New Agents and Techniques for Imaging Prostate Cancer

The successful management of prostate cancer requires early detection, appropriate risk assessment and optimum treatment. Imaging of prostate cancer has become increasingly important to predict which cancers are indolent and which will become aggressive as the treatment may be different for different grades of the disease. Different modalities have been tested to diagnose prostate cancer accurately, stage it and to predict its behavior before and after treatment. This review briefly describes the key clinical issues in prostate cancer diagnosis and therapy and summarizes the various new imaging modalities and agents in use and on the horizon.

Prostate cancer (PCa) is the most common malignancy among men in the United States, with mortality only superseded by lung cancer, accounting for 10% of all cancer related deaths in 2008 (1). PCa is currently characterized by its TNM stage, Gleason score, and prostate-specific antigen (PSA) serum level. PSA testing is the mainstay of PCa detection and has resulted in down-staging of the disease at diagnosis. However, there remains growing concern regarding the potential risk of overdiagnosis and, consequently, overtreatment of potentially indolent disease (2). PSA also lacks the ability to differentiate low grade from high grade cancers. To complicate matters, PCa is not rare among men with PSA levels that are generally thought to be in the normal range (3).

Conventional imaging such as computed tomography (CT), magnetic resonance imaging (MRI), ultrasound (US) and bone scan with [99mTc]MDP have been widely used in different PCa disease states. However, those modalities do not adequately address the overarching problem of distinguishing lethal from non-lethal disease, a prediction of disease prognosis or that of treatment response. A multidisciplinary approach is required to manage PCa. At a recent workshop the National Cancer Institute proposed intervention at four different levels (4). The role of imaging in (i) initial diagnosis, (ii) staging, (iii) disease recurrence after treatment and (iv) assessment of response to therapy was discussed. Also discussed were the multiple new molecular agents that are being tested and can be incorporated into the current paradigm of diagnosis, treatment and a prompt diagnosis of recurrent disease. We will address new approaches to imaging PCa in the context of those four levels of intervention.

Pre-diagnosis.

The current standard for diagnosing PCa is transrectal ultrasound (TRUS) guided sextant biopsy with or without additional biopsies. PCa is the only malignancy where the diagnosis is made from tissue obtained on a blind biopsy. Also, the histological grade is underestimated on such biopsies. The heterogeneous nature and multifocality of the tumor renders a blind biopsy inadequate in assessing the grade of the disease. Furthermore, multiple cores only sample a small percentage of the prostate. More comprehensive data are thus required prior to tissue sampling for a more targeted approach. MRI provides higher spatial and contrast resolution than TRUS and CT but lacks specificity. Magnetic resonance spectroscopy (MRS) provides a non-invasive method of detecting molecular biomarkers of low molecular weight within the cytosol and extracellular spaces of the prostate. MRS relies upon the loss of a normal citrate peak from the peripheral zone and an increase in the choline peak, an indirect marker of cell death. The ratio of (choline + creatine)/citrate (CC/C) in PCa exceeds the mean ratio found in healthy prostate tissue. Pulsed field gradients are generally used for localization using volumes of interest and include point resolved spectroscopy (PRESS) and stimulated echo acquisition mode (STEAM), well summarized in an excellent review by Mueller-Lisse et al (5). MRI combined with MRS are superior to sextant punch biopsy with a sensitivity and specificity of 56% and 82%, respectively, with improved diagnosis in the apex, the most difficult area to reach by punch biopsy (6). Additionally, MRS imaging measurement of prostate tumor CC/C and tumor volume correlate with Gleason score. A promising new indication of this method is detection and localization of PCa in previously negative TRUS-guided biopsy. Additionally, MRS at 7T minimizes the chemical shift artifact between citrate and choline to 5% and can be used to include the detection of spermine next to citrate, creatine, and choline (7).

Despite the limited ability of US to delineate cancer, it has the advantage of low cost, availability and speed over MRI-guided interventions. A recent study demonstrated the feasibility of TRUS-MRI fusion-guided prostate biopsy, with the entire procedure, including fusion, requiring approximately 10 min (8). Furthermore, with the use of an ultrasound 3-D navigation system, such as that developed by Bax et al. (9), needle guidance can be improved for sampling small lesions. Tests of the accuracy of biopsy needle guidance in agar prostate phantoms showed that the mean error was 2.1 mm and the 3D location of the biopsy core was recorded with a mean error of 1.8 mm, with less than 5% error in volume estimation. Addition of MRS data to that multimodality approach can improve detection of PCa by a method that can be used by urologists in their office.

Albers et al. (10) recently demonstrated very promising results by quantifying differences in hyperpolarized 13C-labeled pyruvate and its metabolites (lactate and alanine) between the various histological grades of PCa using the transgenic adenocarcinoma of mouse prostate (TRAMP) model (see below). It was previously difficult to observe lactate and alanine with in
They also showed that delayed imaging with [18F]fluorocholine did not result in the ability to differentiate malignancy from normal tissue or benign processes. The evaluation of [11C]acetate PET for primary PCa localization has not been reported.

