EGF receptor</td>
<td>Non-small cell lung</td>
<td>Growth regulation</td>
<td>Ab225, 528</td>
<td>(5)</td>
</tr>
<tr>
<td></td>
<td>ER</td>
<td>Breast cancer</td>
<td>Growth regulation</td>
<td>FES</td>
<td>(6)</td>
</tr>
<tr>
<td></td>
<td>HER-2/neu</td>
<td>Breast, ovary</td>
<td>Growth regulation</td>
<td>Ab4D5</td>
<td>(7)</td>
</tr>
<tr>
<td>Gastrin-releasing</td>
<td>Bombesin GRP</td>
<td>Small cell lung carcinoma</td>
<td>Growth regulation</td>
<td>Anti-GRP</td>
<td>(8)</td>
</tr>
<tr>
<td>peptide receptor</td>
<td>Interleukin 2</td>
<td>Lymphoma T cells</td>
<td>Activation</td>
<td>Anti-TAC</td>
<td>(9)</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>Somatostatin</td>
<td>Neuroendocrine</td>
<td>Growth regulation</td>
<td>111In-labeled</td>
<td>(10)</td>
</tr>
<tr>
<td>receptor</td>
<td></td>
<td></td>
<td></td>
<td>pentetreotide</td>
<td></td>
</tr>
<tr>
<td>Transporter</td>
<td>p-Glycoprotein</td>
<td>Biologically derived</td>
<td>Detoxify MDR</td>
<td>Sestamibi; AbMRK-16</td>
<td>(11, 12)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>drug/toxins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glucose transporter</td>
<td>Monosaccharides</td>
<td>Glucose uptake</td>
<td>FDG; anti-GT1</td>
<td>(13, 14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transferrin</td>
<td>Transferrin</td>
<td>Iron and trace metal</td>
<td></td>
<td>AbD65.30, A27.15</td>
<td>(15)</td>
</tr>
<tr>
<td>receptor</td>
<td></td>
<td>transport</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

normal tissues and in tumors. mAbs themselves can be used to develop novel targeting radioligands, but optimization of their behavior as radiopharmaceuticals may require protein engineering.

The technology of nuclear medicine has advanced to the point where the stage is set for important advances in our understanding of the biology of human tumors based on noninvasive imaging approaches. The recent advances in the molecular and cell biology of cancer now point the way toward development of radiopharmaceuticals targeted to specific receptors or metabolic characteristics of tumors, and imaging based on these agents may provide new insights into the cause and prevention of cancer.

Workshop Summary, Analysis of Current Research and New Directions, and Recommendations for Further Investigation

Somatostatin Receptor Imaging of Neuroendocrine Tumors

Workshop Summary (Presentations by Thomas M. O’Dorisio and Larry K. Kvols). "Somatostatin Receptor Imaging" was presented by Thomas M. O'Dorisio (Ohio State University Hospitals, Columbus, OH), who reviewed the use of this receptor system in tumor imaging, utilizing long-acting somatostatin analogues (21, 22). Various neuroendocrine tumors, such as carcinoids, vasoactive intestinal peptide-secreting tumors, insulinomas, glucagonomas, and gastrinomas, contain somatostatin receptors, and somatostatin and its analogues have been used in the treatment of clinical endocrine syndromes that result from these tumors. The usefulness of somatostatin itself (a tetradecapeptide) as a therapeutic agent or in vivo probe, however, has been limited by its very short plasma half-life.

Sandoz Inc. developed a long-acting somatostatin analogue, the modified octapeptide octreotide (SMS 201–995, Sandostatin; Fig. 1), which is far more resistant to enzymatic degradation than somatostatin; its plasma half-life is 60 min compared with 2–3 min for somatostatin. Octreotide has been shown to be efficacious in the treatment of gut endocrine tumors, and Lambert et al. (10, 23) have utilized a modified form of octreotide in which the phenylalanine residue at position 3 is replaced with a tyrosine residue radiolabeled with $^{125}$I or $^{111}$In; Fig. 1) to detect somatostatin receptors in human endocrine tumors. $^{125}$I-Tyr$^3$octreotide scintigraphy localized primary pancreatic tumors, gastrinomas, and many other endocrine tumors, and identified previously unknown metastases (Fig. 2, A and B).

The group at Ohio State (22) has used $^{125}$I-Tyr$^3$octreotide in conjunction with a hand-held probe to measure the activity of areas of suspected tumor mass for the intraoperative localization of neuroendocrine tumors. In seven of seven patients with known or suspected neuroendocrine tumors (localized by CT scanning), the tumor mass accumulated labeled octreotide at a ratio of 2:1 compared with adjacent normal tissue, whereas in four of the five patients with clinically suspected tumors, no tumor was found. The Ohio State group suggests that intraoperative localization utilizing iodinated octreotide can be useful for both tumor detection and for determining which patients may benefit from octreotide therapy.

