

Clinical Molecular Imaging

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This review summarizes the rapidly growing field of molecular imaging, the spatially localized and/or temporally resolved sensing of molecular and cellular processes *in vivo*. Molecular imaging is used to map the anatomic locations of specific molecules of interest within living tissue and has enormous potential as a powerful means to diagnose and monitor disease. Molecular imaging agents comprise a targeting component that confers localization and a component that enables external detectability with an imaging modality, such as PET, SPECT, MRI, optical, and ultrasound. The advantages and disadvantages of each of these modalities are discussed in regard to spatial resolution, temporal resolution, sensitivity, and cost. Molecular imaging agents can be divided into three categories, Type A, which bind directly to a target molecule, Type B, which are accumulated by molecular or cellular activity by the target, and Type C, which are undetectable when injected but can be imaged after they are activated by the target. The current status of clinical molecular imaging agents is presented as well as examples of some preclinical applications. The value of molecular imaging is illustrated by some examples for diseases such as cancer, neurological and psychiatric disorders, cardiovascular disease, infection and inflammation, and the monitoring of gene therapy and stem cell therapy.

Key Words: Review, tutorial, molecular imaging, molecular probes, smart probes, optical beacons, PET, SPECT, MRI, optical, ultrasound

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Knowledge about the molecular events intrinsic to the normal functioning of cells and tissues and how they are altered in disease states has increased dramatically over the past few decades. At the same time, advances in instrumentation and electronics have led to great improvements in the quality of radiological images and the range of techniques used.

The combination of these advances, together with the creativity of chemists and other scientists, has led to the rapidly growing field of molecular imaging,* which has been defined by the Commission on Molecular Imaging of the ACR as “the spatially localized and/or temporally resolved sensing of molecular and cellular processes *in vivo*.” In other words, molecular imaging maps the anatomic locations of specific molecules of interest within living tissue and how they change over time. Clearly, clinical molecular imaging has enormous potential as a powerful means to diagnose and monitor disease, a potential that is poised to expand into reality over the next few years as new molecular imaging agents and instrumentation become available [1-4].

Although the term *molecular imaging* may be new, the con-

cept has existed for many years [2,3,5]. The first molecular imaging method of clinical importance was the use of radioactive iodide, $^{131}\text{I}^-$, for the diagnosis of thyroid gland disease and the assessment of thyroid function. The scintigraphic detection of the molecular events of iodide uptake within thyroid cells and its subsequent incorporation into thyroid hormones fits the definition of “spatially localized and temporally resolved sensing of molecular processes *in vivo*.” In fact, many nuclear medicine procedures embody the concepts of molecular imaging. Some nuclear medicine units have changed their names to reflect this.

Another important example from nuclear medicine is the use of the fluorinated glucose derivative [^{18}F]-fluorodeoxyglucose ([^{18}F]-FDG). This molecule is an analog of glucose. It is taken up into cells by glucose transporters and is phosphorylated intracellularly by the enzyme hexokinase, the rate-limiting first step of glycolytic metabolism. Once phosphorylated, [^{18}F]-FDG is not metabolized further, and it cannot pass through the cell membrane and leave the cell (Fig. 1). Thus, it accumulates within cells. As a result, an [^{18}F]-FDG scan depicts the spatial distribution of high rates of glucose metabolism, which in turn is closely linked to many important diseases and conditions. For example, many tumors are characterized by increased glucose utilization, and [^{18}F]-FDG imaging is now widely used in clinical practice for cancer imaging [6].

Thus, molecular imaging should be regarded not as a mystery but as a familiar concept that has now been extended from its roots in nuclear medicine to include applications in magnetic resonance (MR), optical, and ultrasound (US) imaging. Most MR, optical, and US molecular imaging applications

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*It should be made clear that molecular imaging in this context does not mean creating images of the molecules themselves. Rather, it is imaging the distribution of endogenous biomolecules or biomolecular activity *in situ*, either by direct detection or with the aid of a specific chemical probe (i.e., a molecular imaging agent).

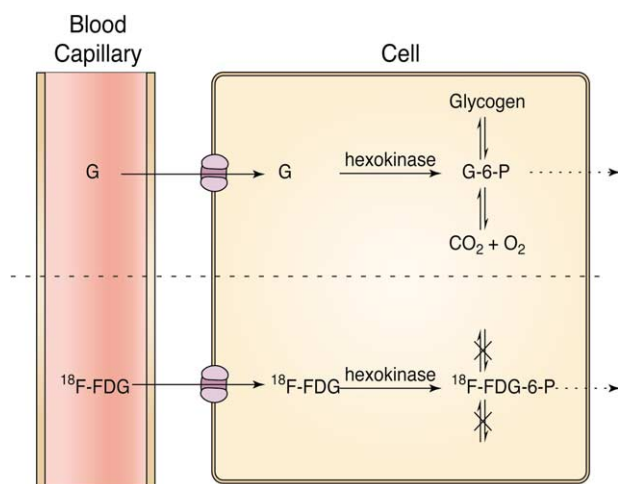


Fig. 1. Schematic of [^{18}F]-fluorodeoxyglucose (FDG) metabolism and intracellular accumulation. Both glucose (G) and FDG leave the blood stream, are carried into the cells by glucose transporter proteins, and are phosphorylated. Unlike glucose-6-phosphate (G-6-P), fluorodeoxyglucose-6-phosphate (FDG-6-P) is not metabolized further and is trapped within the cell because it is unable to pass through the cell membrane. Adapted from Phelps et al. [21].

involve the use of pharmaceutical agents, just as nuclear medicine employs radiopharmaceuticals. However, a common terminology has not yet been adopted to describe these agents,

which are variously called *molecular probes*, *enhancement agents*, or *molecular imaging agents*. In optical imaging, they are sometimes referred to as *optical beacons*.

All chemical agents used in molecular imaging, whether the modality is nuclear medicine, MR imaging (MRI), or optical imaging, must meet the same requirements. That is, each agent must have a targeting component that confers localization through molecular interactions within the tissues and a component that enables its external detection by one of the respective imaging modalities. In the case of $^{131}\text{I}^-$, the chemical characteristics of iodide confer molecular uptake and localization in the cells of the thyroid gland, and the use of a radioactive isotope of iodine confers external detectability.

Table 1 lists a number of molecular imaging agents to illustrate this fundamental concept of a targeting or tissue-localizing component or moiety and a component that allows external detection. These few examples illustrate how molecular imaging may be used for many different diseases and symptoms using a range of imaging modalities.

In some cases, it is possible to make radiological images of intrinsic biological molecules, that is, to take advantage of molecules present in the body.[†] However, in this primer, we

[†]Spatial variations in the oxidation state of hemoglobin can be measured by both blood oxygen level–dependent functional MRI [7] and diffuse optical tomography [8], and MR spectroscopy can be used to image natural or administered substances that have characteristic MR spectra and are present in sufficient quantities [9,10].

Table 1. Examples of molecular imaging agents

Molecular Imaging Agent	Clinical Application or Potential	Target Biomolecule/Physiological Process	Detectable Component	Modality of Detection
<i>Type A: agent bound directly to target</i>				
[^{111}In]-monoclonal endoglin antibody	Angiogenesis (cancer)	Endoglin receptor	[^{111}In]	SPECT (47)
[^{11}C]-raclopride	Parkinson's disease	Dopamine receptors	[^{11}C]	PET (61)
[^{18}F]-FDDNP	Alzheimer's disease	β -Amyloid protein	[^{18}F]	PET (72)
Annexin V-CLIO	Monitoring cancer treatment	Phosphatidyl serine (cell membrane lipid indicative of apoptosis or cell death)	Iron oxide	MRI (29)
Pamidronate-IRDye78 conjugate (Pam78)	Skeletal disease, coronary calcification	Hydroxyapatite	IRDye78	Optical (NIRF) (34)
Microbubble-peptide conjugate	Thrombus detection	GpIIb/IIIa receptor (binds fibrinogen to activated platelets)	Microbubble	US (40)
<i>Type B: agent accumulation through molecular or cellular activity of the target</i>				
$^{131}\text{I}^-$	Thyroid disease	Sodium iodide transporter	^{131}I	X-ray film, SPECT (2)
[^{18}F]-FDG (fluorodeoxyglucose)	Cancer, infection, inflammation, brain function, myocardial viability, Parkinson's disease	Glucose transporter and hexokinase	^{18}F	PET (6,44,54)
[$^{99\text{m}}\text{Tc}$]-sestamibi	Drug-resistant tumors	ATP-dependent transport proteins that confer drug resistance	$^{99\text{m}}\text{Tc}$	SPECT (52,73)
[^{131}I]-Altopane	Parkinson's disease	Dopamine transporter	^{131}I	SPECT (74)
[^{11}C]-palmitate	Myocardial viability	Enzymes responsible for fat metabolism	^{11}C	PET (62)
CLIO-Tat	Stem cell therapy	Phagocytosis	Iron oxide	MRI (23)
USPIO or MION particles	Metastasis to lymph nodes	Macrophage phagocytosis	Iron oxide	MRI (23)
IDDC-octreotate	Cancer	Somatostatin receptor and endocytosis	Cyanine dye	Optical (NIRF) (75)
<i>Type C: agent conversion to a detectable form through target enzyme activity</i>				
Near-infrared fluorescent (NIRF) probes	Cancer, inflammation, thrombosis	Protease enzymes	Fluorochrome	Optical (NIRF) (32)
EgadMe	Gene expression marker	β -Galactosidase	Gd	MRI (17)

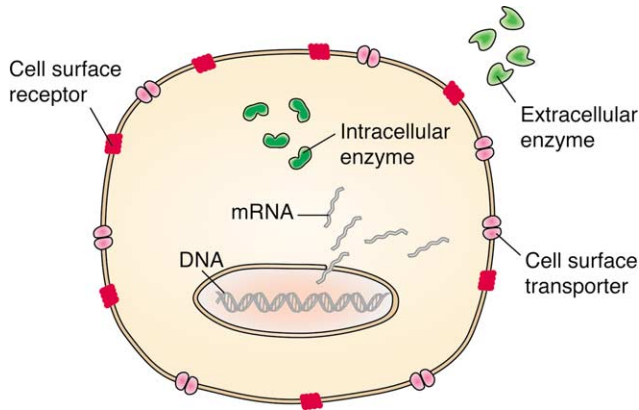


Fig. 2. Targets for molecular imaging.

have chosen to focus on imaging applications that use administered molecular imaging agents, target a specific molecular site or activity, and enable the noninvasive imaging of that site. When restricted in this way, molecular imaging can be regarded as a modern form of *in vivo* histopathology, whereby the chemical characteristics of an agent or probe are equivalent to a stain, and the imaging modality takes the place of a microscope. Thus, molecular imaging has an advantage over conventional anatomic imaging in that it provides noninvasive information about biological processes *in vivo* at the molecular level.

A plethora of new molecular imaging agents and probes have been developed over the past few years [3,11-13]. Many are still being applied only in the realm of research but promise considerable clinical advantages over some present radiological methods as well as other diagnostic methods, such as blood chemistry or invasive biopsy procedures. Other molecular imaging methods, such as bioluminescence (e.g., luciferase) and protein fluorescence (e.g., green fluorescent protein), require genetic manipulation and are not suitable for human use,

although they can be used to image interactions between proteins in living animals, and they may have medically valuable applications in the phases of drug development that require experimentation in animals [14].