Initial experience with the positron emission tomography (PET) agent, anti-1-amino-3-18F-fluorocyclobutane-1-carboxylic acid (anti-[18F]FACBC), a synthetic L-leucine analog, in 15 patients with either newly diagnosed or suspected recurrent PCa, has been encouraging (16). In newly diagnosed patients, anti-[18F]FACBC correctly identified tumor in 40 of 48 prostate region sextants, with seven out of nine patients demonstrating pelvic nodal uptake concordant with the clinical follow-up. Anti-[18F]FACBC was positive in all four patients with proved recurrence (negative ProstaScint. scans in three of these patients) and was instrumental in directing biopsy to prove neoplastic recurrence in one patient in whom lymph nodes were not obviously enlarged.

**Initial staging.**

The TNM system describes the extent of the primary tumor within the gland as well as within extraglandular tissue, spread to lymph nodes and the presence of more distant metastases. Patients with organ-confined disease tend to do better than those with extraglandular disease. The approach of combining dynamic contrast-enhanced MRI with T2-weighted MRI at 1.5T and high-spatial-resolution has a specificity of 95% for the detection of extracapsular disease with a sensitivity of 86% (17).

The presence of pelvic lymph node metastases is considered the strongest predictor of disease recurrence and progression, and the presence of metastases often means the difference between local and systemic therapy. The conventional criteria based on size and shape of the nodes cannot determine the presence of micrometastases within the lymph nodes. The use of lymphotropic ultrasmall superparamagnetic particles of iron oxide (USPIO) as a contrast agent for MRI enables reliable detection of metastases in normal-sized pelvic lymph nodes of patients with PCa. USPIO particles are consumed by macrophages in normal lymph nodes resulting in a signal decrease on T2/T2*-weighted MRI sequences and demonstrate extremely high sensitivity (100%) and specificity (96%) for lymph nodes measuring between 5 mm and 1 cm in diameter, dropping to 41% for nodes less than 5 mm (18) in diameter. However, the interpretation of this technique is time consuming, since a node-by-node comparison must be made between the native MRI and a second MRI after USPIO administration.

**Diffusion-weighted imaging (DWI).**

Diffusion-weighted imaging (DWI) is an MRI technique based on the detection of random movement of water molecules that is theoretically lower in cancers showing high cellular density, higher Gleason score (19) or tissue heterogeneity. An apparent diffusion coefficient (ADC) is calculated on the basis of the combination of this measurement, which is lower in PCa compared to the normal tissue. In patients with low-risk, localized disease, tumor ADC may be a useful marker of PCa progression and may help to identify patients who stand to benefit from radical treatment. There is a recent trend toward the use of two or more functional imaging sequences, especially since the advent of DWI, which is fast and does not require exogenous contrast. Studies that combine DWI with
T2-weighted, postcontrast and MRS techniques show promising results (20). Diffusion-weighted sequences are typically T2/T2*-sensitive and potentially allow combination of cellular information with USPIO uptake in the same examination. The combination of reduced diffusion together with relatively unchanged T2/T2* after USPIO administration in malignant lymph nodes leads to hyperintense signal suggesting that USPIO-enhanced MRI in combination with DW-MRI is an accurate method for detecting pelvic lymph node metastases. Xu et al. (21) recently obtained diffusion tensor imaging measurements of PCa performed in vivo in patients undergoing radical prostatectomy, and later, ex vivo, in the same patients’ prostatectomy specimens. The results showed that the ADC contrast parallels the T2W contrast with ADC being more specific than T2W contrast for peripheral zone PCa. Diffusion anisotropy provides a unique contrast that differentiates stromal benign prostatic hypertrophy from PCa in the central gland, while its utility for PCa in the peripheral zone is limited.

Disease recurrence after therapy.

Biochemical recurrence occurs in 20-40% of patients within 10 years of ‘definitive’ PCa therapy, often preceding clinically detectable disease (22). Accurate delineation of local versus distant metastatic disease is imperative to determine appropriate therapy. PCa grows slowly and is rarely avid for [18F]fluorodeoxyglucose (FDG). Also, the bladder produces a very strong FDG signal near the prostate. The role of [11C] choline, as previously described, is limited in detecting early recurrence due to its inability to detect microscopic foci of metastatic PCa (23). Although higher urinary excretion by the fluorinated analogs represents a disadvantage in imaging PCa, [18F]fluorocholine PET-CT has demonstrated a sensitivity of 71% in localizing recurrent disease (24). [11C] Acetate has also been evaluated for detection of tumor recurrence and overall has demonstrated results comparable to those of [11C]choline, as reviewed by Morris et al. (25).

