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Review Article

The imaging continuum: bench to biomarkers to diagnostics†

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Abstract: Innovation in basic and applied science has brought radiotracers to fruition as diagnostics. Non-invasive, longitudinal, and quantifiable molecular imaging is the key to diagnosing and monitoring numerous illnesses, with more to come from characterization of the clinical relevance of findings from genomics research. Radiotracers enable real-time *in vivo* studies of the effects of drug candidates on receptors, pathways, pharmacodynamics, and clinically relevant endpoints, thereby providing both early detection of pathophysiology to enable early intervention, and then monitoring of treatment responses to enable individualization of treatment regimens. We review developments which have translated imaging from 'bench to bedside', or 'biomarkers to diagnostics'. Notable developments include (1) synthesis methods for rapid ^{11}C labeling of biomolecules to high specific radioactivity; (2) ligand-binding assays for screening molecular imaging agents rather than drugs; (3) *in vivo* imaging of radiotracers in animals; (4) discovering the imaging advantages of ^{99m}Tc, ^{11}C, and ^{18}F; (5) co-registration and automated quantitative assessment of high spatial resolution CT and MR images with molecular images from PET for longitudinal studies of treatment effect.

Keywords: positron emission tomography; biomarkers; radiodiagnostics; radiopharmaceuticals; radiotracers

Introduction

Only in the last decade have radiotracers come fully to fruition as diagnostic imaging agents. Ventilation–perfusion scans and hydroxy iminodiacetic acid (HIDA) scans were early wins for 'translational medicine', taking basic science tools into medical research as 'biomarkers' and then clinical practice as diagnostics with clear implications for individualized therapeutic intervention at early stages of disease. Since then there have been many ^{99m}Tc-based and other single photon emission computed tomography (SPECT) tracers introduced into clinical practice, enabling the expansion of measurements from anatomic to physiologic parameters. But in order for the pioneering work on [^{18}F]FDG positron emission tomography (PET) in glucose metabolism, which began at the end of the second World War, to directly contribute to patients' health required the proverbial 'Act of Congress.' In the Food and Drug Administration (FDA) Modernization Act of 1997, Congress required the FDA to establish GMP manufacturing guidelines and an approval process for PET commercial diagnostic imaging agents.

This watershed event opened the door to clinical scientific information on targets, and pathways in drug development to be quantified by PET biomarkers.[1] PET biomarkers dramatically enhanced applied research for detecting pathophysiology and assessing mechanisms of action and clinical benefits of experimental treatments. Conversely, enabling real-time studies of receptors and pathways, especially in the living human brain and tumors, enhanced development and validation of pre-clinical *in vivo* and *in vitro* models of human disease using a range of imaging modalities. Molecular imaging is particularly well suited to these applications. It is non-invasive, and therefore can be used in longitudinal *in vivo* studies in similar disease models in

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animals and humans. Furthermore, 'tracer' doses using 'organic' isotopes can quantitatively assess pathophysiology with minimal perturbation of systems being studied.

It took four decades for ^{99m}Tc and three for ^{18}F to progress from basic research to clinical applications. A 'best practices' review of how we got here can reduce the time required for the next generation of tracers to improve patient outcomes by means of early detection and hence early intervention in pathophysiology, followed by individualization of therapy by imaging-based monitoring of treatment benefit. Investments in radiochemistry and imaging research tools for animal and clinical models of disease have enabled studies of novel therapeutics for previously untreated neurodegenerative and cardiovascular diseases and cancer. These studies have confirmed pathophysiology and mechanisms of action such as accumulation of beta-amyloid in the brain and vulnerable plaque in the vessels, and angiogenesis, migration, proliferation, and apoptosis in tumors. These breakthroughs have required innovation in business decision-making for portfolio management and regulatory review for product approval, including the co-development of therapeutics and diagnostics.

Information technology has also contributed innovations, synthesizing information across platforms and species. Information technology enables co-registration of high spatial resolution images (CT, MR) with others of high molecular information content (PET), and unbiased, automated, and quantitative algorithmic assessment of resulting images. Linking imaging data across disparate platforms, including gene expression profiling, will improve diagnoses and initial choices of therapy, and individualize treatment regimens via monitoring benefits after therapy has begun; that is to say, converting information to insight.

These new diagnostic tools can optimize genomics as a risk assessment tool via individualized screening programs. Mammography in BRCA1/2 positive women is an example. When early detection in high-risk patients is followed by early intervention (rather than prophylactic removal of healthy tissue) and then monitoring of breast cancer treatment effects, regimens can be tailored for individual patients to gain maximum treatment benefit with minimum risk of adverse events. This yields better outcomes and greater efficiency in the delivery of health care.

^{11}Carbon in molecular imaging: future perspectives

Claude Bernard, the great French physiologist (1813–1878) said,[2] 'Un jour nous saurons la physilogie lorsque nous pourrons suivre pas à pas une molecule de carbone ou d'azote, faire son histoire, raconteur son voyage dans le corps d'un chien.' ('One day we will understand physiology when we can follow a molecule of carbon or nitrogen step by step, to tell its story and travels in the body of a dog.') By enabling us to view life processes using molecules labeled with positron-emitting radionuclides ^{11}C, ^{13}N, and ^{15}O, PET has brought us a giant step closer to Bernard's vision. In particular, ^{11}C has been of central importance to PET clinical molecular imaging over the last almost 30 years.

One of the major advantages with ^{11}C labeling is the vast literature in carbon chemistry, which significantly increases synthesis opportunities relative to other PET radionuclides. Among these opportunities is differential or position labeling with ^{11}C for elucidating *in vivo* biochemistry. As a key element of life, carbon is of special interest for labeling compounds endogenous in man as well as other naturally occurring biomolecules. Although ^{18}F-labeled analogs of many endogenous compounds are available and clinically useful as diagnostic imaging tools, they have different biochemical properties and kinetics *in vivo* compared with endogenous compounds labeled with ^{11}C. For example, L-[^{11}C]dopa and L-6-[^{18}F]fluoro-L-dopa have different *in vivo* decarboxylation rates as determined by comparing L-[^{11}C]dopa labeled in the carboxylic and β positions.[3]

Examples of endogenous compounds used as ^{11}C-labeled tracers are shown in Table 1.

There are several methods for ^{11}C production. The most common uses a nitrogen target and the ^{14}N(p,α)^{11}C nuclear reaction. The target products are [^{11}C]carbon dioxide or [^{11}C]methane, obtained in high specific radioactivity. Both of these one-carbon units are useful starting materials for the labeling synthesis of complex organic compounds. At present, [^{11}C]carbon dioxide is the dominant target-produced precursor. It is also very useful to incorporate ^{11}C into one-carbon synthetic intermediates for use late in a preparation sequence. These synthetic intermediates greatly increase flexibility in planning synthesis strategy. Scheme 1 presents some of the one-carbon reactive intermediates used so far.

Of the ^{11}C tracers found in the literature today, many were synthesized using labeled methyl reactive intermediates, including the majority of specific receptor ligands and enzyme substrates. In recent years other labeling strategies have been explored. Of these, [^{11}C]carbon monoxide-based strategies have been the most important because they make possible labeling of many functional groups. ^{11}C also figures importantly in PET microdosing clinical studies, which may reduce drug development costs. In drug development, ^{11}C-labeled one-carbon compounds allow formation of

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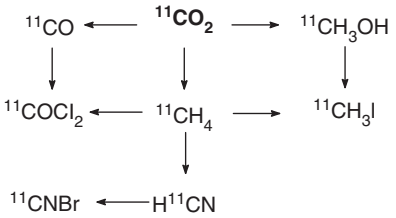
Table 1 Endogenous compounds labeled with ^{11}C

Fatty acids	Amino acids	Peptides	Miscellaneous
Acetic acid	Methionine	Enkephalin	Thymidine
Palmitic acid	Alanine	Met-enkephalin	Prostaglandine E2
Arachidonic acid	Dopa	Substance -P	Acetylcarnitine
Octanoic acid	Tyrosine		Carnitine
	Phenylalanine		AcetylCoA
	5-Hydroxytryptophan		Glucose
	Tryptophan		Lactic acid
	Valine		Pyruvate
	Ornithine		Choline
	Glutamic acid		Acetylcholine
	Aspartic acid		Dopamine



Scheme 1

small subsets of labeled tracers for specific targets. This is important for creating tracer libraries. Position labeling, PET microdosing, and tracer libraries with ^{11}C are discussed later in this section.

Time and concentration in ^{11}C-labeling chemistry

In syntheses using short-lived radionuclides, time is more important than in conventional synthetic work.[4–6] The ^{11}C decay curve and the yield curves of Figure 1 indicate the importance of optimizing synthesis time in relation to labeled product formation. Optimizing time when developing methods to produce radioactive products thus adds another strategic consideration to those of chemical yield and purity.

When analyzing kinetic equations to include the decay correction, it is clear that the concentration of the non-labeled reactants and eventual catalysts can be used to optimize radiochemical yield. In labeling chemistry it is thus important to remember that the ^{11}C-labeled precursors are produced in very low amounts. Most labeling syntheses formally follow second-order kinetics. However, considering the very low concentration of the labeled reactant, a pseudo first-order kinetics is obtained and increasing the amount of the non-labeled reactant can significantly speed up product formation. The low mass of the product also explains why this technique has unique properties with regard to sensitivity (see PET microdosing).