PHARMACEUTICALS FOR MOLECULAR IMAGING

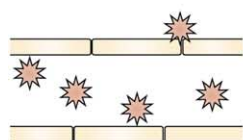
A major challenge to overcome in the development of molecular imaging agents is the extraordinarily low concentrations of most biomolecules normally present in tissues (in the picomolar [10^{-9}] to nanomolar [10^{-12}] range). Molecular imaging agents must be highly specific in their interactions, must reach their targets and remain there in sufficient quantities and for sufficient time to be detectable, and at the same time be minimally detectable in other regions (i.e., the agents must achieve high target-to-background concentrations). The uptake of an imaging agent within the tissue of interest (tissue specificity) is achieved through the design and synthesis of agents that interact with specific molecules (targets) characteristic of different diseases. Detectability is enhanced by the selective accumulation or activation of the imaging agent, whereas the agent is rapidly washed out in areas where disease is not present.

Molecular imaging targets are generally products of gene expression. Theoretically, they could include the first product of gene expression, mRNA, but in practice, there are not sufficient amounts of mRNA for detection *in vivo* using current technology. However, thousands or even millions of copies of some proteins—the products of RNA transcription—can be present in individual cells. Thus, proteins are more practical targets for detection with molecular imaging agents. The target proteins can be structural elements of cells, receptors, or enzymes (Fig. 2).

The molecular interactions that confer localization of a molecular imaging agent may be due to agent bound to a target molecule, accumulation through molecular or cellular activity of the target, or conversion to a detectable form through target

Contrast Agent

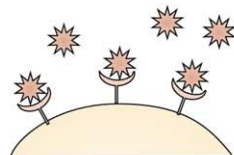
No Specific Binding



Blood volume, tissue perfusion

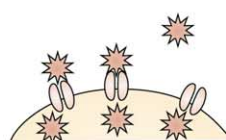
Molecular Imaging Agents

a. Agent Bound to Target Molecule
Low sensitivity, high background noise



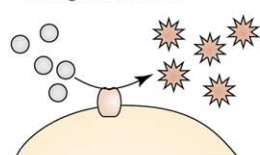
Locate proteins, determine structure

b. Agent Accumulated by Target Molecule Activity
High sensitivity, significant background noise



Locate proteins, determine function

c. Agent Converted to Detectable Form by Target Enzyme Activity
High sensitivity, minimal background noise



Locate proteins, determine function

Fig. 3. Classes of contrast and molecular imaging agents. Contrast agents generally have compartmental distributions and can be used to image physiological processes such as changes in blood volume, perfusion and blood flow in angiogenesis. Molecular imaging agents can (a) bind directly to the target molecule, sometimes with the aid of antibodies (background noise can be fairly high); (b) accumulate by cellular uptake and/or enzyme activity (signal-to-noise ratios are good); or (c) depend on activation by their target to become detectable (very high signal-to-noise ratios). Agents in group c are sometimes known as “smart probes” and have been developed for optical and magnetic resonance imaging and used extensively in animal studies. Adapted from Weissleder [20].

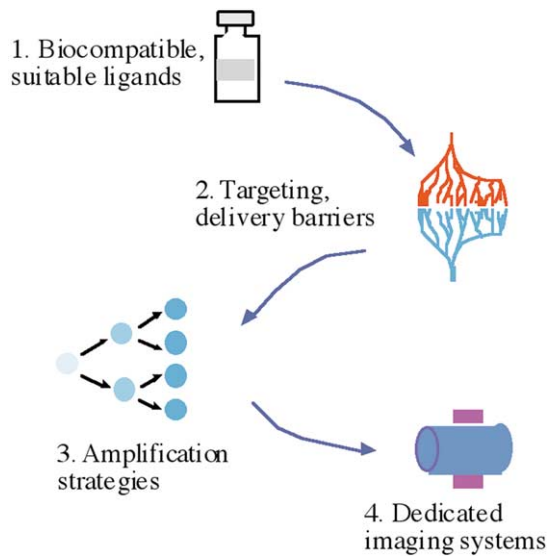


Fig. 4. Prerequisites for clinical molecular imaging. From Weissleder [1], with permission.

enzyme activity (Fig. 3). When an imaging agent is retained in tissue by simply binding to a target molecule, the amount of imaging agent that accumulates is typically limited to one molecule of imaging agent bound to one target molecule. Because a certain amount of unbound agent is generally present, the signal-to-noise ratio is limited. When the imaging agent is accumulated by molecular or cellular activity, thousands of molecules of imaging agent can be retained by the action of one target molecule, increasing detectability and improving the signal-to-noise ratio. Such accumulation has been accomplished by co-opting the process of cellular transport in certain types of cells, bringing in many molecules of the molecular probe (molecular imaging agent), an approach that has been successfully used with positron emission tomography (PET), single photon emission computed tomography (SPECT), MRI, and optical techniques (Table 1). For example, the thyroid can accumulate many thousands of iodide molecules through the action of transport proteins and subsequent enzyme activity, which has been used as the basis for a very sensitive method of thyroid imaging for many years.

The enzymatic activation of molecular imaging probes is a superior method of amplification because a single enzyme molecule can act on many individual molecules of a molecular imaging agent that cannot be detected in the form in which it

is originally administered. For example, some molecular imaging agents are activated by protease enzymes that cleave specific peptides, releasing fluorescent components that can then be visualized with near-infrared fluorescence (NIRF) [15,16]. Another example in this category is the MRI molecular probe EgadMe, which contains gadolinium in a chelated form that does not enhance MR images. The enzyme β -galactosidase cleaves EgadMe, causing the transition of the agent to an active state [17].

Several other features that are necessary for clinically useful molecular imaging agents are summarized in Figure 4. The agents must overcome biological barriers to delivery, including vascular, interstitial, and cell membrane barriers. They must have a long circulation time to allow sufficient time to reach and interact with their targets before being degraded or excreted. And they must have low immunogenicity [18]. Although these requirements are challenging, a number of new molecular imaging agents are now in use in clinics, and many more are in the phase of animal and preclinical studies.

MOLECULAR IMAGING MODALITIES

As described above, the earliest molecular imaging agents were radiopharmaceuticals. PET and SPECT remain the most commonly used detection modalities, but advances in research are also bringing MRI, near-infrared optical imaging, and US imaging to the forefront. Each imaging modality has different strengths and weaknesses in terms of spatial resolution, temporal resolution, sensitivity, and cost [2,19,20]. Among these modalities (Table 2), MRI has the best spatial resolution, and in the clinical setting, MRI can routinely image structures in the millimeter range. However, MRI is not very sensitive in terms of the concentration of the molecular imaging probe needed for detection, with a limit in the micromolar range without the use of signal amplification methods. In contrast, PET, SPECT, optical fluorescent, and US methods have relatively low spatial resolution but high sensitivity to molecular concentration, allowing mere trace amounts of a molecular imaging agent to be detected.

Radionuclide Imaging

The molecular probes or imaging agents that have been in use longest are radiopharmaceuticals that use γ -emitting radionuclides, such as ^{123}I or $^{99\text{m}}\text{Tc}$. Thus, SPECT remains the most widely used nuclear imaging technique for clinical molecular imaging. The radionuclides that are commonly used for

Table 2. Comparison of clinical imaging modalities for molecular imaging agents

Modality	Sensitivity	Resolution			Cost
		Spatial	Temporal	Contrast	
MRI	+	10–100 μm	msec	+++	+++
MRS	+	1 cm	min/h	+	+++
PET	+++	3–4 mm	min	++	+++
SPECT	++	8–12 mm	min	+	++
Optical fluorescence	+++	1–2 mm	msec	+++	+
US	+++	50 μm	msec	++	++

Data from Piwnica-Worms (19), Pomper (2), and Weissleder (20). +++, high; ++, medium; +, low.

Table 3. Radionuclides used in SPECT

Radionuclide	Radioactive Half-life	Molecular Imaging Applications
^{99m} Tc	6 h	Tumor detection and characterization, cardiac infarction detection and monitoring thrombolytic therapy, renal function studies
¹³¹ I	8 d	Thyroid function and tumor detection, renal function studies, receptor binding studies, transporter function
¹²³ I	13.2 h	
¹¹¹ In	2.8 d	Inflammatory disease detection, neuroendocrine tumor detection, receptor binding studies

SPECT have half-lives (Table 3) that range from 6 hours to 8 days, long enough to allow the radiopharmaceuticals to be shipped, avoiding the need for individual medical centers to have cyclotrons and chemical laboratory facilities nearby. These and other radionuclides have been incorporated into many agents for different diseases, including Parkinson's disease and cancer, as well as for monitoring gene therapy [3]. The major limitation of SPECT is that only a small proportion of the γ -rays emanating from a patient are detected because of the need for collimation, which greatly limits its sensitivity. In addition, radiation scattering lowers spatial resolution because it is not possible to locate precisely the origin of scattered photons in the body.

Positron-emitting radionuclides, such as ¹⁸F, which are visualized through PET, have also been incorporated into numerous molecular imaging agents. As noted above, the most frequently used is the fluorinated glucose derivative [¹⁸F]-FDG, which is taken up preferentially by cells with high rates of metabolic activity (glycolysis) [21]. PET imaging is more sensitive and has better resolution than SPECT because of the unique physics of positron decay. After a positron is emitted, it collides with an electron, usually within a few millimeters of its point of origin, and is annihilated. In the process, two photons are released in opposite directions, at 180° from each other. PET detectors record the simultaneous emission of two photons, eliminating the need for the bulky and heavy collimators used to exclude scattered photons in SPECT. Typically, PET cameras have multiple rings of detectors arranged in a circle around the body to detect the emitted photons. PET reconstruction procedures produce images that have threefold to fourfold higher resolution with greater sensitivity (down to the nanomolar range) than those obtained with SPECT [22].

However, PET does have its disadvantages compared with

SPECT, including the very short half-lives of most positron-emitting radionuclides (Table 4) and comparatively expensive instrumentation. The most commonly used positron-emitting radionuclide, ¹⁸F, has a half-life of <2 hours. [¹⁸F]-FDG is now widely available on a commercial basis in the United States. However, a hospital must have access to a nearby cyclotron and a chemistry laboratory to make radiopharmaceuticals with ¹¹C, ¹³N, or ¹⁵O. This significantly adds to the cost of PET and limits its availability.

Magnetic Resonance Imaging

The very high spatial resolution that is attainable with MRI makes it an attractive method for molecular imaging, except that unenhanced MR images can detect substances only in the millimolar concentration range. Unenhanced MR images depend on the paramagnetic characteristics of the nuclei of elements that contain odd numbers of protons.

MRI contrast agents enhance the signal because they have magnetic properties themselves and alter the relaxation rate of nearby paramagnetic nuclei. Because one molecule of contrast agent can perturb many nuclei, the effects of nanomolar concentrations of contrast agent can be visualized, a fact that makes molecular imaging with MRI possible. Agents that contain gadolinium slow T1 relaxation time and are particularly valuable in regions where there are large amounts of rapidly tumbling free water molecules, such as in tumors or blood. Agents that contain ferromagnetic substances, such as iron oxides, shorten the T2 relaxation times of nearby hydrogen atoms and enhance the contrast of T2-weighted images.