The prostate-specific membrane antigen (PSMA) is upregulated in PCa, particularly in advanced, hormone-independent and metastatic disease. PSMA is an integral membrane protein with an enzymatic active site in an extracellular domain, which makes this an excellent target for imaging and therapy. ProstaScintTM, a monoclonal antibody-based PSMA imaging agent, was previously developed but has demonstrated limited clinical utility (26). Other PSMA-binding antibodies are under development for imaging, and may prove superior to ProstaScintTM (27). Urea-based low molecular weight agents of high affinity and in vivo selectivity for PSMA have been synthesized using a variety of radionuclides for positron emission tomography (PET) and single photon computed tomography (SPECT) (28), (29). Compounds of this class demonstrate a very high target to non-target ratios and rapid washout kinetics in both preclinical models (Figure 3) and in a phase I study (30). Initial indications for the use of these agents would be for staging and in patients who have undergone prostatectomy who later present with a rising PSA.

Therapeutic monitoring.

1-(2'-Deoxy-2'-fluoro-3-α-D-arabinofuranosyl)thymidine (FMAU) is a thymidine analog that can be phosphorylated by thymidine kinase and incorporated into DNA. This agent has been evaluated for imaging PCa in three patients. FMAU demonstrated low uptake in a previously radiated prostate bed. Standardized uptake values ranging from 2.89 to 4.49 were seen in the tumor. Bone metastasis also showed high radiotracer uptake. Low urinary bladder radioactivity was seen despite renal excretion (31).

16β-[18F]Fluoro-5β-dihydrotestosterone (FDHT), an analog of 5β-dihydrotestosterone, has been evaluated to study the binding selectivity of FDHT in patients with PCa (32). Patients with a positive study underwent a repeat examination after the administration of flutamide, an antiandrogen, and demonstrated interval decrease in tumor uptake, suggesting FDHT to be potentially useful for evaluating the availability and functional status of tumor androgen receptors to monitor therapeutics that target them. Future studies are needed to investigate whether androgen receptor availability as assessed by FDHT-PET is predictive of response to hormonal therapy.

There has been a renewed interest in the use of [18F]NaF PET imaging for early detection of metastases. A recent study has shown this agent to be superior to [99mTc]MDP planar bone scan, with or without SPECT, in detecting metastatic disease (33).

Molecular genetic imaging provides a way to monitor gene therapy, especially to determine the level and volume of therapeutic gene expression that can be correlated with clinical outcome. Barton et al. (34) recently conducted a phase 1 study demonstrating the feasibility of adenovirus-mediated gene therapy for PCa. They developed a methodology based on the sodium iodide symporter (NIS). Patients with clinically localized PCa were administered a replication-competent adenovirus, Ad5-yCD/utTKSR39rep-hNIS, armed with two suicide genes and the NIS gene via an intraprostatic injection. FDHT to be potentially useful for evaluating the availability and functional status of tumor androgen receptors to monitor therapeutics that target them. Future studies are needed to investigate whether androgen receptor availability as assessed by FDHT-PET is predictive of response to hormonal therapy.

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up to seven days. Whole-body imaging showed intraprostate gene expression, without evidence of extraprostate dissemination of the adenovirus by SPECT imaging, demonstrating the feasibility of this technology in humans (Figure 4).

Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating peptide receptor VPAC1 are overexpressed in 100% of human PCa (35). [64Cu]TP3939, a VIP analog labeled with the positron-emitting radionuclide 64Cu, specifically targets VPAC1 receptors for PET visualization of PCa. Copper-64, with a half-life of 12.7 h ([A+] = 665 keV [17.4%]; [A-]C = 573 keV [30%]), is commercially available, has well known chemistry, provides quantitative yields, and permits the use of radiolabeled compounds without further purification (35). [64Cu]TP3939 was tested in PCa xenografts in athymic nude mice and spontaneous PCa in TRAMP mice (see below). The compound demonstrated a high target to background ratio at 24 hours after injection and is a promising agent not only for imaging PCa but also for detecting its recurrence, detecting metastatic lesions, and determining the effectiveness of therapeutic intervention.

Although we have focused on new agents and techniques for clinical use in PCa, preclinical models, which are continually increasing in relevance to human disease, have proved critical in the development of new molecular imaging agents for PCa. The current state of relevant preclinical models to study tumorogenesis and to develop effective prevention strategies and therapeutic interventions has been described by Pienta et al. (36). That description includes the TRAMP model, which shows a histopathologic disease progression and associated metabolic changes that mimic the human disease. Additionally, researchers have applied tools used to study tumor biology, including the insertion of the VEGF-C gene into well known cell lines, which promotes lymphangiogenesis causing the cells to metastasize (37). Those highly

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relevant preclinical models have and will continue to hasten the development of molecular imaging agents for PCa.

**Perspective.**

Anatomic localization and assessment of disease aggressiveness play key roles in determining prognosis and in guiding the therapy of PCa. Multiple, new promising non-invasive diagnostic tools have the ability to provide that information, and are continually emerging. As new, selective biomarkers, such as the recently described stage-dependent urinary marker sarcosine (38), are discovered, coupling them with molecular imaging tools will avert unnecessary surgeries and prolong survival. In the future, disease assessment will depend on a multimodality imaging approach tailored to each patient for maximum therapeutic benefit.