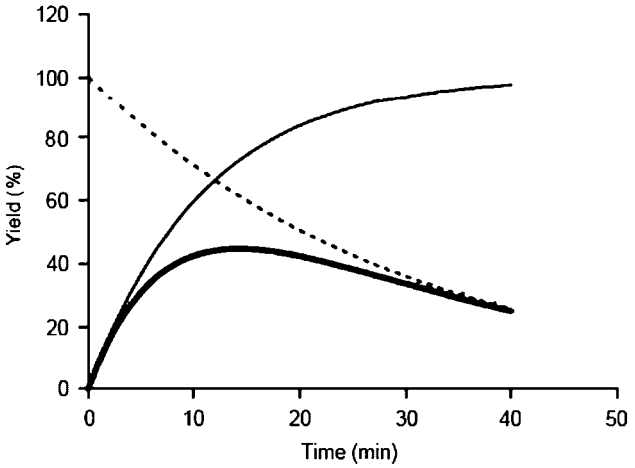


Figure 1 Decay curve for ^{11}C (dotted line), chemical yield in a hypothetical chemical reaction involving only stable nuclides (solid line), and decay-corrected radiochemical yield using the same reaction but including ^{11}C (bold line).

Production of ^{11}C and importance of specific radioactivity

Particle accelerators produce all positron-emitting radionuclides of interest for labeling and PET studies except ^{68}Ga, which is generator-produced. To use PET successfully, it is important to obtain these radionuclides in very high specific radioactivity. High specific radioactivity allows the tracer criteria formulated by De Hevesy[7] nearly 100 years ago to be fulfilled (i.e. the tracer itself has no measurable effect on the biological process being studied) and partly accounts for the successful implementation of PET in biomedical and clinical research. Especially in receptor–ligand interaction studies it is important to minimize the amount of tracer in order to quantify the number of free receptors (i.e. binding potential) following dosing with a drug. If the tracer occupies less than 5% of the available receptors the tracer concept is fulfilled. This

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implies that, depending on the regional concentration of the receptor or enzyme to be quantified, tracers require different specific radioactivities. PET studies using highly potent tracer compounds such as the opioid agonist [^{11}C]carfentanil,[8] is an example of requiring high specific radioactivity in a tracer, in this case to avoid unwanted pharmacologic effects of the tracer itself. Isotopic dilution with the stable element is thus of major concern.

The search for high specific radioactivity has been pronounced in ^{11}C chemistry due to omnipresent stable ^{12}C. The purest nitrogen/oxygen gas mixture used for the production of ^{11}C still contains enough carbon to contribute substantial carbon mass and reduce specific radioactivity by a factor of about 100 from the theoretical value 3.4×10^5 GBq/μmol. Most secondary reactive intermediates are obtained from target-produced [^{11}C]carbon dioxide and these reagents, too, are potential sources of stable carbon dioxide for isotopic dilution. Currently, much work is devoted to reducing isotopic dilution from reagents by designing reaction sequences that avoid using [^{11}C]carbon dioxide. Significant improvements have been made. ^{11}C-labeled cyanide and carbon monoxide are examples of secondary precursors that can be obtained with high specific radioactivity from [^{11}C]carbon dioxide without reagents contaminated with stable carbon isotopes. The same applies for [^{11}C]methyl iodide obtained via the gas-phase method, preferably from target-produced [^{11}C]methane.[9] Today, methylation reactions with [^{11}C]methyl iodide or [^{11}C]methyl triflate can deliver products with specific radioactivity in the range of 200–350 GBq/μmol. [^{11}C]carbon monoxide with specific radioactivity up to 1300 GBq/μmol has been reported.[10]

^{11}C-reactive intermediates

Tracer development was in the beginning of the PET era hampered by the limited number of methods available for labeling synthesis. [^{11}C]carbon dioxide could either be used directly in Grignard reactions or transformed by on-line processes to [^{11}C]cyanide, giving access to carboxylic acids, alcohols, and primary amines.[11] In the mid-1970s, [^{11}C]methyl iodide was introduced as a labeling reactive intermediate.[12,13] Further innovations added [^{11}C]phosgene and [^{11}C]formaldehyde as reactive intermediates.[14,15] [^{11}C]Methyl iodide, however, has had the largest impact on PET. During the 1980s [^{11}C]methyl iodide was used to label many receptor ligands for PET studies. Labeling was mainly by methylation on nitrogen and oxygen nucleophiles followed by, to a lesser extent, reactions with carbon, sulfur, and phosphor anions. Development of labeled reactive intermediates has driven improvements in labeling chemistry. Figure 2 presents examples of labeled reactive intermediates based on [^{11}C]methyl iodide.

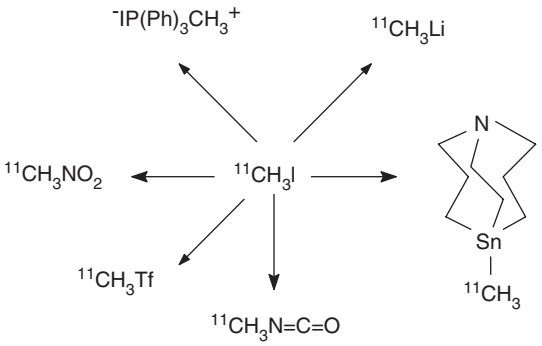

Figure 2 Examples of reactive intermediates obtained from [^{11}C]methyl iodide.

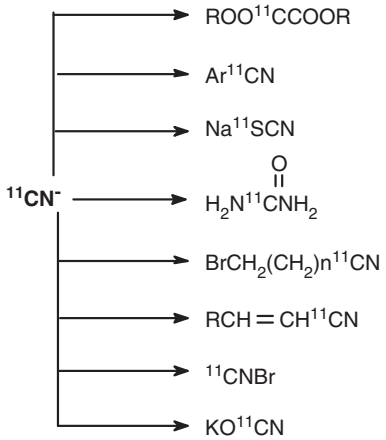

Figure 3 Examples of reactive intermediates obtained from [^{11}C]cyanide.

Many other useful compounds with various functional groups can be obtained from [^{11}C]cyanide, as shown in Figure 3.

[^{11}C]carbon monoxide has a labeling potential that is far from being fully exploited. Carbon monoxide gas has very high vapor pressure and low solubility in most organic solvents. When technology of controlling small amounts of [^{11}C]carbon monoxide was introduced, a new era of ^{11}C-labeling opened and the number of ^{11}C-functional groups that may quickly be produced and the number of new chemical intermediates based on ^{11}CO significantly increased. Figure 4 shows several examples.[16]

Today, numerous ^{11}C-reactive intermediates are available for a multitude of different reaction sequences. Synthetic opportunities with ^{11}C significantly

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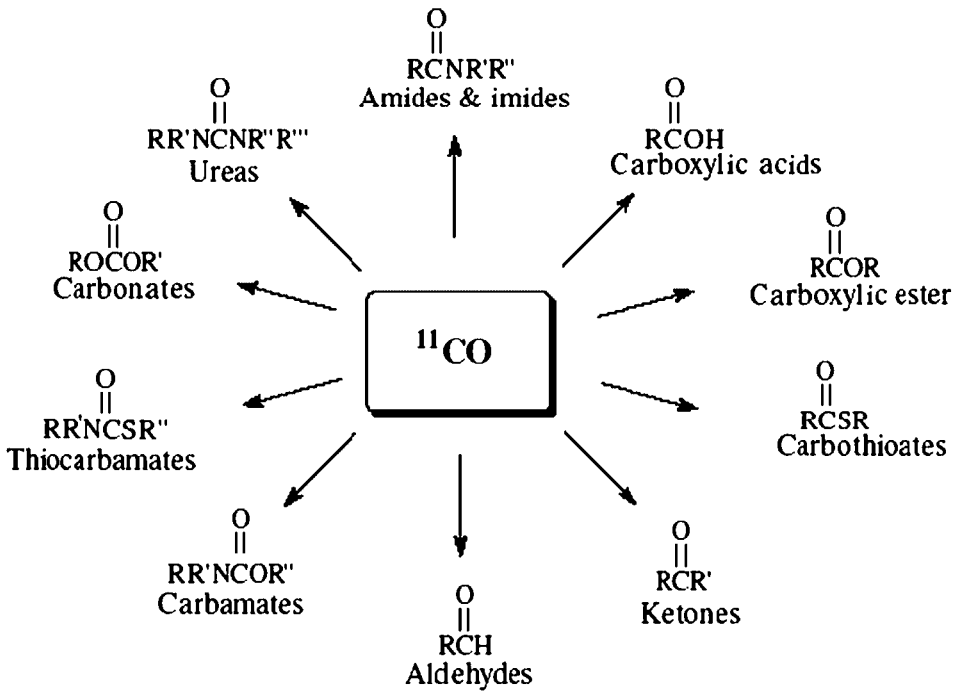

Figure 4 Examples of functional groups that can be produced using [^{11}C]carbon monoxide as a reactive intermediate.

outnumber those of any other positron-emitting radionuclide. From a synthesis perspective, ^{11}C is clearly the most important PET radionuclide.