The effectiveness of ultrasmall superparamagnetic iron oxide and monocrySTALLINE iron oxide nanocompound (MION) preparations in distinguishing between healthy lymph nodes (which take the particles into their cells by phagocytosis) and

Table 4. Radionuclides used in PET

Radionuclide	Radioactive Half-life	Molecular Imaging Applications
¹⁸ F	1.8 h	Metabolic activity (tumor, inflammation, infection), receptor binding, transporter function, enzyme activity
¹¹ C	20 min	Metabolic activity (myocardium, cardiac infarction detection), receptor binding
¹⁵ O	2 min	Metabolic activity (cognitive function)
¹³ N	10 min	Protein synthesis, cell proliferation (mitotic rate)
¹²⁴ I	4 d	Antibody binding

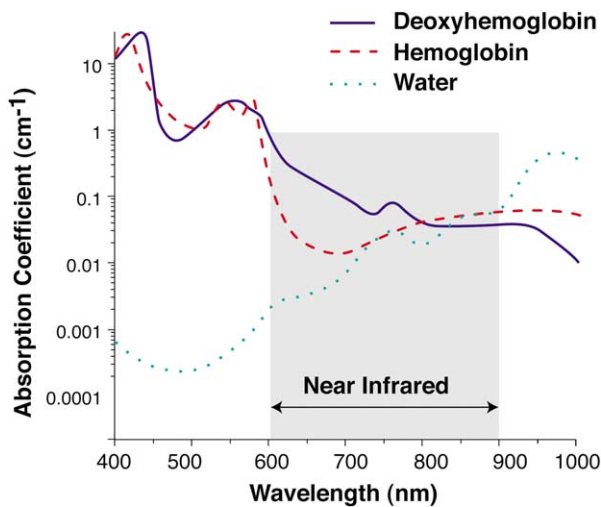


Fig. 5. Light absorption by biological molecules. Adapted from Yodh and Chance [76].

cancerous lymph nodes (which do not) demonstrates the value of iron oxide as an MRI contrast agent [23,24]. At the core of each MION particle is a single crystal of iron oxide about 5 nm in diameter, which is surrounded by a layer of the flexible complex carbohydrate dextran, a few nanometers thick [25]. The overall size of a MION nanoparticle is comparable to that of a protein molecule, so MIONs can readily leave the vasculature through capillary walls. The iron component confers detectability, and under ideal conditions, MRI can detect these particles down to a concentration of 50 nmol (2.8 μg) Fe per gram of tissue [25].

Recently, MION-based MR contrast agents have been modified for use as molecular imaging probes. This has been achieved by chemically attaching agents that interact with target molecules to the dextran coats of MION particles. The

binding agent confers the capacity to localize to specific molecules, which can then be detected through the MRI enhancing effect of the MION particles. In one demonstration of this concept, the human protein transferrin was chemically attached to the MION core and used to detect cells that overexpressed the transferrin receptor in gene transfer experiments [26]. In other experiments, a peptide, Tat, was attached to the cross-linked dextran coat of a related iron oxide-containing contrast agent to form cross-linked iron oxide (CLIO)-Tat. The Tat peptide is naturally found in HIV, in which it plays a role in facilitating the entry of the virus into T-cells. In CLIO-Tat, the peptide facilitates the entry of the imaging agent into hematopoietic stem cells. The labeled cells have been used to track the migration of the stem cells after they were infused into experimental animals [27]. A number of other CLIO imaging agents have recently been developed that target markers of blood vessel development (angiogenesis) [28] and enzyme-regulated cell suicide (apoptosis) [29], processes that are characteristic of diseases including cancer [20]. The MION and CLIO moieties are proving to be very flexible “magneto-labels” for many other applications as well. The value of these molecular imaging agents will no doubt be seen in clinics in a few years.

Optical Imaging

NIRF Imaging NIRF imaging is a recent development that promises to be very valuable for a number of different purposes, including the detection of small tumors, infection, and thrombosis *in situ* [16,30-33]. Fluorescence in the near-infrared spectrum was selected because these wavelengths penetrate tissue farther than other wavelengths in the optical range (Fig. 5). Pigmented proteins such as hemoglobin absorb light within the visible spectrum, and water absorbs a significant amount of light in the infrared range. Fluorescence imaging has long been a valuable tool in the laboratory setting, where a fluorescent

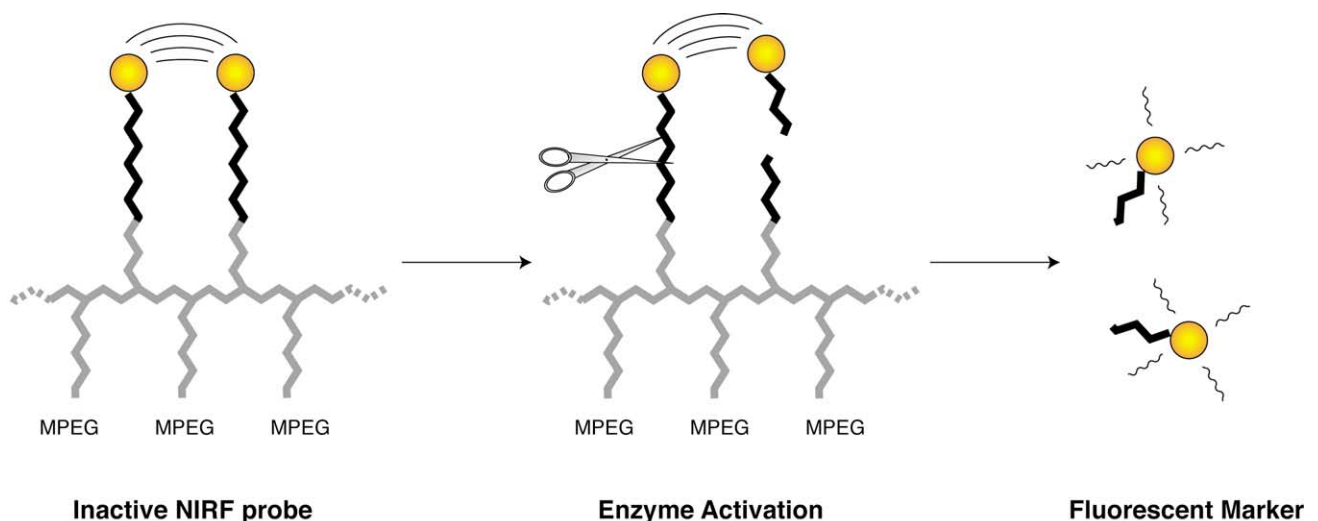


Fig. 6. Schematic of “smart” near-infrared fluorescence (NIRF) molecular imaging agents. Filled circles represent fluorochromes that are spatially near each in the intact imaging agent. Given the close proximity, the fluorochromes are quenched. With specific enzyme cleavage of peptide spacers (black undulating lines), fluorochromes are separated from the backbone and each other and markedly increase their fluorescence. Adapted from Tung et al. [35].

Table 5. Examples of near-infrared protease imaging agents ("smart" NIRF probes)

Specificity/Function	Diagnostic Use
Cathepsin B (lysosomal protease)	Tumor growth and metastasis (colon); inflammation (30)
Cathepsin D (protease)	Cancer (breast) (35)
MMP-2 (gelatinase)	Tumor stage and metastasis (36)
Cathepsin K	Osteoporosis, bone remodeling (32)
Prostate-specific antigen	Cancer (32)
Herpes simplex virus (HSV) protease	Infection (32)
HIV protease	Infection (32)
Cytomegalovirus (CMV) protease	Infection (32)
Thrombin	Thrombosis (63)
Caspase-3	Cancer treatment monitoring (apoptosis or cell death) (32)

agent is used to bind to a molecular site in a sample of cells *in vitro*, and the unbound fluorescence is washed away to increase the target-to-background ratio before imaging. Some agents have been developed that bind to specific sites *in vivo*, such as hydroxyapatite to image bone deposition [34]. However, it is not possible to effectively wash away unbound fluorescent agents *in vivo*, which limits this technique to agents that bind strongly to sites of high capacity.

A recent breakthrough is the design of "smart" NIRF molecular imaging probes that are not significantly fluorescent in the forms in which they are administered [30,32,35]. The essential features of the molecular structures of these NIRF imaging agents (Fig. 6) are a molecular "backbone," to which 10 to 20 peptide stalks are attached, with a fluorescent dye molecule covalently attached to the end of each peptide [30]. In this structure, the fluorescent moieties are held so closely together that they interact, quenching most of the fluorescence. However, on reaching the sites of their targets, the imaging agents are cleaved by enzymatic activity, releasing the fluorescent moiety and thereby allowing their detection with very high signal-to-background ratios (Fig. 6). A series of imaging agents have been designed (Table 5), each of which is sensitive to a different target enzyme, such as those that are characteristically overexpressed in tumors, infection, inflammation, and apoptosis [30,32,35,36]. Because many kinds of peptide chain can be attached, each of which is designed to be cleaved by a specific enzyme, this is an extremely versatile system, useful for making images of many kinds of tumors or tissues that have enzymes with different specificities [32].

NIRF imaging agents have also been designed to overcome some of the barriers to effective delivery to tumors [18]. For example, some NIRF imaging agents are designed to have very high molecular weights and thus cannot easily pass through normal capillary walls, whereas they readily pass through the highly permeable neovasculature of tumors. Second, the imag-

ing agents can be designed to be long circulating to allow sufficient time for them to penetrate tumors before they are excreted.

Near-Infrared Imaging Systems Technically, the easiest method to image NIRF is with a simple reflective system [16] (Fig. 7), which is useful for probing structures up to a depth of 7 mm [32]. The wavelength of light used to activate fluorescence has higher energy than the binding energy of electrons in a fluorochrome (a molecule capable of fluorescence) and causes the displacement of these electrons. As electrons move to refill the electron orbits, fluorescent light of lower energy than the activating light is emitted. In principle, this is identical to the generation of characteristic x-rays following displacement of orbital electrons by x-rays or γ -rays (the photodiode effect), although the energy level required for NIRF is much lower. NIRF reflectance imaging systems, such as the one depicted in Fig. 7, can detect extremely low concentrations (subpicomolar) of fluorochromes and have been used to detect tumors <1 mm in size in living mice [16]. The fluorochrome most commonly used to date is indocyanine green, whose fluorescence is detectable at 700 nm.

Reflective systems could be used to diagnose a number of human epithelial cancers. Colonoscopes, bronchoscopes, endoscopes, and laparoscopes can be used in conjunction with reflective detection systems to diagnose superficial cancers of hollow viscous and cavities. Catheters could be used to image the proteolytic enzyme activity associated with inflammation in vulnerable atherosclerotic plaques [37]. In addition, near-infrared goggles with appropriate filters could be used during surgery to evaluate the tumor margin.

Near-infrared light penetrates tissues relatively well (Fig. 8), and fluorescence molecular tomography (FMT) [32] is a three-dimensional imaging technique capable of imaging lesions at depths of several centimeters, because the intensity of the fluorescence is so well localized when enzyme-activated NIRF imaging agents are used. Even so, optical imaging is limited to depths of 7 to 14 cm by light absorption (the coefficient of absorption is about 1 cm^{-1} in the near-infrared range) and by light scattering (which occurs approximately every millimeter). Currently, FMT can detect nanomolar concentrations of fluorochromes at spatial resolutions of 1 to 2 mm in the case of