In a related presentation, Larry K. Kvols (Mallinckrodt Medical, Inc., St. Louis, MO; formerly Mayo Clinic and Foundation, Rochester, MN) discussed the experience at Mayo Clinic utilizing somatostatin. In 77% of the patients suffering from malignant carcinoid syndrome, administration of octreotide resulted in clinical improvement. A subsequent study showed a
Fig. 2  Radionuclide images of tumors based on receptor binding or metabolism of a radiopharmaceutical tracer. A, somatostatin receptor imaging 24 h after injection of $^{111}\text{In}$-labeled pentetreotide in a 55-year-old man with multiple paragangliomas. The anterior image of the head and neck demonstrates a right glomus tympanicum tumor (T), a left glomus jugulare tumor (J), bilateral carotid body tumors (CB), and a left mediastinal tumor (M) (Courtesy of T. M. O’Dorisio, unpublished data). B, anterior 24-h $^{111}\text{In}$-labeled pentetreotide image of the abdomen in a 74-year-old woman with a 1.5-cm insulinoma (arrow) in the head of the pancreas. Normal uptake of the tracer in the liver, spleen, and kidneys is evident, as is normal excretion of the tracer into the bowel (Courtesy of T. M. O’Dorisio, unpublished data). C and D, PET images obtained with FES (C) and FDG (D) in a 55-year-old woman with a 3- x 3-cm ER-positive carcinoma of the right breast. The images shown are anterior maximum-pixel-intensity volume-rendered reprojection views compiled from a series of transaxial PET scans. Both images show tracer uptake in the primary tumor (long arrow) and in ipsilateral palpable axillary nodes (short arrow). The FES image shows normal intense tracer uptake in the liver (L); the FDG image shows normal activity in the myocardium (M) and the liver (L), and excreted tracer in the left renal collecting system (K) (Courtesy of F. Dehdashti and B. Siegel, unpublished data).

correlation between the presence of tumor somatostatin receptor, assayed by autoradiography with $^{[123]}\text{I-Tyr}^3$octreotide, and the response to octreotide in patients with advanced, metastatic, neuroendocrine tumors. Receptors were detected in carcinoid tumors in 16 of 20 patients, and all but 1 of these 16 patients improved clinically after treatment with octreotide. In contrast, only one of the four patients with receptor-negative carcinoid tumor showed clinical improvement. All eight patients with metastatic islet cell carcinomas were positive for somatostatin receptors and showed improvement with octreotide treatment, while three patients with medullary carcinoma of the thyroid were receptor negative and showed no response. Thus, the presence of somatostatin receptors in malignant neuroendocrine tumor tissue appears to correlate with the response to octreotide therapy.

The Mayo group evaluated $^{[123]}\text{I-Tyr}^3$octreotide in vivo to detect known carcinoid and islet cell tumors and to correlate tumor uptake with the presence or absence of somatostatin receptors. Tumors were best seen on scans obtained 1–4 h after injection. Of the 28 patients, 22 had positive scans with uptake in tumors, 3 showed photon-deficient uptake in regions of known tumors, and 3 had negative scans; 17 patients in whom results of the tumor biopsy were positive for somatostatin receptors had positive scans, and 1 patient in whom results of the biopsy were negative for somatostatin receptors had a negative scan. Previously unsuspected lesions were detected on the $^{123}\text{I}$-labeled octreotide scans in 4 of the 28 patients. Thus, $^{123}\text{I}$-labeled octreotide appears to be a useful tracer for the localization of neuroendocrine tumors and, most likely, other soft tissue tumors as well.

Analysis of Current Research and New Directions (Moderators: Michael J. Welch and Larry K. Kvols; Contributions by Mario Maggi, Edward Deutsch, and J. Lister-James). The presentations and discussions in this panel focused on the identification and characterization of the somatostatin receptors in tumors, as well as approaches that have been taken to image these receptors with novel radiopharmaceuticals. As was reviewed above, somatostatin-based tumor-imaging agents are the most developed members of this class. Clinical studies, which have been performed in many institutions using labeled oct-
reotide (Sandostatin), have demonstrated the utility of this receptor probe in identifying tumors and predicting their responsiveness to therapy with somatostatin analogues (23, 24).