Methods for synthesis of ^{11}C-compounds

[^{11}C]Methyl iodide has been the workhorse for labeling ^{11}C-compounds for 25 years and still accounts for the main labeling reactions. Figure 5 shows several methylation reactions with different nucleophiles that produced PET tracers important in the development of PET as a molecular imaging modality. R-[^{11}C]PK11195, [^{11}C]nicotine, and S-[^{11}C]Raclopride were developed for studying peripheral benzodiazepine, nicotinic acetylcholine and dopamine D_2 receptors, respectively.[17–19] L-[^{11}C]methionine, one of the first labeling syntheses published using [^{11}C]methyl iodide, has been used for 20 years to study brain tumors. [^{11}C]Methylspiperone[20] was the first ligand for visualizing and studying dopamine D_2 receptors *in vivo*. Although [^{11}C]methylspiperone was later replaced by the more dopamine D_2 receptor-selective [^{11}C]Raclopride, it is still a useful PET tracer for studying 5-HT_{2A} receptors.[21]

The formation of carbon–carbon bonds is naturally a key issue in ^{11}C-labeling. Nucleophilic substitution reactions with [^{11}C]cyanide and Grignard reactions with [^{11}C]carbon dioxide were the first successful attempts, followed by [^{11}C]methyl iodide alkylations on carbanions, to produce amino acids. The search for other C–C formations that could be accomplished within the half-life time frame of ^{11}C took advantage of developments in metallo-organic chemistry. The Stille and Suzuki couplings attracted special attention. Scheme 2 shows examples of transition metal-mediated reactions.[22] The ketone synthesis in Scheme 2 points to a very important approach in molecular imaging: labeling the same molecule in different positions.[23]

Endogenous tracers and position labeling

In tracers labeled for *in vitro* studies (e.g. frozen tissue autoradiography), the position of the label is not critical because tracer metabolism is not expected. In contrast, tracer metabolism does occur in PET studies and therefore the position of the label in the tracer is important and may be critical for interpreting PET data. A labeling position should be chosen that avoids labeling tracer metabolites that confound PET images with background radioactivity not associated with the biological process under study. To achieve a good image contrast, the labeled compound should be metabolically stable during PET investigation, or, alternately, the label should be positioned so that labeled metabolites are hydrophilic and quickly eliminated.

Dopamine and serotonin are two important neurotransmitters in the human brain. Neither penetrates the blood–brain barrier (BBB). Thus, studying functional aspects of dopaminergic and serotonergic neurons

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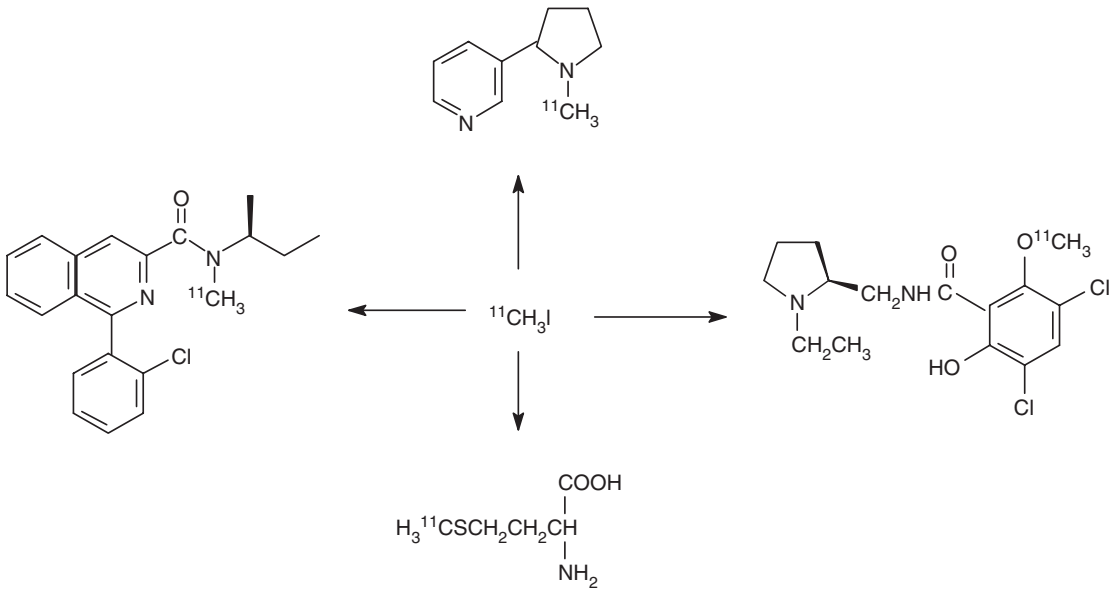
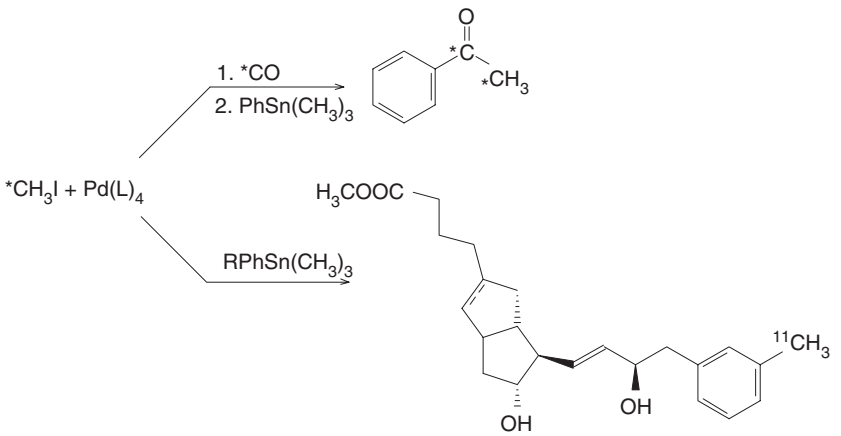


Figure 5 Examples of reactions using [^{11}C]methyl iodide on different nucleophiles.



Scheme 2

requires labeling of other molecules, such as the endogenous precursors for dopamine and serotonin, L-dopa and 5-hydroxy-L-tryptophan (HTP), respectively, which do pass through the BBB. L-[^{11}C]dopa and [^{11}C]HTP provide tools to study neurological disorders related to deficiencies in dopaminergic and serotonergic neuronal activity. L-dopa is synthesized from tyrosine, an aromatic amino acid. Tryptophan, from which HTP derives, is also an aromatic amino acid. Scheme 3 presents multi-step chemo-enzymatic syntheses of ^{11}C-labeled aromatic amino acids HTP at the top and L-dopa at the bottom.[24–26]

As presented in Scheme 3 the common synthon, alanine, labeled either in the carboxylic or 3-position, was synthesized from [^{11}C]cyanide and [^{11}C]methyl iodide, respectively. The aromatic amino acids were then obtained from labeled alanine via pyruvate by multi-enzymatic procedures, and thus aromatic amino acids can be labeled in either the carboxylic or β position. Position labeling has been used to study *in vivo* decarboxylation of HTP and L-dopa to the corresponding neurotransmitters, serotonin, and dopamine. Figure 6 shows the rate of dopamine synthesis in the brain of the same rhesus monkey within 3 h, as investigated using L-[β-^{11}C]dopa and L-[carboxy-^{11}C]-dopa. Figure 6(a), the upper image, shows ^{11}C concentrated in the striatum, presumably as [^{11}C]dopamine. The lower image of Figure 6(b) is different because the carboxylic label ends up as [^{11}C]carbon dioxide. The assumed action of the aromatic

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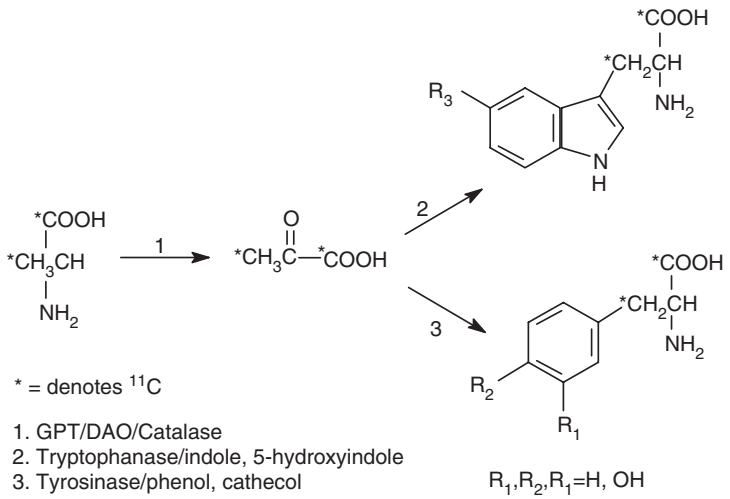