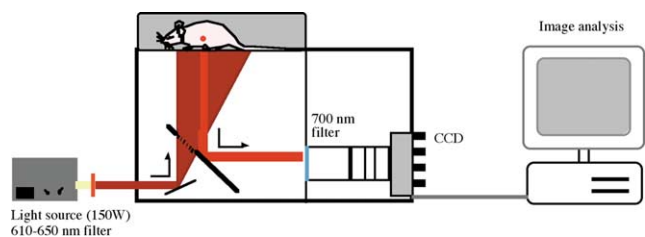
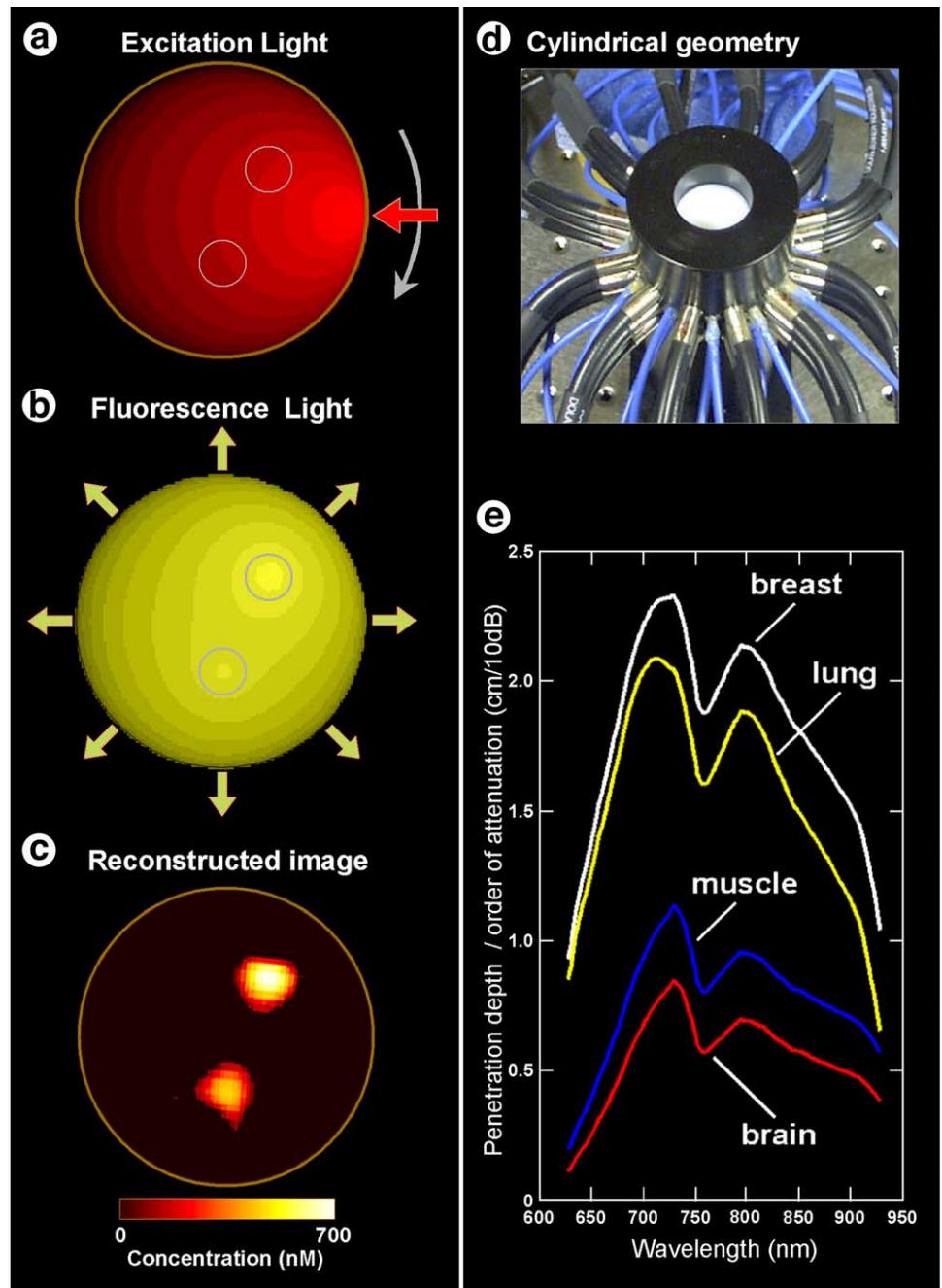


Fig. 7. Near-infrared reflectance imaging system. Filtered light homogeneously illuminates the animal with 610 nm to 650 nm excitation photons. Fluorescent photons are selected with a 700 nm longpass filter, optimized for the fluorochrome under study. The emission signal is focused with a zoom lens and recorded with a cooled charge-coupled device (CCD) camera. From Mahmood et al. [16], with permission.

Fig. 8. Fluorescence molecular tomography (FMT). This imaging technique involves principles similar to X-ray computed tomography (CT). (a) As a single point source of light penetrates the tissue, a given distribution of fluorochromes are excited. (b) The light source then rotates around the boundary, effectively illuminating the fluorochrome distributions at different projections. Excitation and fluorescence light are both collected from multiple points of the surface, using appropriate filters. (c) The measurements are tomographically combined to yield quantitative maps of fluorochrome distribution. (d) A cylindrical FMT imaging system for mouse imaging. (e) Modeling of the distance that near-infrared light can propagate into different tissues before it attenuates by an order of magnitude. Fluorochromes can be detected up to 7 to 14 cm depth. From Weissleder and Ntziachristos [32], with permission.



small animals [20]. The techniques and computations used in FMT are similar to those used in x-ray computed tomography (CT) but are adapted to allow for the diffuse pattern of light transmission. Multiple optical fibers encircle a portion of the anatomy, illuminating the tissue from many different directions and simultaneously collecting the emitted fluorescence from all around the tissue. The data are reconstructed into three-dimensional images [32].

In 2000, near-infrared tomographic techniques were used to image tumors with a resolution of about 5 mm within the human breast, using the fluorescent agent indocyanine green, which binds nonspecifically to proteins in the blood [33]. The enzyme-activated NIRF imaging agents have been used only in animal

research to date. However, they have considerable promise as clinical agents. Moreover, given the high target-binding specificity of these imaging agents, the spatial resolution of FMT is more than adequate to localize and characterize lesions.

Ultrasound

Molecular imaging with agents detectable by US is now being explored in animal experiments. These molecular imaging agents are modified US contrast agents, micrometer-sized gas-filled microbubbles, whose shells are composed of lipids, protein, or polymers. They can contain various gases depending on the degree of impermeability of the shell. The microbubbles vibrate strongly in response to the high-frequency sound waves

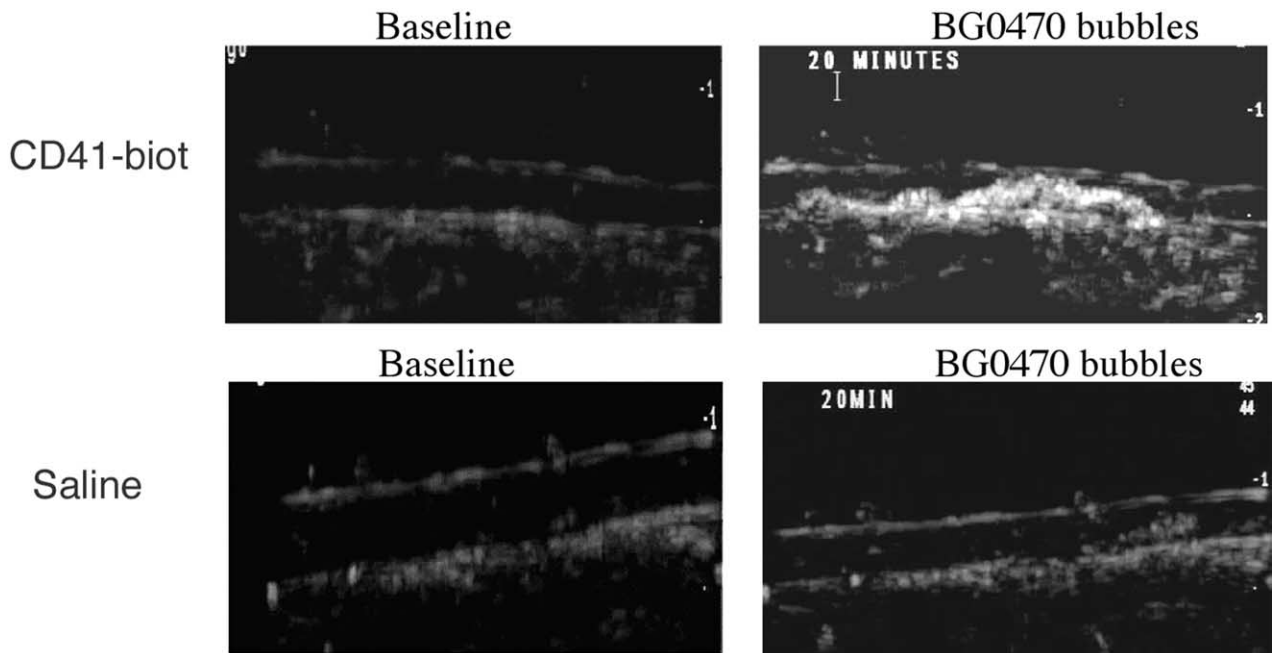


Fig. 9. Ultrasound imaging of a thrombus after administration of biotinylated antibody (CD41) or saline followed by avidin-coated BG0470 microbubbles. The avidin-coated bubbles bind to the biotin-antibody complex and can be seen as intense ultrasound reflectivity at the location of the thrombus. From Tardy et al. [39], with permission.

used in diagnostic US imaging, making them several thousand times as reflective as normal tissue. Thus, the amount of contrast agent needed is exceedingly small, and it is possible to detect picogram quantities of agent. Indeed, a single bubble with a diameter in the micrometer range can be seen, indicating that this method has the promise of being both very sensitive and inexpensive, but with low spatial resolution.

Microbubbles do not normally leak out of blood vessels into the interstitium, unlike the smaller molecular probes used in MR, nuclear, or optical imaging. This limits the use of US molecular imaging agents to targets within the vasculature in most cases. However, microbubble agents can be taken up nonspecifically by phagocytosis into normal cells, such as those of the liver and spleen. In this case, their absence in a region of an image can be used to diagnose focal lesions in these tissues [4,38].

Recently, microbubbles have been chemically modified to make them into molecular imaging agents that target specific biomolecules. Because these agents typically remain within the confines of the vascular space, they are suitable for targeting markers of thrombus, endothelial cells, and blood cells [13].

For example, two different molecular imaging agents have been designed to target thrombus via the GPIIb/IIIa receptor and to be detected with US. The GPIIb/IIIa receptor is found on activated platelets, where it serves to bind fibrinogen as a blood clot develops. This receptor is the most abundant marker present in thrombi [39,40]. One of these agents targets the receptors indirectly through a biotin-tagged antibody to the GPIIb/IIIa receptor, which binds to the thrombus. Once the antibody has bound to the receptor, avidin-conjugated microbubbles are injected and bind to the biotin, providing bright enhancement in US images in the region of the thrombus in experimental animals (Fig. 9) [39]. The second of these molecular imaging agents is a molecular chain consisting of a ligand that binds to the GPIIb/IIIa receptor, a tether that allows steric freedom, and an anchor moiety that holds the chain in place in the lipophilic membrane of the microbubble. These targeted microbubbles have been shown to bind to thrombi in living animals by intravital microscopy and to enhance US images in a phantom model [40].

The importance of the role of inflammation in atheroscle-

Table 6. Ultrasound imaging of intravascular inflammation

Microbubble Structure	Molecular Target or Physiological Action	Site
Albumin	Opsonization and phagocytosis	Activated leukocytes
Phosphatidyl serine/lipid	Opsonization and phagocytosis	Activated leukocytes
Monoclonal antibody/lipid	Intercellular adhesion molecule-1 (ICAM-1)	Inflamed endothelium, attracts leukocytes
Antibody/lipid	P-selectin	Inflamed endothelium, attracts leukocytes
Data from Lindner (41).		

Table 7. PET as a tool for staging cancer; comparison with CT

	PET Sensitivity (%)	CT Sensitivity (%)	PET Specificity (%)	CT Specificity (%)
Lung cancer Mediastinal staging (7 studies, 29-74 patients)	76-100	43-75	82-99	63-94
Colon cancer Staging of recurrence (5 studies, 24-115 patients)	91-95	47-86	86-100	58-97
Head and neck cancer Nodal staging (3 studies, 48-60 patients)	72-90	67-82	82-98	85-97

Adapted from Bar-Shalom et al. (43).

rotic disease has inspired several approaches to imaging inflamed plaques. The US-based molecular imaging strategies (Table 6) take advantage of the molecular changes that occur in inflammation, in activated leukocytes that have been recruited to the site of inflammation, and in the increase in P-selectin and other endothelial cell adhesion molecules (ECAMs) [41]. Both air-filled albumin microbubbles and lipid microbubbles containing the cell membrane phospholipid phosphatidylserine adhere to activated leukocytes. Using monoclonal antibodies to target ECAMs and P-selectin is particularly attractive because they are expressed in relatively high concentrations on the surface of inflammatory plaque, neovessels within the plaque, and adventitial vessels.