A Sandostatin analogue labeled with $^{111}$In via a diethyl-enetetramine pentaacetic acid bifunctional chelate, $^{111}$In-labeled pentetreotide, initially developed in collaboration between Sandoz and investigators at the University Hospital Rotterdam (25), is being commercialized by Mallinckrodt Medical, Inc. under the trade name OctreoScan. As discussed by Edward Deutsch (Mallinckrodt Medical, Inc.), this agent is being evaluated in several European centers where clinical trials are being carried out, aimed at establishing its efficacy in diagnosing a variety of diseases states, including neuroendocrine tumors, lymphomas (both Hodgkin’s and non-Hodgkin’s), breast cancer, small cell lung cancer, and various other cancers (Fig. 2, A and B). The use of $^{111}$In-labeled pentetreotide in detecting neuroendocrine tumors was approved by the U.S. Food and Drug Administration in June 1994, and U.S. trials to establish the utility of this agent in detecting lymphomas are under way. Dr. Deutsch also pointed out that other radiolabeled forms of octreotide are being developed in academia and industry; these include $^{18}$F, $^{68}$Ga, and $^{64}$Cu compounds for PET imaging and $^{90}$Y, $^{186}$Re, $^{64}$Cu, and $^{67}$Cu forms for radiotherapy.

The development of new $^{99m}$Tc-labeled somatostatin analogues was discussed by J. Lister-James (Diatech, Inc., Londonderry, NH). $^{99m}$Tc has advantages over other radionuclides due to its ready availability and the fact that, in many cases, radionuclides of rhenium can be substituted for technetium to develop agents for radiotherapy. A lead compound that has a high affinity for the receptor ($K_v = 0.15$ nM as the rhenium complex) and shows high uptake in rat tumors will soon be ready to undergo clinical trials.

The presentations in this panel showed the current interest in the studies of somatostatin and nonmetabolizable peptide analogues, both for diagnosis and for therapy, utilizing both the native peptide and the peptide labeled with various radionuclides. These many evident successes notwithstanding, there are fundamental questions regarding the basic biology of somatostatin receptors that remain unknown. For example, as discussed by M. Maggi (University of Florence, Florence, Italy), at least five types of somatostatin receptors have been identified, all of which have been cloned, and somatostatin and octreotide display different binding affinities to these five sites (24, 26, 27). Somatostatin$_{1}$ receptors, present in three of eight neuroblastoma cell lines, bind with low capacity and very high affinity, whereas somatostatin$_{2}$ receptors have a high capacity and are widely distributed in all of the cell lines investigated. The active interplay between clinical studies utilizing labeled octreotide in various tumors and basic studies on the nature and distribution of somatostatin receptor subtypes may further improve both the diagnostic and therapeutic uses of these receptor probes, and advance our fundamental understanding of the receptors for this growth-regulating peptide.

**Recommendations for Further Investigation.** The recommendations are:

- Investigate whether it is possible to quantify the number of receptors from radiolabeled somatostatin analogue uptake and thereby predict whether patients would benefit from therapy with a somatostatin receptor-directed $\beta$-emitting, Auger-emitting, or $\alpha$-emitting radionuclide.
- Develop agents with potential for therapeutic applications that have less accumulation in normal tissue, particularly in the organs involved in their metabolism and excretion.
- Examine the effect of various treatment regimens, such as chemotherapy, immunotherapy, and hepatic dearterialization, on the expression of somatostatin receptors through sequential imaging with a somatostatin analogue.
- Correlate receptor subtype with tumor type to see whether the content of the five known subtypes of receptor vary with the type of tumor known to contain somatostatin receptors.
- Develop agents specific for the various receptor subtypes.

**Steroid Receptors and Imaging Hormone-responsive Cancers**

**Workshop Summary** (Presentations by Farrokh Dehdashti and Richard Wahl). The use of a clinically refined receptor system for PET imaging was presented by Farrokh Dehdashti (Mallinckrodt Institute of Radiology), who summarized the Saint Louis experience with “Estrogen Receptor Imaging in Breast Cancer.” ERs are found at quite high levels in biopsy samples of certain breast cancers, and the tumor response to hormone therapy often correlates quite well with ER levels. Using FES (Fig. 1) as a probe for ERs (28), this group has shown the following: (a) it is possible to identify the ER-positive breast tumors by PET with FES (29); (b) FES localization in ER-positive distant metastases from breast carcinoma (e.g., bone and soft tissue) is receptor mediated, as the uptake is significantly blocked by antiestrogen treatment (6); (c) FES uptake is quantitatively related to the ER concentration (Fig. 3), but not to cell metabolism measured by FDG uptake; (d) FES uptake is selective and occurs only in ER-positive malignant tumors, whereas FDG uptake is increased in both ER-positive and ER-negative malignant tumors (Fig. 2, C and D), and abnormal uptake of either probe does not occur in benign breast tumors; and (e) FES uptake indicates hormone status and, therefore, potentially predicts hormone responsiveness, while FDG metabolism may predict response to chemotherapy (30). These studies appear to provide a “proof of concept” that quantitative tumor receptor imaging has the potential to contribute useful clinical information regarding prognosis and selective therapeutic response in patients with breast cancer.