Scheme 3

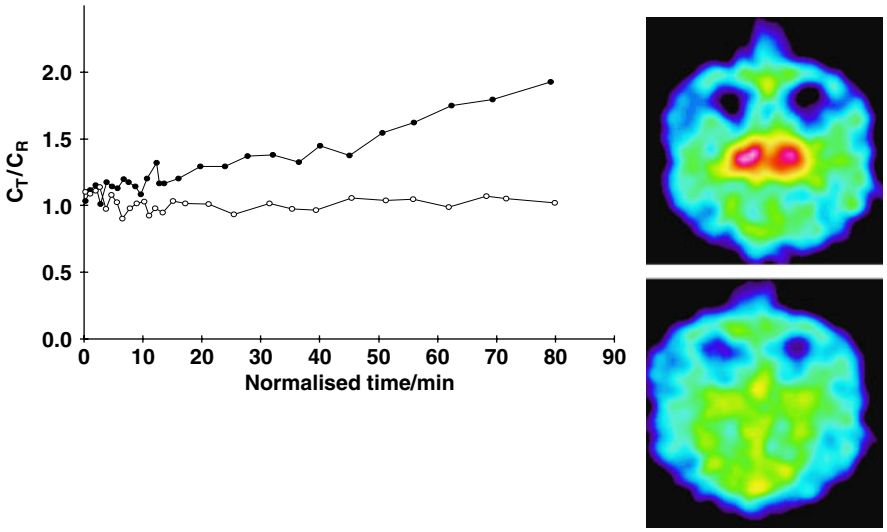


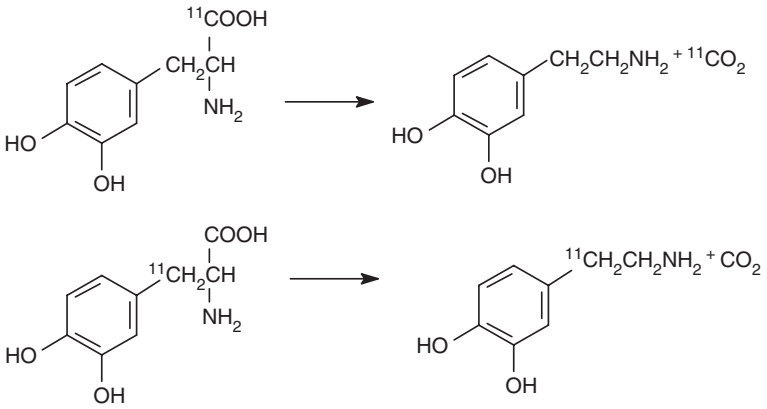
Figure 6 In color are summation PET brain images using (a) L-[β-^{11}C]dopa (upper image), (b) L-[carboxy-^{11}C]dopa (lower image). Closed circles represent the time activity curve for L-[β- ^{11}C]dopa and open circles represent the time activity curve for L-[carboxy-^{11}C]dopa. Vertical axis (CT/CR): ratio of radioactivity in tissue to radioactivity in reference region (cerebellum). This figure is available in color online at www.interscience.wiley.com/journal/jlcr

amino acid decarboxylase on the two different labeling positions is shown in Scheme 4.

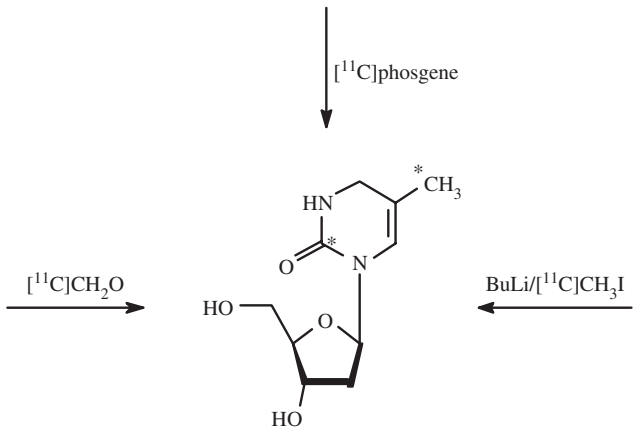
^{11}C-labeled neurotransmitter precursors have proved to be clinically useful. L-[^{11}C]HTP is an excellent imaging tool for studying neuroendocrine tumors.[27,28] L-dopa can also be applied to measure the *in vivo* synthesis rate of dopamine and thus be used for Parkinson's disease diagnosis and measuring loss of dopaminergic neuronal activity. [^{11}C]Thymidine is another example of an endogenous compound that can be labeled in different positions. [^{11}C]Thymidine can be used to measure tumor proliferation and has a long history as a PET tracer in oncology.[29] Scheme 5 shows three methods for [^{11}C]thymidine synthesis.

PET microdosing

Costly failures[30] of new chemical entities in three out of four clinical trials and dramatically increased costs for drug development (costs borne by patients and society) have initiated proposals to make drug development more effective. There are also demands to reduce preclinical animal experiments. A number of scientifically valid



Scheme 4



Scheme 5

proposals have been published recently to address these problems.[31–35]

Some of these proposals promote early screening of new drug candidates in humans using PET imaging.[36–38] Non-invasive PET imaging has the potential to determine drug distribution and concentration *in vivo* in man using labeled drugs.[39] Rapid synthesis of organic compounds labeled with positron emission radionuclides now allows many new drug candidates to be labeled for use as PET probes. Therefore, early PET studies, performed with drug doses (microdoses) far lower than therapeutic doses can be used to select compounds for further clinical trials or terminate clinical development based on *in vivo* performance in man.

Since only very low drug doses are used in PET microdosing studies, safety requirements should be reduced in relation to the safety requirements for therapeutic doses.[39] In terms of assuring safety there is no major difference between studying a new drug candidate using PET microdosing and validating a new PET tracer for biochemical and physiological studies. PET microdosing has the additional benefit that far lower amounts of compound need be manufactured. Conventional Phase I clinical trials require synthesis of several grams of a new compound, which takes considerable resources and time. For PET microdosing studies, the amount of compound needed would be of the order of a few milligrams.

PET microdosing data can be used with drug distribution data in monkeys to generate models for the relationship between plasma and target tissue concentration. Since biodistribution in humans may differ from a tracer microdose as compared with a pharmacologic dose, modeling data should include biodistribution data from a bridge study using intermediate doses in non-human primates. PET microdosing studies can also evaluate drug interaction with a target by utilizing a PET tracer specific for the target. Drug interaction models can be further developed to include aspects of receptor occupancy, backed up with monkey dose escalation studies including observation of receptor occupancy. For the final refinement of PK/PD modeling, human receptor occupancy studies are used. Combining a PK/PD-driven process with mechanistic information will help make drug development more cost effective. Thus, PET microdosing can help drug development become less empirical and more mechanistic, predictive, and cost effective. These advantages necessitate more rapid development of PET methodology and its validation in humans.

Tracer libraries and fine-tuning molecular properties

Labeling methods using ^{11}C now make it possible to create small tracer libraries where molecular properties can be fine-tuned by varying substrates and reactants. Fine-tuning can be achieved by alkylations on heteroatoms with ^{11}C-labeled alkyl and fluoroalkyl halides.

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The implementation of carbonylation reactions with [^{11}C]carbon monoxide further increases the ease with which tracer libraries can be created. In preclinical screening using *in vitro* and *in vivo* techniques, biological properties of tracer candidates can be investigated very quickly. If a compound exhibits suitable tracer characteristics in screening assays, validation of important parameters such as binding properties, metabolism, and distribution is performed. When tracer libraries are combined with microdosing, the time to achieve proof of concept in man can be very short.

There are also other methods to fine-tune tracers; for example, kinetic isotopic effects (KIEs). A number of reaction mechanism studies have shown that primary KIEs from ^{11}C/^{12}C are negligible in connection with biological studies. However, in mechanistic chemistry KIEs have become an important tool.[40–42] The substitution of hydrogen with deuterium may significantly change the rate of a reaction by primary or even secondary KIEs, and can be a tool for fine-tuning molecular properties in biological applications. For example, replacing the hydrogen atoms in the propargyl group in the mono-amino oxidase B inhibitor L-[^{11}C]deprenyl with deuterium reduced the reaction rate and changed the rate-limiting step from distribution (flow dependent) to the irreversible reaction between enzyme and substrate.[43] The induced KIE thus gave the opportunity to measure the regional distribution and concentration of mono-amino oxidase B in the brain. Another example of double-isotope labeling is the use of deuterium, via a KIE, to reduce metabolic loss of ^{18}F in the norepinehrine transporter antagonist *(S,S)*-[^{18}F]FMeNER-D$_2$.[44]

Technological aspects

PET chemistry is associated with high amounts of radioactivity. Successful development of labeling synthesis methods goes hand in hand with improvements in technology to increase safety. Remote-controlled synthesis devices are a natural consequence. Most PET laboratories have developed their own automated synthesis equipment. In addition, several automated synthesis systems are commercially available; for example, FDG synthesizers, gas-phase methyl iodide systems and systems for loop synthesis of acetate. Automated synthesis systems add reproducibility and may reduce synthesis time. These systems are also beneficial for quality assurance. PET radiopharmaceuticals for human use must be Good manufacturing practice (GMP) produced, and here especially remote-controlled automated procedures are to be preferred (Table 2).