Neovasculature has been targeted using lipid-shelled microbubbles with antibodies to α_v -integrin, a marker for angiogenesis [42]. These agents will have uses that extend beyond imaging plaque inflammation, because angiogenesis is also associated with tumor growth.

However, there are practical issues related to the use of microbubble US molecular probes. For example, microbubbles are best detected at frequencies below 2.5 MHz, which is relatively low power for vessel imaging [41].

MOLECULAR IMAGING OF DISEASE

In the previous section, we discussed the range of imaging techniques that can be used for molecular imaging, many of which have not yet been applied to human disease. In this section, we discuss some clinical applications of molecular imaging for diagnosis and treatment, emphasizing those used currently but including some promising future applications. To date, most of the molecular imaging agents or probes that are in clinical use are those suited to PET or SPECT imaging. Some molecular agents that are detected by MRI are in the final stages of clinical trials. It is likely that some of the more promising NIRF and US agents will reach the stage of clinical trials in the foreseeable future.

Cancer

A significant factor for decreasing the rate of death among patients with cancer is early specific diagnosis allowing prompt effective treatment. Today, radiological screening depends on

anatomical imaging techniques, which are limited in both their specificity and their sensitivity. For example, although screening by mammography has been shown to decrease breast cancer deaths by 50%, as many as 75% to 80% of the lesions biopsied because of concerns raised by mammography prove to be benign. Benign pulmonary nodules in the lung account for as many as 40% of the abnormal nodules seen on CT images but are indistinguishable from tumors. With more sensitive and specific molecular imaging techniques, the accurate detection and diagnosis of smaller tumors will become possible, as will the rapid and accurate assessment of their response to treatment.

Molecular imaging holds much promise for improving the ability to detect and diagnose cancerous tumors, which have a number of features that differentiate them from normal tissues. For example, cancerous tumors commonly have an unusually high metabolic rate, and they frequently overexpress genes that code for proteolytic enzymes (proteases), growth factors, cell surface markers, DNA-binding transcription factors, and cell cycle regulators, among others. Many molecular imaging probes have been designed that target these gene products. Most of these are still in the preclinical phase of research but promise to be extremely useful diagnostic tools in the clinic.

[¹⁸F]-FDG PET for Cancer Patients Imaging with [¹⁸F]-FDG PET is a widely used application of molecular imaging for cancer, and it clearly illustrates the advantages of molecular imaging over conventional imaging. [¹⁸F]-FDG PET targets the enzymes and cellular processes involved in metabolic activity, which is typically faster in tumors compared with normal tissue. Therefore, [¹⁸F]-FDG PET is not confounded by scar tissue, benign lesions, or the residual fibrotic mass that may be present after cancer treatment, all of which have a low metabolic rate. [¹⁸F]-FDG PET has been shown to be more accurate than CT for the diagnosis of several cancers (Table 7) and to be a particularly sensitive diagnostic tool for non-small-cell lung cancer, recurrent colon cancer, lymphoma, breast cancer, head and neck cancer, and melanoma [43]. It is likely that this list will be extended as studies continue.

Breast cancer can be accurately detected, staged, and re-staged with PET imaging, which has been shown to be superior to conventional imaging for predicting outcomes in previously treated breast cancer patients [44]. The sensitivity, specificity,

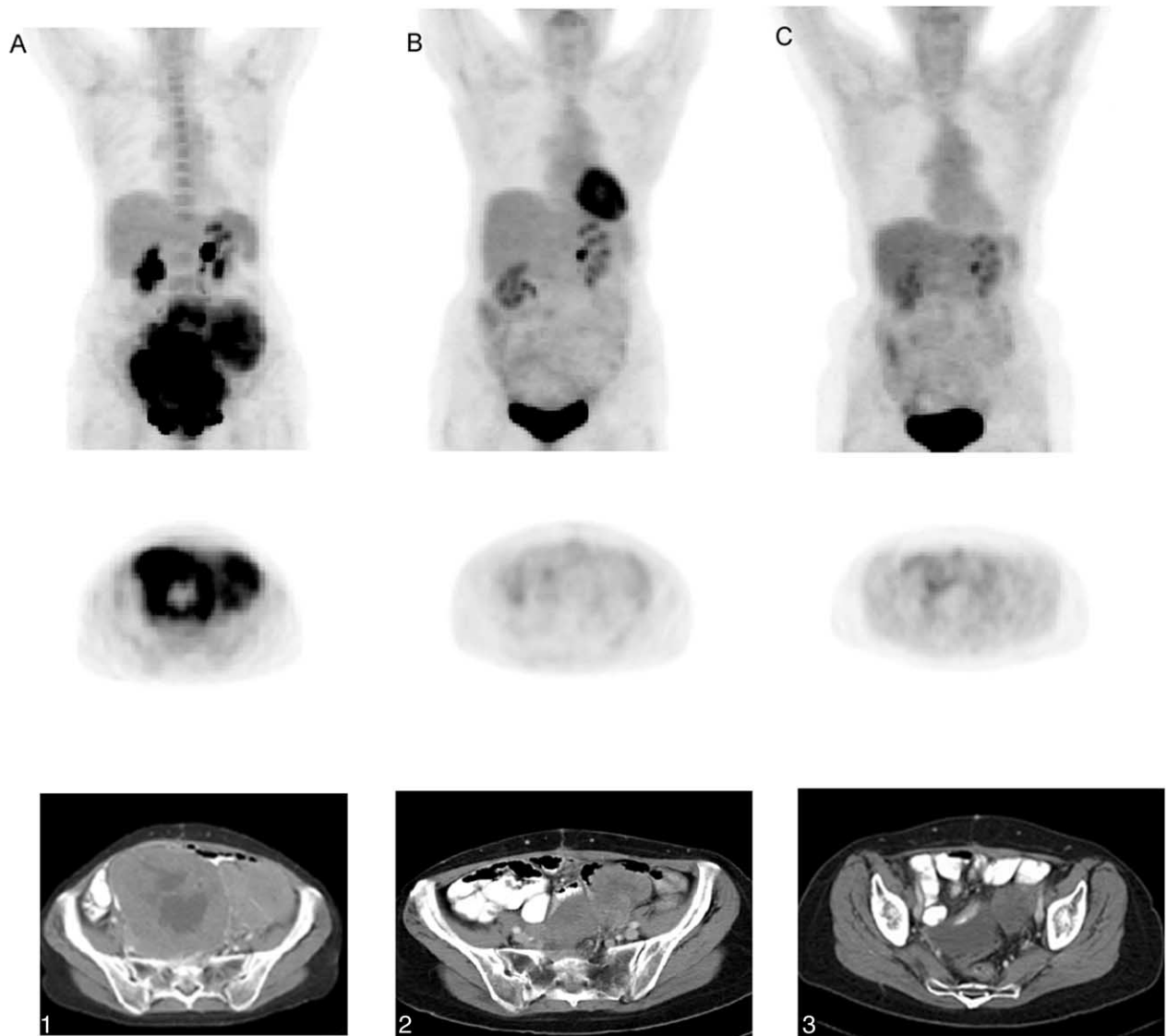


Fig. 10. Sequential [^{18}F]-fluorodeoxyglucose positron emission tomography (PET) scans in the same patient with gastrointestinal stromal cancer. (A) Before treatment; (B) 1 month after treatment began; and (C) after 16 months of continuous treatment. (1) Two-dimensional PET scan of the body; (2) axial PET scan through the site of the pelvic tumor; (3) correlating computed tomography from same level as (2). From Demitri et al. [77], with permission.

and accuracy of [^{18}F]-FDG PET for detecting tumors in the breast is around 90% overall. [^{18}F]-FDG PET has been shown to be a valuable tool for detecting and staging recurrent colon cancer in the form of metastases in the liver and elsewhere. In cases in which elevated levels of carcinoembryonic antigen suggested the presence of recurrent disease but the patients were asymptomatic, [^{18}F]-FDG PET has been used to confirm and localize disease recurrence with high specificity and sensitivity and to guide further therapy, including additional surgery [43].

PET images show the presence of tumors throughout the body, providing important information for the staging of disease that in turn determines the course of treatment for individual patients. This is particularly valuable for patients with cancers such as lymphoma in which there may be tumors at multiple sites and unpredictable locations [43].

Furthermore, [^{18}F]-FDG PET is proving useful in managing patients with cancer, because changes in glucose metabolism can be used to assess the effectiveness of chemotherapy or radiation therapy [43]. The successful treatment of cancer kills cancer cells, resulting in a decrease in the rate of [^{18}F]-FDG uptake. This measure of tumor response to therapy can be seen within the first few days, much earlier than a decrease in tumor size, typically seen by conventional anatomic imaging. In lymphoma patients, it has been possible to predict the tumor response from PET scans after only one or two cycles of chemotherapy [43], and in patients with glioma, responses to radiation and/or chemotherapy have been observed within 1 day of the start of treatment [43]. The dramatic response to treatment of gastrointestinal stromal cancer is shown in Fig. 10. However, at this time, it is not clear whether the best ultimate measure of successful treatment is tumor shrinkage or a cessation of [^{18}F]-FDG uptake in the region of the tumor. Nor is

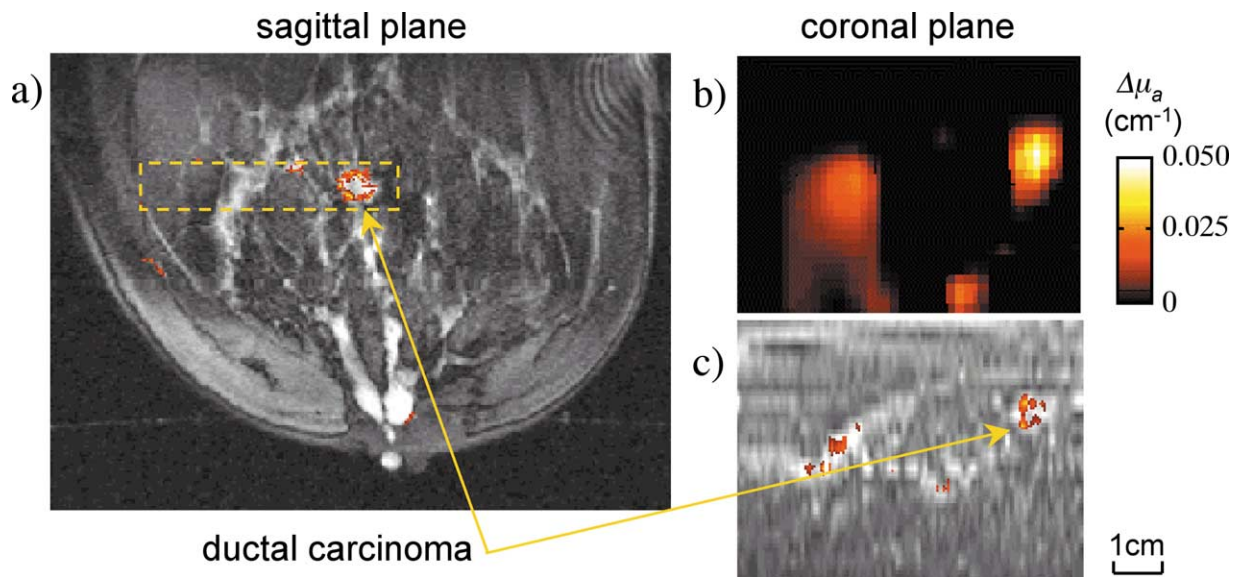


Fig. 11. Optical and magnetic resonance (MR) imaging of ductal carcinoma of the breast. (a) Functional sagittal MR image after Gd contrast enhancement, passing through the center of the cancerous lesion. (b) Coronal diffuse optical tomography image, perpendicular to the plane of the MR image in (a), for the volume of interest indicated on (a) within the interrupted line box. (c) Functional MR coronal reslicing of the volume of interest with the same dimensions as (b). From Ntziachristos et al. [33], with permission.

it clear whether a positive PET scan after treatment justifies further treatment in an otherwise asymptomatic patient. The answers to these questions will be resolved by further research.