The use of metabolic imaging in cancer diagnosis was reported by Richard Wahl (University of Michigan Medical Center, Ann Arbor, MI) in his presentation “Tumor Imaging With PET.” PET imaging using FDG (Fig. 1) has demonstrated the potential for noninvasive tumor staging, including the detection of lymph node metastases from breast and lung cancers as well as from melanoma and head and neck carcinoma, based on the increased glucose utilization in these malignant tumors (31). For tumor staging and assessment of treatment response, PET appears to have advantages over purely anatomical imaging methods in that it can separate, in many cases, scar and other nontumorous masses from viable tumor. Similarly, detection of...
small tumors also appears to be possible with PET in some settings in which CT and other morphological methods are insensitive. Dr. Wahl believes that primary uses for the FDG probe in cancer will be to differentiate a benign from a malignant mass, to determine the tumor growth rate, to differentiate necrosis from recurrent or residual tumor, and to monitor response to therapy in individual patients (13, 32).

Analysis of Current Research and New Directions

[Moderators: John A. Katzenellenbogen and Barry A. Siegel: Contributions by C. Kent Osborne (University of Texas Health Science Center, San Antonio, TX), Eugene DeSombre (University of Chicago, Chicago, IL), Donald Tindall (Mayo Research Foundation, Rochester, MN), Mark Schoenberg (Johns Hopkins University, Baltimore, MD), E. Brad Thompson (University of Texas, Galveston, TX), John Katzenellenbogen (University of Illinois, Urbana, IL), and Michael Welch (Mallinckrodt Institute of Radiology)]. In the area of steroid hormone-responsive cancers, the dual potential of radiodiagnostic imaging (a) to assess or characterize function by quantification of tumor receptor levels or metabolic activity, and (b) to locate tumors based on these functions or activities is beginning to be realized. Two general applications can be envisioned: (a) imaging of tumor function or location to assist in patient staging or prognosis in order to make a more appropriate selection of alternative treatments, and (b) imaging of tumor function to make an early assessment of the effectiveness of therapy.

Breast Cancer. ER and PR are well established as markers that are useful as both predictive (response to hormonal therapy) and prognostic (tumor aggressiveness) factors (33, 34). Patients with receptor-negative tumors have a more rapidly progressive clinical course and more frequent visceral metastases; they show a low, but still significant response rate to tamoxifen treatment. Patients with receptor-positive tumors have a more indolent course and more frequently benefit from endocrine therapy such as tamoxifen (especially with tumors having high receptor content; Refs. 35 and 36). Since drugs like tamoxifen have little toxicity, many patients are treated after primary surgery even if there is only a small chance of benefit; yet, the drug is costly and does have side effects, as is becoming increasingly evident in major recent tamoxifen trials (37), so that prospective identification of patients with no real chance of benefit from tamoxifen treatment would be useful clinically.

In their overview presentations, Drs. Wahl and Dehdashti demonstrated in preliminary clinical studies that primary and metastatic tumors (that included axillary and mediastinal lymph nodes and distant lesions) could be clearly imaged based on ER uptake of FES and/or elevated FDG uptake (13, 30, 31). The latter method enabled a reliable distinction between benign and malignant disease (31, 32), and the former, a reliable assessment of ER positivity (6, 29, 30). In addition, a good correlation was found between tumor FES uptake and ER levels by quantitative assay (Fig. 3; Ref. 29). FES uptake decreased after tamoxifen therapy (6), and FDG uptake was reduced after chemotherapy (13). More extensive studies are needed to investigate the relationship between tumor FES and FDG uptake and the clinical outcome of hormonal or chemotherapy.

Other potential applications include the following: (a) The concurrent assessment of tumor metabolic activity (with FDG) and ER content (with FES) in multiple metastases in a single breast cancer patient might provide an internally controlled assessment of tumor heterogeneity; this might be particularly important in recurrent disease or in situations where tumor sites are inaccessible for ER assay. (b) To the extent that FES or FDG imaging can provide a reliable assessment of the involvement of lymph nodes, these noninvasive methods might obviate diagnostic lymph node dissection in some patients; the specificity of imaging in providing a negative assessment of lymph node involvement would need to be established carefully, since microscopic metastases may be important prognostically. (c) These imaging methods might enable a rapid assessment of the potential effectiveness of hormonal therapy prior to surgery or other treatment. In a "preoperative tamoxifen challenge test," the effectiveness of hormonal therapy might be evident after even a brief (1–3-week) course of tamoxifen treatment through an alteration in tumor metabolic activity. The transient agonist activity that may precede the onset of effective tamoxifen antagonism, that is manifest in some patients as a tamoxifen flare (38), could result in a transient elevation of tumor FDG uptake followed by an eventual suppression as tamoxifen antagonism begins to dominate. The results of such a test might provide a more reliable means for selecting hormonal therapy in the primary or adjuvant setting, and could reduce the cost and risk of ineffective or superfluous treatment.