Table 2 Table of medical and pharmaceutical terms and abbreviations

CMS	The Centers for Medicare and Medicaid Services (CMS) measure quality and costs for the Medicare and Medicaid health service systems in the United States
GMP	Good manufacturing practice regulations, promulgated by the FDA, strictly govern the manufacture of pharmaceuticals and are intended to assure that medicines are effective, safe, and free of contaminants
HIDA scan	A Hydroxy Iminodiacetic Acid scan (also called a gall bladder scan or cholescintigraphy) uses [^{99m}Tc]HIDA to evaluate cystic duct obstruction, a disorder associated with diseases including gall bladder infection, gallstones, hepatobiliary cancer, and rejection of liver transplants
NICE	The National Institute for Health and Clinical Excellence (NICE) is an office within the British National Health Service. NICE recommends treatments for adoption by the NHS, based on evidence of treatment outcomes weighed against economic costs
Ventilation–perfusion scan	A combination of blood flow and airflow scans of the lungs used to detect blood clots. After a patient breathes an aerosol of a ^{99m}Tc-labeled compound, the ventilation scan monitors evenness of ^{99m}Tc distribution in the lungs. A perfusion scan monitors pulmonary blood flow by detecting a ^{99m}Tc-labeled compound injected into a vein. A mismatch between lung images of the injected and inhaled compounds may indicate a pulmonary embolus

Future perspectives

PET techniques as tools in drug development are becoming more recognized, especially due to the development of new labeling methods and techniques.[45] Although ^{11}C-labeling has reached a level where very high percentages of biomolecules and drugs can be labeled, there still exist unexplored areas of ^{11}C-labeling chemistry and structures that cannot be labeled with present reactions. We also anticipate that developments in PET technology will continue to have a major impact on the clinical PET studies. In particular, we foresee combining a particle accelerator and synthesis equipment to create the 'radiopharmaceutical coffee machine,' where the operator selects the radionuclide and tracer from a menu and the apparatus produces the tracer automatically by running a GMP protocol. While new synthetic methods and techniques are essential, it is perhaps even more important to continue to develop tracers of relevance for biological targets and defined clinical needs.

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We foresee more opportunities to combine radiotracers using the same or different radionuclides. This is an area where the short half-life of ^{11}C (20.4 min) actually turns into an advantage by allowing repetitive administration of tracers in the same individual during a single day of PET examinations. We also foresee more dual tracer combinations in the future. For example, a recent study[46] combined [^{11}C]PIB and [^{18}F]FDG in a longitudinal study of Alzheimer's disease patients in order to improve the detection of stabilized cerebral metabolism in response to therapy. [^{11}C]PIB showed the senile plaque load. [^{18}F]FDG showed the reduction in energy consumption. The combined information proved to be synergistic for the interpretation of Alzheimer's disease status.

Early-stage discovery of molecular imaging agents

Discovering molecular imaging agents shares much with standard drug discovery practices. For example, target validation, identification of suitable candidate compounds with high affinity and uptake at the target site, adequate clearance and low-potential toxicity are key considerations for both therapeutic and imaging compounds.[47] There are similar standard stages such as hit identification and lead generation. However, there are also differences in early-stage imaging agent discovery practices that can be critical to the ultimate success of an imaging agent.

Standard drug discovery often begins target validation. This means that there is evidence that the drug target plays an important mechanistic role in the disease process such that inhibiting the target may modify the disease.[48] Therefore, the drug will have a functional impact. A validated target serves as the basis for early-stage screening of potential drug compounds. Typical functional screens target kinases or receptors.[47] An important distinction between screening for drugs and imaging agents is this: Although imaging agents should be aimed at targets that play critical roles in disease, they do not necessarily need to affect target functions. Instead, imaging agents are also valuable for quantitation of receptor distribution and density/overexpression in disease states. There are several instances where non-functional targets manifest as biomarkers of the disease process, such as extracellular matrix proteins, membrane lipids, structural proteins, or extracellular deposited peptides.[49–52] Thus, for discovering imaging agents, identifying direct binding interaction is often preferred and a more relevant screening assay is a ligand-binding assay.

The ligand-binding assays of choice are scintillation proximity assays (SPAs).[53,54] Their definite advantage is the ability to measure binding interactions without the need for mechanical separation of free from bound probe. SPAs enable measurement of saturation binding, binding constants (K_d) and the amount of binding sites (B_{max}). B_{max} is a critical measure because image quality can be affected by the ratio of B_{max} divided by the binding constant (K_d). In general B_{max} should exceed K_d.[55]

Although SPAs are the gold standards for measuring binding affinity of imaging agents, a potential drawback is the necessity to radiolabel compounds, which may be cumbersome and costly if several different compounds are to be analyzed. Further, radiolabeling may be particularly problematic for macromolecular imaging agents such as antibodies or antibody fragments. Labeling conditions for proteins or peptides can be harsh and caution is needed to not adversely affect their natural secondary or tertiary structures. Further, non-site-specific radiolabeling chemistries such as NHS-amine chemistry can often react within the binding site and thus negatively affect affinity. It would be advantageous to evaluate all of these possible complications prior to radiolabeling so that an optimal approach can be employed. Recently, advanced non-labeled approaches to measure binding and affinity such as surface plasmon resonance (SPR; Biacore, GE Healthcare) have gained in popularity, particularly because such techniques can also generate real-time kinetics.[56,57] Additionally, SPR can measure the affects of a variety of different labeling chemistries performing under cold conditions, thereby determining the optimal approach prior to radiolabeling. As with SPA, SPR can be used to screen for direct target–ligand interactions and measure binding affinity. Although SPR is most robust for protein–protein interactions, it can be used to measure small molecule or peptide interactions using either affinity solution techniques or direct binding.[56,58–60]

Another aspect of target validation is determining the amount of target accessible so as to provide high imaging signal to noise. The absolute amount of accessible target *in vivo* is therefore important. With a highly specific imaging agent, saturation binding conditions can be used to calculate B_{max} empirically. This has been particularly useful for well-understood receptor–ligand interactions. However, as stated above, complex non-receptor targets are sometimes examined. For example, imaging beta-amyloid plaques requires ligand binding to a complex heterogeneous target with multiple independent binding sites.[49,61,62] In addition to the complexity of the target binding sites, some imaging targets, such as beta-amyloid, have a complex

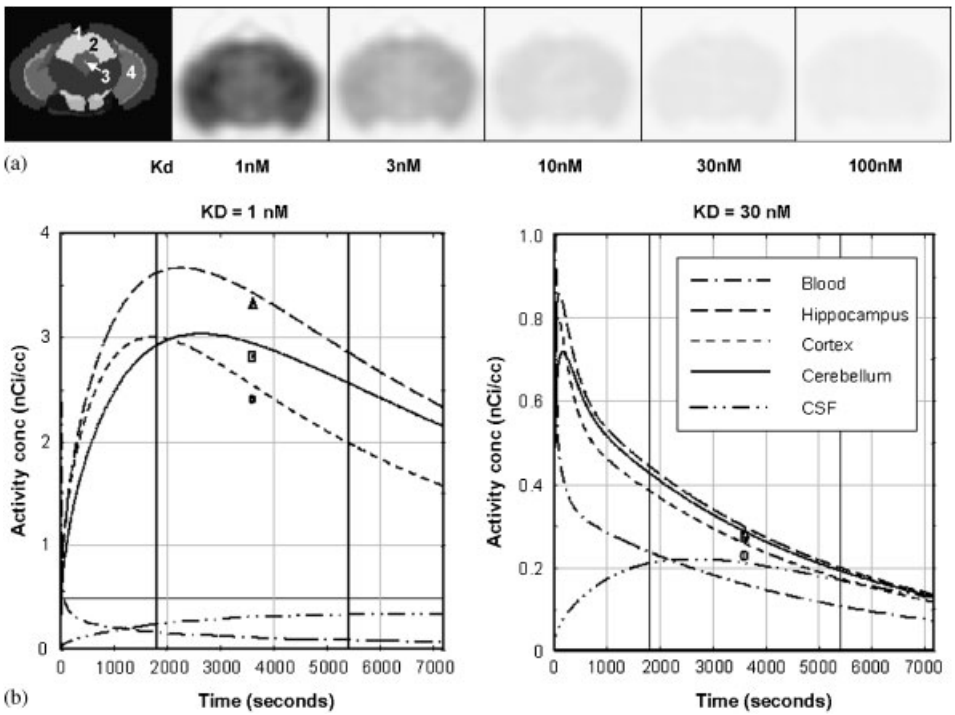

Figure 7 PET and tracer simulation model for mouse brain. (a) Segmented brain regions of an MRI phantom, within which regions of interest were manually drawn (1 = cortex; 2 = cerebellum; 3 = CSF; 4 = hippocampus). Images simulate intensity at differing binding strengths of 'virtual' tracer. Image rendering was held constant to illustrate the differences in raw binding uptake. (b) Comparison of quantitation derived from the simulated images with input time-activity curves for a virtual tracer. The points show the computed integrated activity from the image ($n = 5$ for each affinity) at the indicated time of acquisition. [With kind permission of Springer Science and Business Media.]

trafficking pattern *in vivo*.[63,64] In such cases, computational and systems biology may provide useful guidance. This approach was recently applied to understand the relationship between the heterogeneous microenvironment of plaque and imaging kinetics.[65,66] Further, recent data by our group[67] indicate that combining models of pharmacokinetics with physiological models of beta-amyloid production and clearance has value in understanding the relationship between target concentrations, affinity, and image quality (Figure 7).