The diagnostic capabilities of [^{18}F]-FDG PET are limited by a few factors. For example, false negatives can occur if the tumor is small (<1.5 cm). Hyperglycemia decreases the sensitivity of PET because the greater availability of glucose lowers the uptake of [^{18}F]-FDG. False positives can occur because of inflammatory disease such as tuberculosis. Furthermore, it should be noted that some cancers, such as lobular carcinoma of the breast, which accounts for 7% to 12% of all breast cancer, have lower metabolic activities than ductal carcinoma [44] and may result in false-negative PET scans. Therefore, a breast biopsy may be necessary even if a PET scan is negative because of the risk involved in delaying treatment [45].

Imaging Lymph Node Metastases MRI molecular imaging agents are now emerging as useful agents for the diagnosis of lymph node metastases. The agent Combidex (Ferrumoxtran 10, Advanced Magnetics Inc, Cambridge, MA), designed for this purpose, is a MION suspension made from iron oxide crystals 5 nm in diameter coated with dextran [25]. The particle size was designed to take advantage of the physiological behavior of macrophages, which pick up minute foreign particles and carry them to the lymph nodes. Thus, 24 hours after the intravenous injection of Combidex, the particles have collected in the lymph nodes, changing the way the nodes appear in MR images. Because lymph nodes that have been invaded by cancer cells do not accommodate macrophages, the normal lymph nodes can be distinguished from those that have been invaded by cancer. Clinical trials of Combidex have demonstrated it to be a highly sensitive method of detecting metastasis in breast cancer, prostate cancer [23], and head and neck

cancer [46]. This method of detecting metastasis will be a major advance over the current practice, the removal of sentinel lymph nodes for laboratory study.

Imaging Angiogenesis As cancer develops, growth is accompanied by angiogenesis, the development of new blood vessels within a tumor. Therefore, the introduction of a novel antiangiogenesis drug, Endostatin, was accompanied by much fanfare. However, even though Endostatin has had some remarkable success, it has also met with failure. Molecular imaging may provide an explanation for these inconsistent results. Agents that target molecules associated with angiogenesis could provide valuable images not only of the presence of growing tumors but also their responses to this and other antiangiogenic drugs. One suitable target for molecular imaging is the receptor endoglin, which is overexpressed in proliferating but not normal human vascular endothelium. Molecular imaging agents for endoglin have now been constructed in the form of a monoclonal antibody tagged with ^{111}In for SPECT imaging [47].

Another angiogenic target is E-selectin, a proinflammatory protein that is present in large amounts in proliferating endothelial cells. A molecular imaging agent for E-selectin has been developed for the MRI of angiogenesis, using a monoclonal antibody linked to an iron oxide-based CLIO particle [28].

The excessive vascularization of tumors has also been used in a clinical study to demonstrate the potential for optical imaging in detecting breast cancer with a nonspecific perfusion fluorochrome, indocyanine green. This proof-of-principle study demonstrated that near-infrared light penetrated the tissue sufficiently to highlight a region of fluorescence that corresponded to a lesion seen in MRI, demonstrating the presence of an 8 mm ductal carcinoma (Fig. 11) [33].



Fig. 12. Near infrared fluorescence imaging. A 2 mm tumor in the mammary fat pad of a mouse fluoresces brightly (right) after intravenous administration of a cathepsin B-selective protease probe. White light image (left) for anatomic correlation. From Weissleder et al. [30], with permission.

Imaging Abnormal Enzyme Activity Although the study described above demonstrates the feasibility of using optical imaging in breast tissue, the use of targeted NIRF imaging agents for the detection of abnormal enzyme activity in cancerous tumors will be far more valuable [32]. Several NIRF imaging agents (Table 5) have been developed that target certain proteolytic enzymes (proteases). These enzymes are present in abnormally high levels in tumors, even at early stages, presumably in adaptation to the rapid rate of cell division, the need to penetrate and invade tissue, and for angiogenesis.

Cathepsin B, a lysosomal protease involved in cellular protein turnover, is present in abnormally high levels in many tumors as well as in host cells associated with tumors. High levels of this enzyme correlate with aggressive tumor progression and with low patient survival [15]. Two-fold to 50-fold increases in a related enzyme, cathepsin D, have been reported in breast cancer, with high levels associated with higher metastatic potential [48].

A NIRF imaging agent that targets cathepsin B has been used in mouse experiments to detect the presence of lesions as small as 50 μm , identified by a two-fold to four-fold increase in fluorescence, when colon tissue was illuminated with infrared light. Many of these lesions were not seen by visual examination using normal white light [49]. The clinical use of such molecular image-enhancing tools could increase the sensitivity and specificity of detecting and evaluating colonic cancerous and precancerous lesions. Repeat colonoscopy studies have shown that as many 24% of adenomas may be missed, especially those <1 cm in size [50].

The same NIRF agent, injected into mice, has been used to demonstrate the presence of tumors as small as 1 mm in living mice (Fig. 12) through the use of fluorescence reflective imaging [30].

The abnormal expression of matrix metalloproteases (MMPs), enzymes that are secreted from cells and degrade the

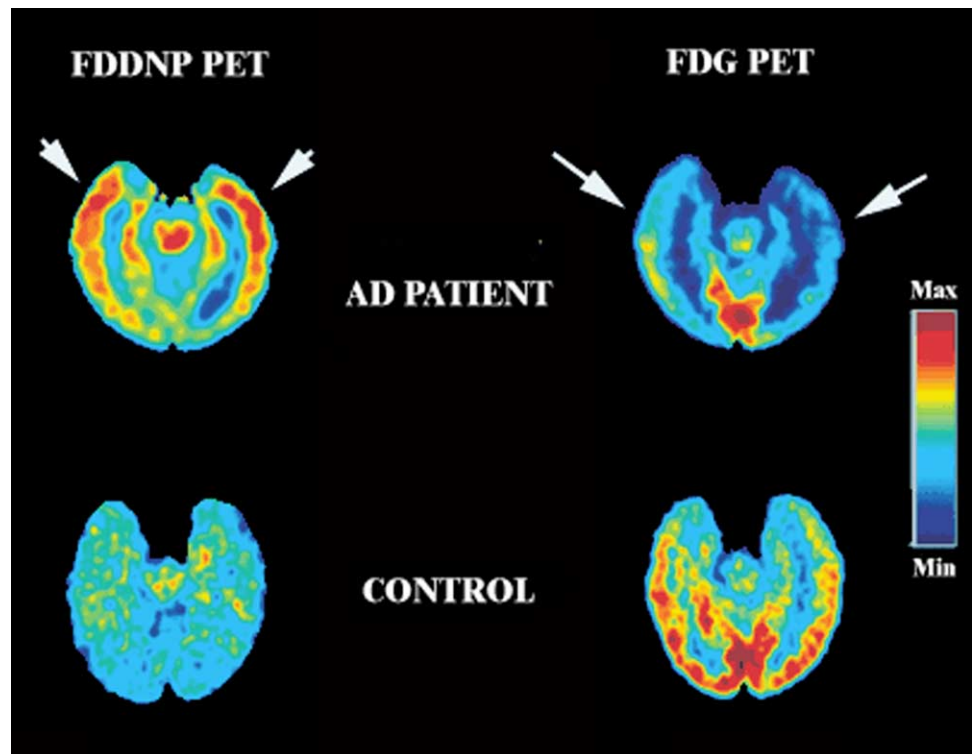
extracellular matrix, has also been correlated with tumor stage and metastasis, with high levels associated with poor disease outcomes. A number of drugs that inhibit MMPs have been developed for cancer therapy, some of which are in phase III clinical trials. A NIRF imaging agent that targets MMP-2 has been constructed, and although it has not yet been approved for human use by the US Food and Drug Administration, it has the potential of providing information about the effectiveness of these drugs in clinical trials [36]. In mice, these NIRF imaging agents have been used to demonstrate MMP-2 inhibition within hours of the start of treatment.

Monitoring Therapy Successful chemotherapy often induces cell death through the process of apoptosis, which is characterized by the activation of several enzymes, including the caspases, and the external appearance of a phospholipid that is normally found on the internal surface of cell membranes. Several molecular imaging agents are being developed that detect apoptosis as a means to test for the effectiveness of a particular treatment. For example, $^{99\text{m}}\text{Tc}$ -annexin has been used to target the cell membrane phospholipid, phosphatidyl serine, which becomes externalized as a cell dies. In a recent phase I clinical trial, the uptake of $^{99\text{m}}\text{Tc}$ -annexin was shown to increase in patients who responded to chemotherapy but not in those who progressed [51]. Another annexin-based imaging agent has been developed for the MRI of apoptosis [29], and a NIRF imaging agent detects apoptosis through the activity of a caspase enzyme [32].

Multidrug resistance is a major problem in cancer therapy. In many cases, resistance results from an increase in the expression of ATP-dependent efflux pumps belonging to a family of structurally similar transporters, the ATP-binding cassette (ABC) transporters [52]. These pumps expel a wide array of toxic substances, including many chemotherapeutics, such as paclitaxel, vinblastine, and doxorubicin from the interior of cells, thus rendering them harmless. At least 11 members of this family of ABC transporters are known to confer drug resistance. Increases in various members of the family have been correlated with drug resistance in acute myelogenous, chronic lymphocytic, and prolymphocytic leukemia, and there are reports of their probable role in drug resistant cases of breast cancer, ovarian cancer, and small-cell lung cancer [52]. The molecular imaging agent [$^{99\text{m}}\text{Tc}$]-sestamibi is recognized by some of these ABC transporters, which actively expel it from cells. Thus, SPECT images that show high levels of retained [$^{99\text{m}}\text{Tc}$]-sestamibi correlate with low activity of these proteins and predict that drug resistance is less likely to be problematic. Thus, [$^{99\text{m}}\text{Tc}$]-sestamibi promises to be a valuable tool in patient management to optimize chemotherapy selection [3].

Abnormal Chemistry of Tumors The detection of abnormal levels of biomolecules that result from cancer is one of the most promising uses of MR spectroscopy (MRS) of the brain. For example, single-voxel MRS may be a useful means of distinguishing the two most common brain tumors, gliomas and metastases, which appear very similar in other radiological images. In a small study of 31 patients, all but one of the high-grade gliomas showed a creatinine peak and an intense choline peak. In contrast, most metastatic tumors had no mea-

Fig. 13. Positron emission tomography (PET) imaging for Alzheimer's disease (AD). PET images comparing temporal lobe uptake of [^{18}F]-FDDNP, an amyloid-binding radiotracer, and fluorodeoxyglucose (FDG), a marker of glucose metabolism, in a patient with AD (left) and a control subject (right). There is increased uptake and retention of [^{18}F]-FDDNP (arrowheads) in temporal lobes of the patient with AD, compared with that in the control subject. The patient with AD still demonstrates typical findings of decreased temporal (arrows) and parietal (not shown) FDG uptake. From Shoghi-Jadid et al. [57], with permission.



surable creatinine. In the few metastases that did have measurable creatinine, the ratio of another marker, N-acetyl-aspartate, to creatinine could be used to distinguish between the two types of tumors with confidence [53].