In the ER and other steroid receptor systems, efforts are being made to develop SPECT imaging agents, and there are published clinical SPECT studies with two iodoestrogens in breast cancer (39–41). These agents, as well as ones labeled with 99mTc (42, 43), could take advantage of the greater availability associated with SPECT compared to PET imaging. The potential for ER-directed therapy (44) based on Auger electrons emitted from 123I is being explored as an approach to localized radiotherapy of ER-positive cancers.

PR is also a potentially useful target in breast cancer. While PR-based imaging agents have been prepared (45), none has yet
proved useful in humans (46). If a good agent was available, it could be used for tumor receptor imaging in patients on tamoxifen therapy (that would result in full occupancy of ER), and the rise in PR levels reported early after the start of tamoxifen treatment (47) might offer another means for establishing the effectiveness of hormone therapy.

**Prostate Cancer.** Although AR is detectable in most prostate cancers, it has not proven to be a reliable predictor of response to hormone therapy (48, 49). Nevertheless, imaging based on the uptake of a suitable radionuclides ligand or elevated metabolic activity by the primary tumor or metastatic sites might provide an effective means for staging the cancer. In particular, a more definitive determination of disease dissemination or pelvic lymph node involvement would assist in the decision to undertake radical prostatectomy. The need for improved staging is becoming more acute, because the sensitive detection methods of transrectal ultrasonography, magnetic resonance imaging, and prostate-specific antigen are resulting in increasing numbers of patients identified at early stages (50). In advanced prostate cancer, an AR imaging probe could also be of value to assess the likelihood of response to a second endocrine manipulation after failure of initial androgen deprivation (orchiectomy). Although they have not yet been studied in humans, some 18F-labeled androgens have been used to image the prostate in baboons with PET (51); a SPECT agent is under investigation (52).

**Ovarian Cancer.** This cancer commonly spreads i.p., and present imaging techniques are inaccurate in identifying the presence of the metastases. Although diagnostic imaging of peritoneal metastases using radionuclide labeled steroids might be limited by the presence of gut activity from hepatobiliary excretion of the metabolites, a hand-held detector might aid the surgeon in locating small receptor-positive lesions. Furthermore, metabolic imaging with FDG appears to be promising in detecting ovarian metastatic disease. Receptor-directed radiotherapy with Auger electron-emitting radionuclides might provide a more effective therapy, especially for micrometastases, than is currently available (44).

**Leukemias and Lymphomas.** While the application of tumor imaging based on GR must await the development of effective radiopharmaceuticals (53), the correlation of GR levels with response to therapy could make diagnostic imaging a useful prognostic tool (54, 55). GR-based imaging or metabolic imaging using FDG could assist in the location of lymphoid tumors, and GR imaging in certain nonlymphoid tumors where glucocorticoids are of benefit could also be of value, as would receptor imaging methods that can surmount the difficulties in GR evaluation in sequestered sites (e.g., central nervous system, bone marrow, and pancreas).

**Recommendations for Further Investigation.** The recommendations are:

**Breast Cancer.** Establish clinical correlations between the uptake of FES and FDG and the outcome of hormone therapy and chemotherapy.

Determine whether tumor heterogeneity in patients with multiple metastases can be determined by combined FES and FDG imaging and related to the success of therapy.

Develop an effective PR-based imaging agent.

Identify optimal ligands and imaging times to assess the potential of SPECT imaging for breast cancer.

Establish whether the assessment of lymph node involvement by radionuclides estrogens or metabolic markers is sufficiently reliable to reduce the need for axillary node dissection.

Investigate whether the potential success of hormone treatment can be assessed preoperatively with a tamoxifen challenge test that would involve a rapid, functional assessment of tumor response based on changes in the uptake of FDG or a PR ligand.

Investigate the possibility of developing imaging agents for other markers of steroid-induced responses (e.g., growth factors or growth factor receptors, other steroid-induced specific gene products, as well as general metabolic markers) that might be used in functional assessment of endocrine therapy. Such a predictive test would also be useful in deciding between chemotherapy and hormonal therapy in patients with advanced metastatic breast cancer.

**Prostate Cancer.** Determine whether an AR-based imaging agent or FDG can provide (a) a definitive assessment of disease dissemination or pelvic lymph node involvement to assist in the selection of therapy, and (b) an estimation of the likelihood of response to a second endocrine manipulation after failure of initial androgen deprivation.

**Ovarian Cancer.** Determine whether novel methods for receptor-directed radiotherapy can be developed based on the selective distribution of suitable receptor ligands.