Target concentrations are often determined with simple biochemical methods such as Western blotting or ELISA. Recently, such an approach was used to correlate PET image quality to receptor density.[68] Although useful, such approaches do not consider the complex cellular biology of the target and should be evaluated cautiously. For example, it was recently demonstrated that the receptor tyrosine kinase c-met, which is upregulated in many cancers (and presumed to be membrane spanning), can have altered proteolytic processing that leads to accumulation in the cytoplasm and nucleus.[69] Thus, target concentration as measured by western blot or ELISA may not always accurately represent the amount of target accessible to an imaging ligand. Recently, fully quantitative immunohistochemistry approaches that consider subcellular compartmental expression have been developed.[70,71] Such techniques may improve the understanding of relationships between membrane accessibility and image quality.

Another critical consideration is the potential effects of background binding, a key determinant of imaging signal to noise. Typically, imaging agent background is determined by *in vivo* pharmacokinetic measures using either reference regions or target regions within naive animals. In standard drug discovery practice, predictive *in vitro* assays such as Caco-2 permeability assays have achieved large time and cost savings.[72] It is possible that *in vitro* assays may predict *in vivo* tracer binding. Recently, Patel *et al.* developed an *in vitro* 'wipe assay' that predicts the background binding of tracers to brain tissue *in vivo* with high accuracy.[73] *In vitro* assays that predict brain uptake, tumor penetration, vascular leakage, interstitial retention, and clearance would all improve cost-effectiveness of imaging agent discovery practices.

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In conclusion, early-stage discovery of molecular imaging agents differs notably with standard drug discovery by the importance placed on direct binding assays and kinetic analysis of ligand–target interactions. Looking forward, imaging agent target validation should assess complex target microenvironments, sub-cellular localization, and trafficking, which is increasingly possible through novel systems biology approaches and advances in automated and quantitative tissue analysis.

The evolution of radiolabeled molecules for pre-clinical imaging

Pre-clinical imaging of radiolabeled molecules bridges *in vitro* determinations of binding properties in early-stage discovery of radiolabeled molecules and *in vivo* imaging of radiotracers in humans. Radiotracer specificity, selectivity, binding capacity, and localization in complex biological milieu have been determined *in vitro*, *ex vivo*, and *in vivo* in animals for decades. The last four decades of imaging radiolabeled molecules in animals began with static methods of imaging using autoradiography. These methods dictated the types of radionuclides (e.g. ^{3}H, ^{14}C) for labeling as well as the classes of molecules to be labeled (e.g. endogenous biomolecules and drugs). Later, adoption of clinical SPECT and PET scanners enabled researchers to image large animals in order to visualize drug derivatives and biomolecules labeled with gamma- or positron-emitting radionuclides. The imaging modality again dictated the type of label (e.g. ^{99m}Tc, ^{18}F, etc.) used to image specific biochemical and physiological changes *in vivo*, such as chemically lesioned non-human primate models of human disease. As biomedical research focused on molecular markers of disease, the role of mouse models with genetically modified cells led to adapting *in vitro* gene expression reporters into *in vivo* imaging reporters. Mouse models of disease in fact motivated the development of high-resolution, high-sensitivity pre-clinical scanners.

Static imaging of radiolabeled biomolecules and drugs

Autoradiography was the gold standard for visualizing radiolabeled molecules in human and animal tissue prior to the advent of *in vivo* nuclear imaging scanners. The simplicity of *in vitro* and *ex vivo* autoradiography stems from the fact that tritiated or [^{14}C]labeled nucleic acids, amino acids and other molecules, including [^{3}H]thymidine, [^{3}H]uridine, [^{3}H]leucine, and [^{3}H]cytidine, are chemically the same as the endogenous biomolecules. Autoradiographic localization of labeled biomolecules therefore reflects the behavior of corresponding non-radioactive biomolecules. Early on, labeled biomolecules proved useful for localizing RNA, DNA, and proteins in microorganisms.[74–76] More complicated quantitative measurements of local glucose metabolism in the rat brain were performed by Sokoloff *et al.*, who developed a seminal tracer kinetic method using 2-deoxy-D-[^{14}C]glucose autoradiography.[77] Tissue-specific autoradiography of animals also increased understanding of neuronal development and cell migration in mice and rats.[78–80] The imaging of analogs to ^{3}H- and ^{14}C-radiolabeled molecules has come full circle with the invention of PET and SPECT scanners. For instance, quantitation of local cerebral metabolic rates for glucose using [^{18}F]FDG PET adapted Sokoloff methodology for autoradiography to *in vivo* imaging, without perturbing endogenous glucose metabolism.[81,82] Using ^{18}F-radiolabeling, sub-cellular, cellular, and tissue distributions of biomolecules have been revisited in the last decade with non-invasive small animal PET imaging, focusing particularly on gene and protein expression.

Autoradiography is still useful in early drug discovery. Biodistribution of a drug is traditionally determined by measuring the radioactivity of ^{3}H- or ^{14}C-labeled drugs in necropsied organs. The same radiolabeled lead compounds are also used in imaging large tissue sections, from mice to non-human primates. These studies provide drug information related to tissue distribution, site-specific drug localization and retention, penetration into specific targets, tissue binding, and interspecies kinetics.[83] For instance, whole body autoradiography of rats aid selection of lead candidates by screening tissue pharmacokinetics of ^{3}H drug entities, including brain penetration, tissue retention, and routes of elimination.[84] Examples below demonstrate how autoradiography with beta- and positron-emitting radionuclides is also a key in the evaluation and validation of new PET tracers.[85,86]

However, ^{3}H and ^{14}C autoradiography is still labor intensive, requires one animal per time point for kinetic studies, and takes days to image drugs. While qualitative interpretation of autoradiographs is straightforward with molecules labeled with beta-emitting radioisotopes, quantitative autoradiography is achievable[84,86] but most reliable as a relative measure of radioactivity in a tissue section.[76] Nevertheless, radiolabeled compounds for autoradiography laid the foundation for PET and SPECT imaging of animals to visualize molecular, biochemical, and cellular processes in animals. Indeed, autoradiography with ^{3}H and ^{14}C act as high-resolution anatomic correlates for lower resolution PET and SPECT imaging.[87]

Imaging animals using clinically relevant radionuclides and clinical PET and SPECT scanners

Unlike *in vitro*, *ex vivo*, and living slice autoradiography,[88] clinical PET and SPECT scanners provided tools to dynamically quantitate radioactivity *in vivo* and tomographically visualize tracers in animals. Once again, however, clinical hardware dictated what radiochemistry and animal models were compatible with clinical imaging scanners. Pre-clinical imaging with clinical scanners also saw the development and validation of new tracers that were chemically distinct from parent drugs or endogenous molecules.

Imaging of animals with clinical PET scanners offers the opportunity to study pharmacokinetics, pharmacodynamics, and metabolism of ^{11}C- or ^{18}F-labeled drugs. Pharmaceutical companies requiring pre-clinical biodistribution studies of novel drug entities in non-human primates may use ^{11}C- or ^{18}F-labeled analogs of drugs to investigate non-invasively and efficiently the delivery of drug to the site of interest or to determine receptor occupancy for PK/PD modeling, as described earlier. As will be discussed later in this section, the mass effects encountered with rodent imaging of PET probes for neuroreceptors may be less of an issue with large monkeys than with mice.[89] Imaging of labeled small molecule drugs in animals using clinical SPECT scanners is restricted by the inclusion of chelating moieties for radiometals, which drastically alters the drug.[90]

Clinical nuclear imaging cameras have opened the door to non-invasive *in vivo* examination of biochemical pathways in animals. Only trace amounts of radiolabeled molecules are required, so pharmacological effects in animals are avoided. However, the limited spatial resolution of clinical PET and SPECT scanners leads to partial volume effects where a measured concentration of radioactivity is lower than the actual concentration in objects of similar size to the scanner's spatial resolution.[91] Illustrations of pre-clinical tracer development are the imaging probes for the dopaminergic system in large animal models of Parkinson's disease. The animal model that was large enough to overcome partial volume effects of clinical PET and SPECT scanners was the MPTP-treated monkey.[92,93] MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is used as a chemical treatment to cause the hallmarks of Parkinson's due to lesioning of the putamen.[94]

Commonly used radiolabels for PET and SPECT imaging of MPTP-treated monkeys are radiohalogens (e.g. ^{18}F) and radiometals (e.g. ^{99m}Tc). Unlike radiolabeling with ^{3}H and ^{14}C, radiolabeling with halogens and metals may alter the biochemistry of labeled compounds, and therefore radiohalogen and radiometal probes must be validated. For instance, ^{18}F was incorporated into 6-[^{18}F]fluoro-L-DOPA, which is an analog of the DOPA substrate found in dopaminergic neurons. Biochemical comparisons between the PET tracer and ^{3}H-labeled L-DOPA[85] were one of the many steps to validate 6-[^{18}F]fluoro-L-DOPA as a tracer for imaging aromatic L-amino acid decarboxylase activity in Parkinson's disease.