Neurological and Psychiatric Disorders

PET has been found to be particularly valuable for assessing brain function because the rate of glucose metabolism increases significantly in regions of neural activity. Over the past 25 years, [^{18}F]-FDG PET has been used to study a number of degenerative and psychiatric disorders, as well as stroke and trauma [54]. MRS has been used to measure changes in neurochemicals due to degenerative disease [9], and many molecular probes have been made that can assess neurochemical changes due to disease. Thus, molecular imaging has provided considerable insight into the functional deficits in specific regions of the brain in Alzheimer's disease and other dementias, Parkinson's disease, Huntington's disease, schizophrenia, and other psychiatric disorders. With further development, molecular imaging will become a useful tool in the management of these diseases.

High-resolution [^{18}F]-FDG PET has proved useful in determining the focus of epileptic seizures because there is very high metabolic activity during seizures and decreased activity in the interictal period. However, the types of seizure, the time since the last seizure, and medication use can complicate the evaluation of PET scans. Nevertheless, in seizures caused by foci in the medial and frontal lobes, PET is superior to electroencephalography in accurately locating the epileptic foci [54]. Furthermore, PET has been successfully used to select the appropriate surgical resection in partial epilepsy that is refrac-

tory to medication and to identify patients who were previously not considered to be surgical candidates [55,56].

The extent of functional damage following a stroke, which primarily results in an acute loss of metabolism in a particular vascular region, can be visualized with PET right after the infarct and may precede and be more extensive than the findings of x-ray CT. Several studies have correlated functional recovery following a stroke with functional changes in PET scans [54]. Furthermore, the pattern of metabolic deficits correlates with the degree of recovery. For example, patients with aphasia following a stroke were more likely to recover if glucose metabolic activation during speech production tests occurred in the left cerebral cortex compared with those patients whose metabolic activation was restricted to the right. PET has also been used to monitor the success of various treatments, demonstrating that the early reperfusion of poorly perfused but viable tissue can lead to greater recovery.

Alzheimer's disease is notoriously difficult to diagnose, and there is no absolute diagnosis before autopsy. It is also very difficult to determine the rate of deterioration or the effectiveness of treatment; cognitive tests of memory and problem solving are too variable to be reliable. [^{18}F]-FDG PET has been used to detect deficits in metabolism in the temporoparietal region of the brains of Alzheimer's patients. More recently, molecular imaging agents that target the β -amyloid plaques have been developed. One of these, [^{18}F]-FDDNP, was found to accumulate to a greater extent in the temporal lobes of Alzheimer's patients than healthy control subjects, in a study of nine patients (Fig. 13) [57]. Another new molecular imaging agent that targets β -amyloid protein, a ^{11}C -labeled lipophilic thioflavin-T analogue known as PIB, is now in clinical trials in

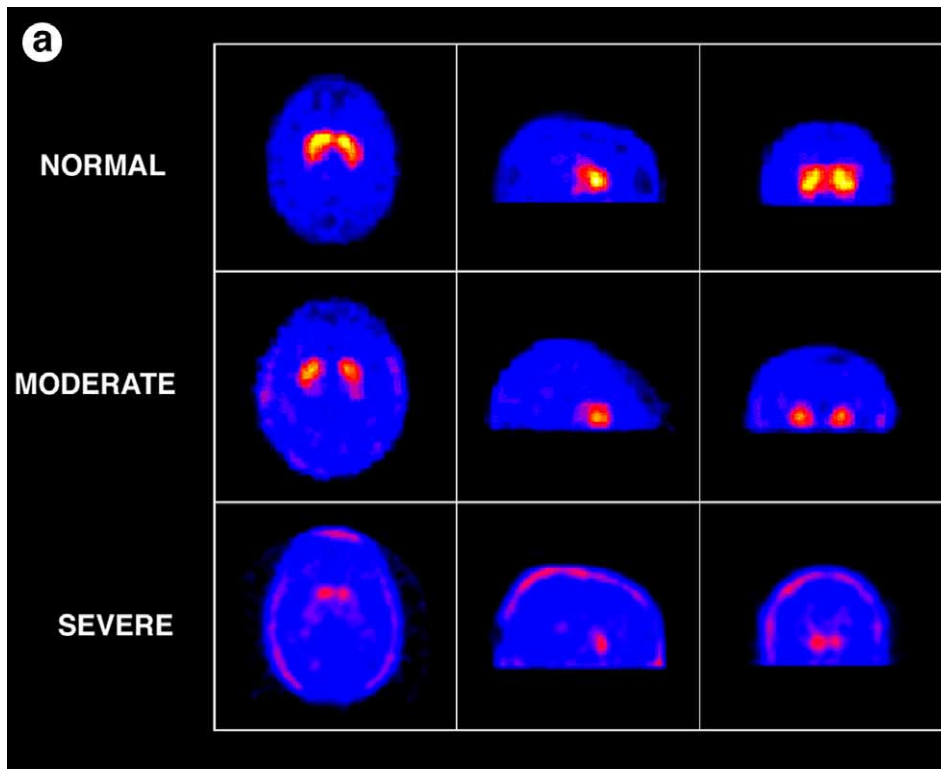
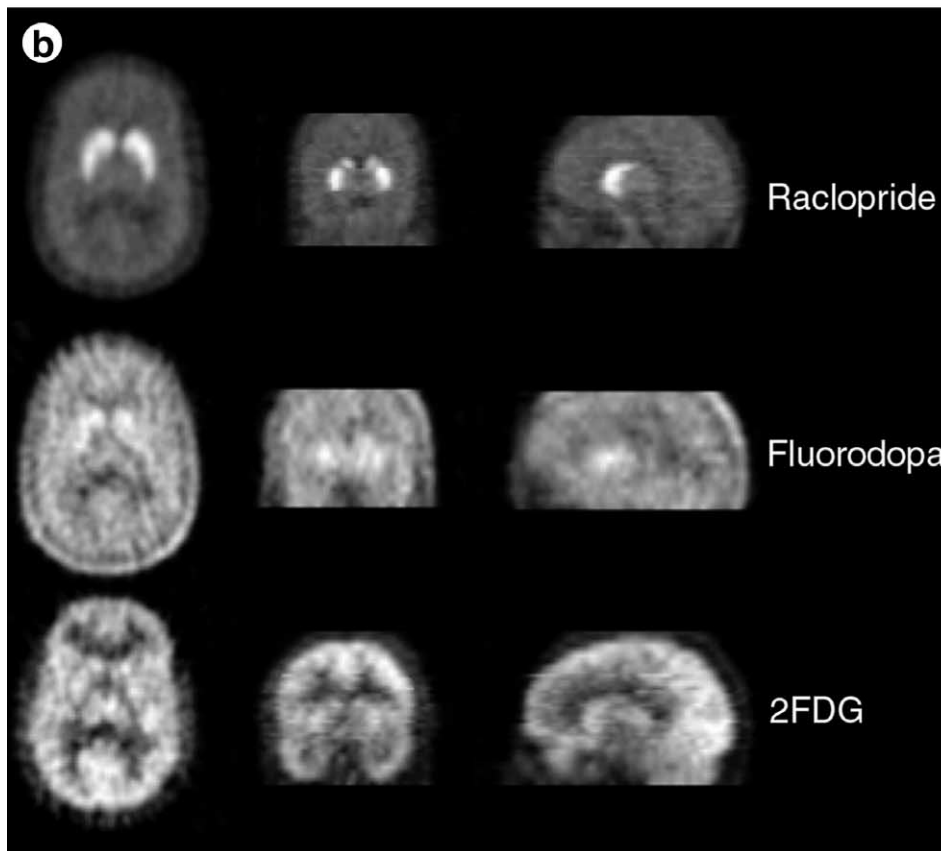


Fig. 14. Molecular imaging in Parkinson's disease. (a) Altopane binding to dopamine transporter density in subjects with moderate and severe symptoms of Parkinson's disease compared with normal subjects. (b) Positron emission tomography scans of [¹¹C]-raclopride binding to the dopamine receptor, [¹⁸F]-dopa uptake, and [¹⁸F]-fluorodeoxyglucose in a patient with moderate Parkinson's disease. In a normal subject, the intensity of [¹¹C]-raclopride binding and [¹⁸F]-dopa would be about equal. A. Fischman, personal communication.



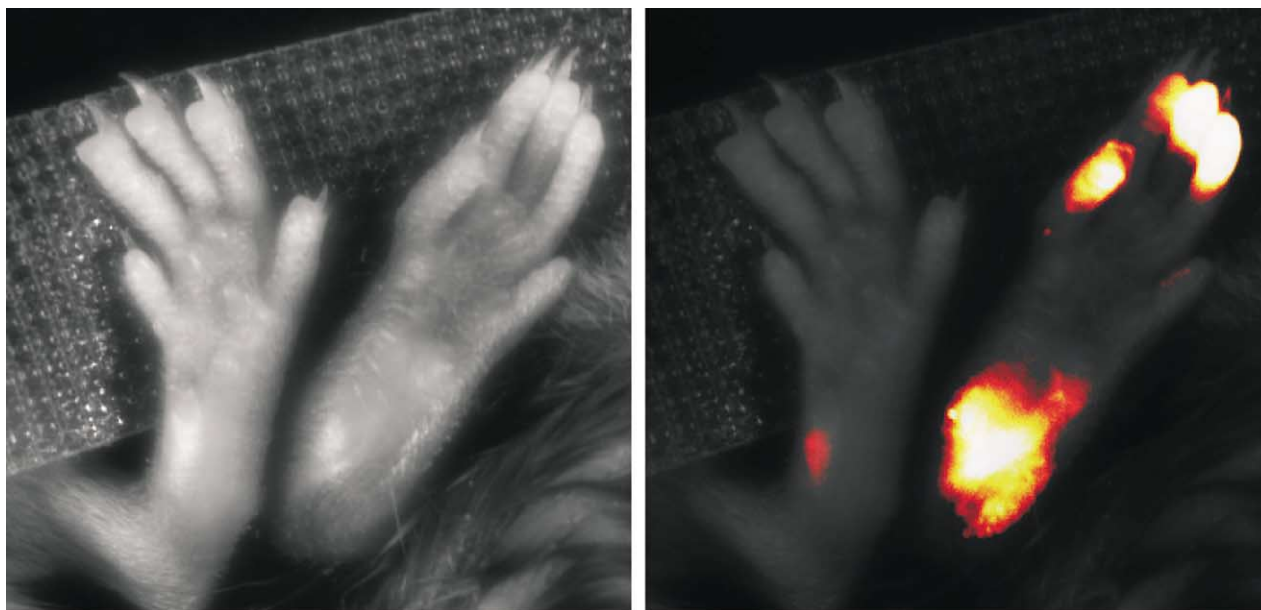


Fig. 15. Near-infrared fluorescence imaging of inflammation. Cathepsin B was used as a biomarker for the detection of inflammation in a rheumatoid arthritis model in the mouse. In each photo, the paw on the left is from a normal control mouse, and the one on the right from a mouse with arthritis. The photo on the left is a light image and on the right is a fluorescence reflective image. From Weissleder and Ntziachristos [32], with permission.

Sweden [58]. A similar agent that is labeled with ^{123}I for detection with SPECT has been also been developed [59]. Not only do these agents promise better diagnosis of Alzheimer's disease, but their usefulness in measuring change will aid the development of an effective drug to prevent progression of the disease.

A progressive loss of dopamine transporter density is a characteristic of Parkinson's disease that has been used as a target

for molecular imaging. [^{131}I]-Altoprane is a cocaine analog that has been demonstrated to have a high affinity and selectivity for the presynaptic dopamine transporter in the human striatum in clinical trials [60]. Another molecular imaging agent, [^{11}C]-raclopride, has been developed and measures the postsynaptic availability of dopamine [61]. These agents illustrate the value of molecular imaging in that distinctions can be made in the presynaptic and postsynaptic changes that occur with advancing disease (Fig. 14). Monitoring these changes is likely to be very valuable in studies of disease progression and in drug development.