**Leukemias and Lymphomas.** Develop GR-based agents suitable for imaging of lymphoid tumors.

Assess the potential for GR- or FDG-based imaging for the detection of lymphoid tumors in nodes and the brain, and develop correlates between imaging and treatment.

**Ligand Development and Methodology.** Develop effective ligands for PR- and GR-based imaging.

Develop and evaluate receptor-based imaging agents labeled with more readily available radionuclides useful for SPECT, such as 67Ga, 111In, 123I, and 99mTc.

Improve an understanding of the relationship between steroid structure and in vitro measures of binding affinity and in vivo uptake characteristics.

Develop reliable methods to assess the comparative metabolism characteristics of imaging agents in animals and humans to improve the value of animal studies in predicting radiopharmaceutical behavior in humans.

Develop methods suitable for receptor quantification in tumors from in vivo imaging data that address the particular problems in tumor systems.

**Tumor Growth Factor Receptors and Cancer Biology: New Targets for Imaging**

**Workshop Summary (Presentations by John Mendelsohn and David Piwnica-Worms).** In his keynote address, "Tumor Receptors: Biology and Cancer Therapy," John Mendelsohn (Memorial Sloan-Kettering Cancer Center) described the advances his laboratory has made in developing antibodies as antitumor therapeutic drugs. TGF-α and EGF are required growth factors for many types of cells, stimulating cell cycle passage from G1 into the S-phase. TGF-α is produced by the tumor cells and stimulates cell growth through binding to EGF receptors, either on the same tumor cell (autocrine action) or near neighbor tumor cells (paracrine action). EGF receptors
have been identified in nearly all renal, squamous lung, and head and neck cancers, and in many breast cancers as well as other cancers. Expression of high levels of EGF receptor and its ligands, EGF and TGF-α, in some tumors is associated with a poor prognosis (56). Although normal cells have a concentration of approximately 10,000 EGF receptors/cell, there are as many 2 × 10⁶ EGF receptors/malignant cell; this concentration differential is a key factor in the potential of this receptor as a target for diagnostic imaging and radiotherapy.

Dr. Mendelsohn’s group has generated anti-EGF receptor mAbs, mAb 225 and 528, that bind to the EGF receptor with an affinity comparable to the natural ligand (Kd = 2 nm), compete with EGF binding, down-regulate the receptor, and block the activation of receptor tyrosine kinase by EGF or TGF-α (57). They also block EGF or TGF-α stimulation of the growth of tumor cells, and in mouse xenograft models they have caused regression of established human tumors when given in combination with chemotherapy (58). The murine anti-EGF receptor mAbs have been labeled with ¹¹¹In and ¹³¹I, and, in collaboration with Dr. Steve Larson and the Nuclear Medicine group at Memorial Sloan-Kettering Cancer Center, the Mendelsohn group has studied the biodistribution of the labeled mAbs by imaging patients with several types of cancers (5). The group has now made human chimeric antireceptor mAbs, and they are poised to initiate studies in humans.

David Piwnica-Worms (Harvard Medical School, Boston, MA) discussed MDR, a tumor phenotype that correlates with the expression of a small family of human multidrug resistant (MDR1) and multidrug resistant-associated genes. The protein products encoded by these MDR genes, including the archetypic MDR1 P-glycoprotein, are plasma membrane transporters that function as energy-dependent efflux pumps of many of the most potent chemotherapeutic drugs used in cancer treatment, including anthracyclines, Vinca alkaloids, actinomycin D, and taxol, with overlapping, but nonidentical substrate specificities. P-glycoprotein is physiologically expressed in many organs involved in transport, excretion, and detoxification. This protein or its mRNA has also been detected at significant levels in specimens derived from all forms of human cancers, including leukemias, lymphomas, sarcomas, and carcinomas, and the transfection of cloned P-glycoprotein is sufficient to confer MDR in experimental systems.

Overexpression of this gene is one of several mechanisms that may be responsible for resistance to a broad spectrum of diverse cytotoxic agents (59, 60) in human cancer, and strategies designed to block expression or to circumvent this form of drug resistance are being actively sought by many laboratories in cancer research (61). Particularly desirable are agents that inhibit P-glycoprotein transport activity at concentrations that alone produce little or no cytotoxic effect. Termed MDR-reversal or -modulation agents, these drugs might be useful in overcoming MDR when administered in combination with other anticaner agents during chemotherapy.