The work with large animal models of Parkinson's disease not only involves imaging biochemistry but also evaluating new therapies. For instance, [^{99m}Tc]TRODAT-1 used to image dopamine transporters in the brain can also diagnose Parkinson's disease. Recently, ([^{99m}Tc]TRODAT-1 and [^{125}I]nortropane were used *in vivo* and *ex vivo*, respectively, to evaluate the protective properties of Rotigotine.[95] Although the partial protection of nerve terminals by Rotigotine was demonstrated by *ex vivo* methods, the clinical SPECT scanner lacked resolution and sensitivity to show therapeutic efficacy.

These examples demonstrate the development of radiolabeled molecules to non-invasively probe biological processes *in vivo* using clinical systems. However, they also reveal the need for biology – not scanner specifications – to dictate what molecules are radiolabeled to understand the molecular basis for disease and drug properties *in vivo*.

Radiolabeled probes specifically designed for pre-clinical imaging

The last decade has seen development of radiolabeled molecules specifically for pre-clinical assays. Dedicated small animal (e.g. mouse and rat) PET scanners improved imaging resolution and sensitivity to match the small volumes and radioactivity injected in rodents. Although the impact of small animal SPECT and PET imaging in drug development has yet to be fully realized, dedicated rodent scanners have already been used for pre-clinical determinations of biodistribution and pharmacokinetics of experimental radiopharmaceuticals. One example of small animal imaging is the non-invasive monitoring of targeting kinetics and dosimetry using a PET analog of the radiotherapeutic monoclonal antibody (mAb).[96] PET imaging of [^{86}Y]trastuzumab ([^{86}Y]Herceptin) showed mAb uptake in human ovarian carcinoma tumors with minimal organ uptake in mice. In turn, the distribution and specific targeting of the radiotherapeutic [^{90}Y]Herceptin is known because the [^{86}Y] and [^{90}Y]mAbs are chemically identical. The relative ease of creating the mouse model and using a clinically exotic positron emitter in the lab suggests the emergence of small animal imaging as a flexible tool in radiotracer development. Furthermore,

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adapting molecular biology techniques for mouse models led to pre-clinical reporter probes for PET. Transgenic mice and mouse models with genetically engineered cell lines allow for reporter genes (e.g. HSV1-tk) to monitor gene expression with molecular imaging.[97–101] *In vivo* reporter probes based on HSV1-tk are unique to small animal imaging.

Radiolabeled nucleosides represent a family of pre-clinical *in vivo* reporter probes for PET and SPECT that are taken up by cells but intracellularly trapped in the cells expressing gene reporters. Retention of these PET reporter probes in genetically engineered cells is due to phosphorylation of the positron-labeled substrate by HSV1-tk inside the cell. Uracil and acycloguanosine analogs are examples of HSV1-tk substrates used as PET reporter probes. The uracil analog, 2′-fluoro-2′-deoxy-1-beta-D-arabinofuranosyl-5-iodouracil (FIAU), has been radiolabeled with multiple radiolabels for autoradiography, SPECT, and PET.[100–103] Even multi-modality optical-PET imaging is possible with novel fusion reporter proteins (e.g. HSV1-tk/GFP fusion) in animals. Serganova and colleagues[104] showed that reporter cells entrapped another FIAU derivative called [^{18}F]2′-fluoro-2′-deoxy-1-beta-D-arabinofuranosyl-5-ethyl-uracil ([^{18}F]FEAU) and activated the optical reporter gene *TKGFP*, enabling facile imaging of transduced cells *in vitro* and *in situ* with fluorescence microscopy and PET imaging.

Two notable acycloguanosine reporter probes are 8-[^{18}F]fluoro-9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl] guanine ([^{18}F]FGCV) and 9-[4-[^{18}F]fluoro-3-(hydroxy-methyl)butyl]guanine ([^{18}F]FHBG).[86,105] [^{18}F]FGCV was initially developed using autoradiography with ^{14}C and optimized using ^{18}F analogs for conversion from *ex vivo* imaging to *in vivo* imaging using small animal PET[86] (Figure 8). Optimized biodistribution, cellular uptake, and clearance resulted in a low background reporter probe called [^{18}F]FHBG for use in human dosimetry studies[105] (Figure 9).

PET reporter probes have brought pre-clinical imaging full circle. Before, ^{3}H and ^{14}C labeling for autoradiography traced biomolecules and localized them in tissues. Today, novel PET probes non-invasively image not only labeled molecules *in vivo* in

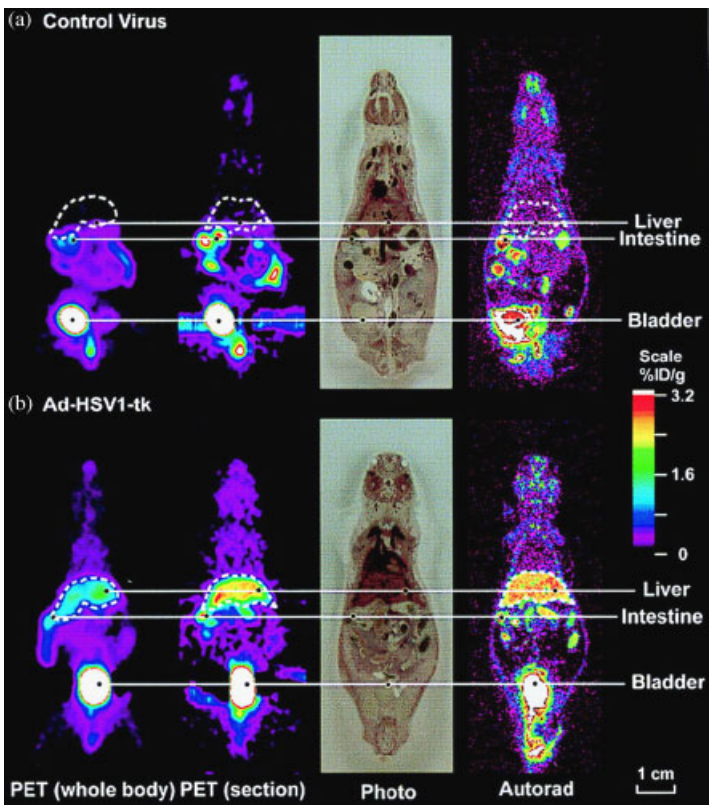

Figure 8 Pre-clinical PET imaging and autoradiography of [^{18}F]FGCV reporter in mice. A control virus was administered to mice (a), and an Ad-CMV-HSV1-tk virus, which was injected into experimental animals (b), was retained in the liver. Subsequent [^{18}F]FGCV biodistribution to the liver in Ad-HSV1-tk mice quantitatively shows HSV1-tk reporter gene expression in the liver (b). Details of imaging experiments are found in Reference 86. This figure is available in color online at www.interscience.wiley.com/journal/jlcr

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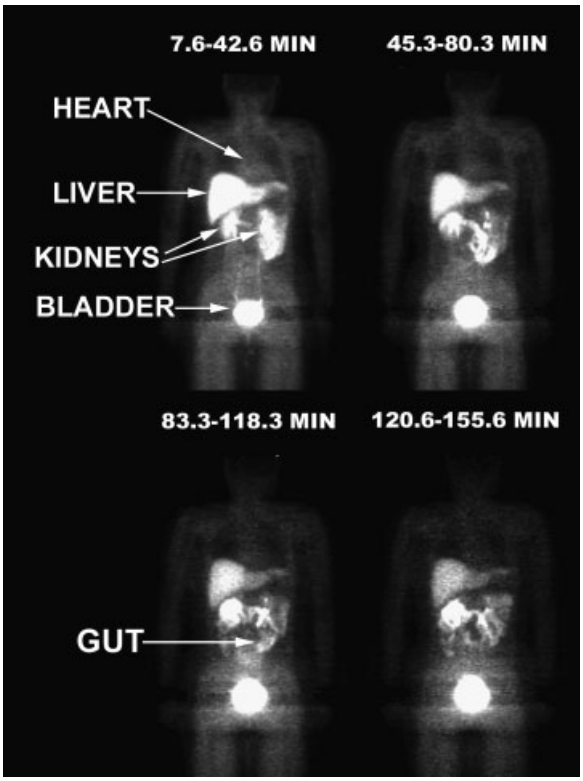

Figure 9 Biodistribution of [^{18}F]FHBG reporter probe in a human subject. A 167.6 MBq administration of [^{18}F]FHBG reporter probe to the healthy human volunteer demonstrated rapid blood clearance, low background signal, and acceptable radiation dosimetry. The desirable pharmacokinetics of [^{18}F]FHBG in humans may allow for imaging HSV1-tk gene expression in human subjects. Details of imaging experiments are found in References 105.

animals, but also image-related molecular processes including gene expression, receptor density, pharmacokinetics, metabolism, and clearance.