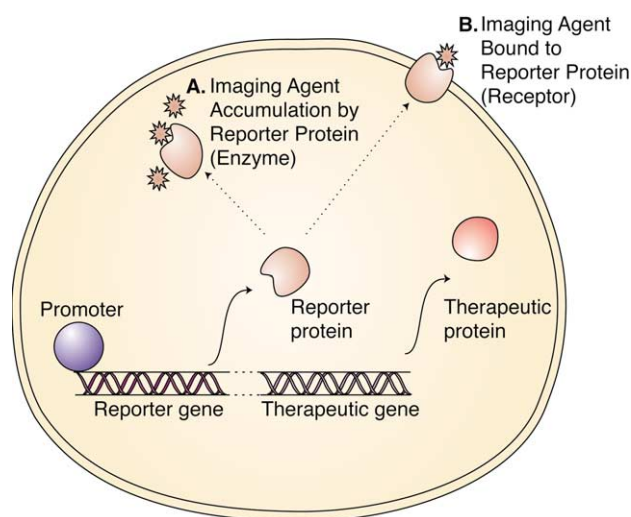


Fig. 16. Schematic of reporter gene concept. A reporter gene is linked to the gene of interest (endogenous gene), and its expression is controlled by the same promoter molecule. The reporter protein may be (A) an intracellular enzyme, such as a viral thymidine kinase, or (B) a cell surface receptor protein.

Cardiovascular Disease

PET has provided much valuable information about energy metabolism in the normal myocardium as well as the characteristic alterations in oxidative metabolism due to coronary artery disease and to infarction. The rate of uptake of [^{11}C]-palmitate, [^{11}C]-acetate, and [^{18}F]-FDG by the normal myocardium depends on several physiological factors, including the availability of glucose, regional perfusion, and oxygenation. For example, in the fasting state, glucose metabolism normally accounts for only 20% to 30% of the total cardiac oxygen consumption; the majority of energy is derived from fatty acid metabolism. These confounding factors make the interpretation of PET scans challenging. Nevertheless, regions of mild to moderate decrease in blood flow (determined by [^{15}N]-ammonia PET) due to ischemia correlate with a decrease in the uptake of [^{11}C]-palmitate and [^{11}C]-acetate and an increase in [^{18}F]-FDG uptake. These changes have been observed in cases of chronic coronary artery disease, even in the absence of clinical symptoms of ischemia, suggesting that they may be due to an adaptive process. In other patients, regional

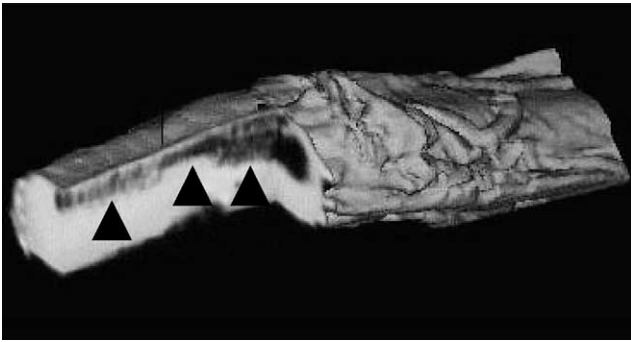


Fig. 17. Magnetic resonance (MR) image of magnetically labeled progenitor cells. Three-dimensional reconstructed *ex vivo* MR image (78 μ m resolution) of myelin-deficient rat spinal cord showing distribution of magnetically tagged oligodendrocyte progenitors 10 days after transplantation. Note the migration along the dorsal column (arrowheads) away from the injection site. From Bulte et al. [69], with permission.

contractile dysfunction is associated with a proportional loss of perfusion and decrease in uptake of [18 F]-FDG.

The relative patterns of perfusion and [18 F]-FDG uptake for a given segment of myocardium have important diagnostic implications and have also been shown to predict the outcome of surgical revascularization. In brief, scans of normal myocardium demonstrate uniform activity for both perfusion and metabolism. Ischemic but still viable and surgically salvageable myocardium demonstrates diminished perfusion, with elevated [18 F]-FDG uptake (discordantly abnormal). Nonviable myocardium shows diminished perfusion and the absence of [18 F]-FDG uptake (concordantly abnormal). These clear differences have led many to believe that the discordance of [18 F]-FDG uptake and blood flow is the gold standard for the determination of myocardial viability [62].

The rupture of vulnerable atherosclerotic plaque, the most frequent cause of acute heart attacks, has been strongly associated with vascular inflammation. Several enzymes are up-regulated (i.e., produced in greater quantity) in inflammatory tissue, including cathepsin B. As mentioned above, a NIRF imaging agent that targets cathepsin B has been developed. This imaging agent could be a useful tool for tomographic imaging as well as the catheter-based screening and profiling of atherosclerotic plaque, providing useful information to guide the preventative treatment of individual patients [37].

Thrombosis is a central pathophysiological feature of a number of life-threatening cardiovascular diseases, including myocardial infarction and pulmonary embolism. Current diagnostic imaging methods measure restrictions in flow and do not distinguish between thrombosis and other obstructions to flow. An activatable NIRF imaging agent has recently been developed for the detection of thrombin, a serine protease that cleaves fibrinogen to form fibrin. The use of this imaging agent could allow the direct diagnosis of thrombosis [63].

Infection and Inflammation

Inflammation and infection are very closely related processes in that infection typically results in an inflammatory response.

Currently available molecular imaging agents for inflammation and infection include [18 F]-FDG PET, because inflammatory sites are typically associated with an increased metabolic rate. However, [18 F]-FDG is not specific; increased [18 F]-FDG uptake can also be indicative of a tumor. Nevertheless, the value of [18 F]-FDG PET for detecting the location of inflammation has been demonstrated in several patient studies with a wide variety of infections. Other imaging agents include chemotactic peptides, such as formyl-Met-Leu-Phe-Lys, which are characteristically produced by bacteria and are bound by high-affinity receptors on granulocytes. SPECT imaging of a 99m Tc derivative of this peptide has been shown to correlate well with granulocyte density in an animal model of pancreatitis [64]. Activatable NIRF molecular imaging agents also show promise for imaging inflammation. For example, an agent that targets the enzyme cathepsin B has been successfully used to demonstrate inflammation in a mouse model of rheumatoid arthritis (Fig. 15) [32].

There are times when infection is present without inflammation (e.g., in immunocompromised patients) and there are times when inflammation is present, but it is not possible to know for certain if the cause is infection (e.g., when antibiotic use prevents the culture of pathogens from a blood sample). Locating the site of infection and/or inflammation and distinguishing between the two is critical for patient management and the selection of the appropriate therapy. Yet the reagents in current use for imaging infection actually target the phenomena of the inflammatory response. Developments in protein and peptide chemistry should lead to molecular imaging agents specific to infection, which will become radiological tools for precise diagnoses [65,66].

Gene Therapy

Imaging gene expression will be an enormous benefit to gene therapy, which has been much heralded for the treatment of many debilitating and fatal diseases. In the case of inherited diseases in which a specific gene is missing or nonfunctional, gene therapy has the potential of correcting the cause of disease rather than treating the symptoms. However, gene therapy to correct inherited disease has not lived up to expectations to date, with the exception of a recent success* in the treatment of severe immunodeficiency disease in infants and some success in gene therapy for the treatment of cancer.

A number of strategies have been devised to improve gene delivery to the target tissue. Molecular imaging is a tool that can demonstrate the effectiveness of these strategies, and new molecular imaging agents for this purpose are now being developed. For example, 111 In labeling of a herpes simplex vector has been used to determine the amount of the vector present after treatment and use this to compare the effectiveness of different methods of gene delivery in an animal model of human disease [5]. This kind of study will be critical to advancing gene therapy in clinics.

In addition to knowing that a gene has reached its target, it

*Although the required gene was successfully introduced, the treatment has been withdrawn because of the development of cancer associated with the treatment.

is also necessary to find out whether it is active there. The addition of a reporter gene, whose product can be targeted with a molecular imaging agent, is often used for this purpose (Fig. 16). Both the detectable reporter gene and the therapeutic gene are included in the same gene therapy vector, and both are "switched on" (expressed or translated) by the same promoter. In other cases, the therapeutic gene may be detectable, obviating the need for a reporter gene. For example, the gene for herpes simplex virus 1 enzyme, thymidine kinase, used in gene therapy for cancer, is therapeutic because it will convert pro-drugs such as ganciclovir into cytotoxic compounds within tumor cells. Thymidine kinase also acts as a reporter because trace amounts of ^{18}F -labeled analogs of these pro-drugs can be used as molecular imaging agents [67]. If viral thymidine kinase is produced by the cells, the prodrugs are phosphorylated, trapping and accumulating the label within the cell. A PET scan can then image the distribution of the viral thymidine kinase and thereby the effectiveness of the gene therapy. Many other molecular imaging agents are under development to assess gene expression using MR, optical, and nuclear imaging [18,32,68].

Stem Cell Therapy

Another much publicized potential treatment of the future, stem cell therapy, will greatly benefit from molecular imaging. Before this form of treatment becomes reality, it will be necessary to optimize the delivery of these cells and to demonstrate that the cells are growing in the desired site and developing properly. Molecular imaging will be helpful for this. For example, a molecular imaging agent designed to be engulfed by cells has been made by attaching the Tat peptide, normally found in HIV, to a CLIO nanoparticle to form CLIO-Tat. The Tat peptide facilitates the internalization of the particles into progenitor cells, which were shown to accumulate up to 30 ng of iron per thousand cells, with no toxicity [27]. When about 1000 magnetically labeled cells were injected into mouse brains as an antitumor therapy, they were clearly seen in MR images [27]. Similarly, images of magnetically labeled oligodendrocyte progenitor cells have been made after their injection into myelin-deficient rat spinal cord (Fig. 17) [69].

Similar Tat peptide-containing molecular imaging probes labeled with $^{99\text{m}}\text{Tc}$ or a fluorescent moiety have been made. The dual labeling enables the direct comparison of quantitative radiometric and qualitative fluorescent data. These probes were internalized into human Jurkat cells and injected into mice, where they were visualized by whole-body imaging [70].

CONCLUSION

From the examples described in this article, it is clear that molecular imaging will make radiologists active and vital participants in the new era of molecular medicine. Increasingly, molecular imaging will be used to diagnose disease and will do so both in symptomatic patients and before symptoms appear. It will be available to monitor treatment immediately after it has been initiated and to guide changes in treatment to suit individual patients' needs [71].

Molecular imaging will also have a very important role to play in drug development. Molecular imaging agents can be

used to assess the response to drugs in a matter of hours in the cases of some cancers. In other diseases, molecular imaging will provide a valuable, objective method of assessing symptoms that will be invaluable in determining the progression of neurodegenerative and psychological disease, for which the present cognitive tests are subject to performance variation.

Although molecular imaging depends on knowledge of molecular biology, it is also an invaluable tool for advancing knowledge. It is used extensively in research in animals, such as mice, that are used as models of human disease [20]. Molecular imaging has been used to track gene and cell therapies, to monitor gene expression [18], and to track processes such as angiogenesis in tumors [47]. New imaging agents and imaging techniques have been developed for imaging in mouse experiments, and some of these may be suitable only for advancing knowledge. Other molecular imaging agents and imaging modalities that are in use today in the laboratory will be developed for clinics. Thus, not only will there be a wide range of novel imaging agents, whole new approaches to imaging will become available to radiologists over the next few years. Together, these advances will have a significant impact on both diagnosis and treatment and will expand the frontiers of the specialty of radiology enormously.

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