Analysis of Current Research and New Directions

Masters: Randall A. Hawkins and Kenneth A. Krohn; Contributions by John Mendelsohn and Steven Larson (Memorial Sloan-Kettering Cancer Center); Napoline Ferrara (Genentech, Inc., South San Francisco, CA); Lee J. Helman and Carol J. Thiele (National Cancer Institute, Bethesda, MD); David Piwnica-Worms (Harvard Medical School); Ira Pastan and Jeffrey Schliom (National Cancer Institute, NIH). Cell surface proteins represent important targets for cancer therapy and diagnosis (cf. Table 1), and innovative applications for functional and anatomical imaging with radiolabeled antibodies, peptides, organic biomimetics, organometallics, and metal-chelate ligands represent a significant new focus for cancer receptor imaging (62).

Growth Factor Receptors and Signal Transduction in Cancer. Based on his extensive studies on the EGF receptor-TGF-α system, John Mendelsohn (Memorial Sloan-Kettering Cancer Center) summarized three important short-term goals for the applications of growth factor receptor imaging in clinical oncology: (a) In Phase I/II clinical trials explore the use of mAbs to treat tumors, including imaging with labeled mAbs to determine critical pharmacokinetic issues such as antibody distribution, uptake in both small and large tumor masses, and retention by tumors as well as normal tissues. If labeled receptor ligands themselves become available, determine the ability of the mAbs to block ligand-receptor interaction in vivo and correlate this with tumor response and normal tissue toxicity. (b) If anti-EGF receptor mAb therapy is shown to be effective for tumors expressing high levels of EGF receptor, use imaging studies with labeled mAbs or the appropriate radioligands to identify patients who are likely to respond to treatment. Also, determine heterogeneity of tumor expression from site to site and correlate this with tumor response. (c) In patients who are started on antitumor therapy, use metabolic imaging with FDG-PET to determine at an early time point (e.g., after 72 h) whether this expensive and prolonged therapy is going to be worthwhile for individual patients.

The biochemistry and tumor biology of other tyrosine kinase receptors, such as those for the insulin-like growth factors I and II (63–65), brain-derived neurotrophic factor (66), and vascular endothelial growth factor (67, 68), are under intense investigation at the basic level at this time. As insight is gained into their respective contributions to tumor growth and angiogenesis, opportunities for application of the radiotracer method may arise.

Thus far, the only agents used to image growth factor receptors have been antibodies, and most mAbs used thus far in clinical trials have been of murine origin (20), typically IgGs with M, 160,000. Such large proteins diffuse slowly into tumors and can cross-react with normal tissues via the Fe receptor. Thus, tumor uptake selectivity is not always high. Being murine in origin, these mAbs will in most cases induce a powerful immune response in humans. Improved behavior may be achieved through genetic engineering. By grafting the murine complementarity-determining regions (which occupy less than 5% of the total IgG) onto a human IgG backbone, humanized mAbs with reduced immunogenicity are produced (69); by joining the ends (Fv fragments) of the IgG with a genetically engineered linker of proper length, one can create a single-chain antigen-binding protein (70) that retains antigen-binding activity but is much smaller than the IgG. These single-chain antibodies have improved targeting characteristics in vivo, with more rapid tumor localization and tissue clearance (71).

MDR. An important controversy in the MDR field is whether identification of tissue expression of P-glycoprotein per
se with mAbs or mRNA probes is sufficient, or whether it is necessary to assay directly the functional transport activity of this integral membrane protein. It was recently reported that the lipophilic cationic radiopharmaceutical \(^{99m}\)Tc sestamibi is recognized and transported by the MDR P-glycoprotein (11) and \(^{[3H]}\)colchicine, a known P-glycoprotein substrate, has been shown to differentiate drug-resistant from drug-sensitive tumors in vivo (72). Intriguing new functional imaging opportunities enabling direct monitoring of the efflux transport function of P-glycoprotein may now be feasible with \(y\)-emitting agents of these classes. Thus, functional diagnostic imaging approaches may allow direct assessment of P-glycoprotein expression and activity in human tumors in vivo. In addition, the effectiveness of modulating or reversal agents in enhancing tumor delivery of chemotherapeutic agents could be monitored prior to a full chemotherapeutic trial, saving patients the morbidity of a futile cycle of chemotherapy. Transport substrates like \(^{99m}\)Tc sestamibi could serve as surrogate chemotherapeutic agents, allowing whole-body and tumor pharmacokinetics to be imaged after modulation therapy. Such a "modulator challenge test" might provide a means to select the most effective reversing agent for each tumor type, and allow patients to be directed to individualized and specific cancer therapies. Because normal expression of P-glycoprotein in liver, kidney, and intestine may potentially interfere with abdominal imaging, these strategies might best be applied to tumors of the head and neck, breast, lung, and extremities.

**Recommendations for Further Investigation.** The recommendations are:

**Growth Factor Receptors.** Develop radioligands suitable for imaging various cancer cell growth factor receptors and associated signaling systems.

Study the relationships between growth factor receptor expression (assessed by specific imaging agents), tumor growth (assessed by FDG metabolic imaging), and prognosis in vivo.

Monitor therapeutic outcome with antigrowth factor mAbs.

**MDR.** Develop radiolabeled binding and transport substrates for the MDR P-glycoprotein.

Establish the relationship between tumor expression of P-glycoprotein, transporter function, and chemotherapeutic outcome.

Investigate whether successful P-glycoprotein modulation therapy can be assessed with a modulator challenge test that would involve the functional assessment of transport inhibition using changes in tumor uptake kinetics of radiolabeled P-glycoprotein substrates as surrogate chemotherapeutic agents.

**Issues in the Development of Tumor Receptor Imaging Radiopharmaceuticals.** [Moderators: Kenneth A. Krohn and Carol J. Thiele; Contributions by Stanimir Vuk-Pavlovik (Mayo Clinic and Foundation, Rochester, MN); Michael J. Welch (Mallinckrodt Institute of Radiology); John A. Katzenellenbogen (University of Illinois); Kenneth A. Krohn (University of Washington School of Medicine, Seattle, WA)] While the focus of the Workshop and this report has been on the application of radiopharmaceuticals to tumor receptor imaging and not on the technical aspects of radiopharmaceutical development per se, a number of important points concerning this latter issue were raised during presentations and discussions.

Although tumor receptor or metabolic imaging (without quantification) can in many cases provide important information on the location of tumors and define certain functional characteristics, the ability to perform tumor receptor imaging quantitatively may determine how useful this information is in making critical diagnostic and therapeutic decisions. The quantification of receptor levels or metabolic activity from in vivo uptake studies is a complex issue that requires the development and validation of pharmacokinetic models; some of these have been worked out in certain systems (73–75), but the uncertainty of permeability barriers, blood flow, and the mix of viable versus necrotic tissue pose further complications in the development of quantitative receptor and metabolism models for tumors.

Animal models that are suitable for the qualitative and quantitative assessment of radiopharmaceutical distribution selectivity and kinetics are critical for the development of tumor receptor imaging agents for humans. However, one can encounter marked variation of radiopharmaceutical metabolism between animals, nonhuman primates, and humans (76–78), and even between the sexes (79), such that the behavior of the agents in humans may be either far worse or considerably better than in rodents (46, 51). Thus, the development and validation of appropriate animal model systems is important. Studies in nonhuman primates can provide an important middle ground, and in vitro systems for studying comparative species metabolism may have predictive value (76–78).

While the mass dose of most tumor imaging radiopharmaceuticals is so low as to have no pharmacological effect, the radiation dose needs to be evaluated carefully. Thus, administered doses need to be set at levels that will ensure that the radiation doses to normal tissue in humans are as low as possible. This is a particular concern for potential radiotherapy applications. If, for example, an analogue of octreotide were to be used, the route and rate of elimination would need to be carefully examined, since the nature and the position of the radiolabel can markedly affect clearance and metabolism: \(^{123}\)I-labeled octreotide is retained in the liver, while \(^{111}\)In-labeled pentetreotide is retained in the kidney (80), so an analogue with faster clearance characteristics would be desirable for therapeutic applications. Although differences in the metabolism and the routes and rates of radiopharmaceutical elimination between experimental animals and humans can complicate such assessments, reasonable estimates of radiation doses to humans can generally be made. As noted above, small alterations in radiopharmaceutical design or method of radiolabeling can have major effects on the routes and rates of elimination (80, 81); factors that affect not only the dosimetry, but also the regions of the body obscured to imaging during the process of elimination.

**Conclusions**

Recent advances in our understanding of tumor hormone and growth factor receptor systems and in our knowledge of biochemical changes that occur during tumor growth have opened up new opportunities for the use of imaging methods in...
clinical oncology and in basic studies of tumor biology. When targeted appropriately to these receptor systems and metabolic characteristics, these imaging methods can facilitate cancer diagnosis, staging, and treatment. Clinical investigations to date have been advanced in only a few systems, imaging based on somatostatin receptors for neuroendocrine tumors, on ERs for breast cancer, on the EGF receptor in various tumors, and on glucose utilization as a general tumor metabolic marker, but there are many opportunities for developing imaging agents directed at other tumor targets, receptor systems, and metabolic changes. Some of these possibilities have been presented and analyzed in some detail in the individual sections of this report. The areas that appear most fruitful for additional research are summarized in the "Recommendations for Further Investigation." The successful development of new tumor receptor imaging agents and protocols will continue to demand that careful attention be paid to aspects of the imaging agent design and labeling, administration, quantification and modeling, and considerations of safety.

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References


Tumor receptor imaging: proceedings of the National Cancer Institute workshop, review of current work, and prospective for further investigations.


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