As reductionism, miniaturization, and specialization in imaging continue, a challenge in the future may be to improve the clinical relevance of pre-clinical nuclear imaging. Perhaps overlooked in the past is the clinical relevance of radiopharmaceuticals for veterinary medicine. Hyperthyroidism, lameness, and hepatic scintigraphy of dogs and horses are common gamma camera or SPECT imaging procedures in the veterinary setting.[106] However, the clinical impact could be improved by utilizing radiotracers for other indications and radiolabeled drugs. A major challenge pre-clinical imaging faces is reducing or accounting for the mass effect encountered in rodent imaging.[89] The mass effect is the higher mCi/kg dose administered to rodents compared with humans in order to increase the count rate in small animal imaging. In neuroreceptor imaging, for instance, the consequence of mass effect may be percentage receptor occupancy in the rodent reaching pharmacologically significant levels.

The future prospect of facile labeling of experimental therapeutics with high specific activity could reduce mass effects in pre-clinical imaging and accelerate screening of new chemical entities in animals and humans. As new biomarkers are discovered, and with them new radiolabeled drugs and probes, an added challenge is to incorporate disparate information (i.e. species differences, multiple tracers, imaging of different markers) into a single model to quickly understand the interplay between mechanism, target, and efficacy biomarkers.[67]

Imaging diagnostics in nuclear cardiology

The advent of myocardial perfusion imaging 30 years ago was a major landmark, heralding the emergence of nuclear cardiology into clinical practice. Since then the power of nuclear cardiology has increased tremendously with the discovery and development of new nuclear tracers, particularly cationic technetium complexes, and advances in instrumentation, especially the transformation from image acquisition with planar gamma cameras to tomographic imaging with single photon emission computed tomography (SPECT) technology.

One of the first radionuclides used for imaging in patients was potassium 43 (^{43}K). Unfortunately, its rather high-energy photons (373-keV peak) made imaging with gamma cameras somewhat problematic.[107] ^{43}K also had a 22.4-h half-life, so that imaging studies had to be separated by a minimum of 4 days to reduce residual activity (e.g. resting imaging) from the first procedure.[108]

The next radionuclide monovalent cation evaluated was rubidium 81 (^{81}Rb), which had myocardial uptake and clearance characteristics similar to those of ^{43}K. This imaging agent was also successful for non-invasive detection of inducible myocardial ischemia in patients with coronary artery disease (CAD).[109] Its limitation was difficulty with image interpretation with pinhole collimation, particularly if the heart was not well centered within the camera's field of view. Both ^{43}K and ^{81}Rb virtually disappeared from further clinical evaluation with the emergence of another potassium analog, thallium 201 (^{201}Tl).[108]

Technetium-based perfusion agents

^{201}Tl became the mainstay of myocardial perfusion from the mid-1970s until the introduction of technetium-based perfusion agents, firstly with [^{99m}Tc]Sestamibi (Cardiolite) in 1991, followed by [^{99m}Tc]

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Tetrofosmin (Myoview) in 1996. Research and development of ^{99m}Tc cations for myocardial perfusion imaging was driven by an interest in exploiting the superior imaging characteristics of ^{99m}Tc over ^{201}Tl.[110] ^{99m}Tc-labeled myocardial perfusion agents provide better image quality because the 140-keV photon energy peak of ^{99m}Tc is optimal for gamma camera imaging. Its relatively short half-life (6 h) provides favourable patient dosimetry and makes it possible to administer a radiopharmaceutical dose 10–15 times greater than ^{201}Tl. Higher count rates for ^{99m}Tc easily permit gated acquisition for the assessment of regional wall motion or regional thickening.[111]

The first documented ^{99m}Tc cation was based on nitrogen macrocyle. But the pioneering studies applying ^{99m}Tc cations to heart imaging were largely based on the work by Drs Edward A. Deutsch and Kenneth A. Glavan (University of Cincinnati) on phosphine complexes, and to a lesser extent, on arsines. Their aim was to develop complexes of ^{99m}Tc that were positively charged and strongly attracted to mitochondria, cellular organelles that are abundant in functional heart tissue.[112]

These early attempts to obtain clinically useful myocardial imaging agents were frustrated by the inadequacy of animal models to predict behavior of lipophilic cations in man. Early Tc(v) diphosphine analogs showed poor heart uptake in animal models and suffered from interspecies variability.[113] The limitations encountered with ^{99m}Tc complexes of simple alkyl or aryl diphosphines were overcome when Amersham International (now owned by GE Healthcare, Inc.) introduced hetero-atomic functions to modify non-target uptake. This innovation led to the development of the cationic ligand Tetrofosmin (Myoview).[114]

Other research groups have exploited alternative ^{99m}Tc ligand combinations, especially Tc(I) complexes including isonitriles, arenes, and phosphites. The initial work on isonitrile compounds by Len Holman and Alun Jones at Harvard Medical School was subsequently developed by DuPont Pharma (now owned by Bristol-Myers Squibb Medical Imaging, Inc.) to give rise to a new cation [^{99m}Tc]MIBI, which is marketed as Cardiolite.[115] The development and commercialization of cationic technetium agents are still characteristics of the synergy between academic and commercial institutions, where fundamental research in chemistry and biology can be used to address problems in applied nuclear medicine. To succeed in future developments of new molecular imaging tracers these relationships must continue.

Myoview and Cardiolite have been used with SPECT imaging technologies for non-invasive evaluation of regional myocardial blood flow, which has enhanced our ability to diagnose CAD, assess prognosis, detect viable myocardium, and evaluate the efficacy of therapies aimed at improving myocardial blood flow. The clear superiority of technetium agents has caused ^{201}Tl use to steadily decline. According to Arlington Medical Resources, a hospital market data firm, in 2006 there were more than 7.75 million myocardial perfusion imaging procedures, of which 95% of patients received a single dose of a technetium agent and only 5% received thallium 201.[116]

Imaging diagnostics: from bench to bedside

Bringing radiolabeled imaging molecules into clinical practice takes a great deal of time, first with demonstrations of proof of concept in animals, and then with clinical trials and regulatory submission. Pharmacoeconomic benefits must also be demonstrated to healthcare payors. In addition, the short half-lives of radiolabeled imaging molecules require radiopharmacies for on-site or nearby dose production. Operating under exacting regulatory standards, radiopharmacies employ 'just in time' manufacturing to deliver imaging molecules on demand, ready for use.

Bringing imaging diagnostics to the bedside is an immensely exciting story of scientific achievement and technological development. The discoveries of ^{11}C and ^{18}F more than 60 years ago are essential to the story, intertwined with development of cyclotron technology during the same era. Although Ernest O. Lawrence won a Nobel Prize in 1939 for inventing the cyclotron, cyclotrons were exceedingly rare before the 1950s. Eventually, cyclotrons became commercial nuclear medicine instruments. In the early 1970s, ^{11}C and ^{18}F were given medical applications when they were chemically incorporated into amino acids and other basic biomolecules for use as radiotracers.[117–119] Without radiotracers, the clinical feasibility and value of these isotopes could never be realized. Nor could it happen without sophisticated imaging equipment, beginning with the first PET instruments in the 1970s. By localizing radioisotopes with spatial resolution, PET converted autoradiography into *in vivo* histology.[120–122]

Advances in imaging instrumentation and computing for kinetics and reconstruction continue to push back the limits for using PET to diagnose new diseases and quantitate functional parameters. In the future, there is hope that motion correction and time-of-flight analysis may increase PET sensitivity to the point that, together with highly specific imaging agents, the cubic millimeter resolution barrier will at last be broken. If this long-sought advance comes to fruition, PET will be

more useful for ruling out disease and periodic screening of asymptomatic patients.[123–125]

Early years of FDG PET diagnostics

Establishing [^{18}F]FDG in PET diagnostics was a halting process. It was initially used for cardiac and brain metabolism studies and found application in tumor imaging as early as the 1980s.[126–129] With the availability of cyclotrons and streamlined methods for [^{18}F]FDG cGMP (pharmaceutical grade) production, the compound seemed poised for adoption into clinical diagnostics. However, a long FDA-imposed moratorium kept it within the domain of research until clinical safety regulations could be provided. In addition, another problem became apparent: Because [^{18}F]FDG never went through the rigors of commercial product development, its imaging techniques were never standardized. Consequently, there was confusion in making accurate measurements and interpreting results. For example, there were difficulties in extrapolating quantification for clinical evaluation of anti-cancer agents. Another consequence of lack of standards was that insurers were reluctant to reimburse for FDG PET services.[130]

Today these difficulties have been resolved and ^{18}F imaging technology has resumed development. High-performance chemistry with dedicated equipment has firmly established [^{18}F]FDG as a PET diagnostics mainstay[131] and more ^{18}F-based molecules are being developed.[132] [^{18}F]FDG production also continues to simplify. The next advance is software-controlled synthesis using versatile chemistry platforms composed of pre-loaded cGMP cassettes.

^{11}C is capable of interrogating a multitude of metabolic pathways. But commercializing ^{11}C and other shorter-lived isotopes will require much faster technologies for producing unit doses in ready-to-inject form.[133] As always for imaging products, quality control for chemistry, manufacturing and control (CMC) will be critical. The amount of time that quality control requires must also diminish in order to be compatible with shorter-lived isotopes. Miniaturization and microfluidics are part of the answer. In particular, biosensors, nanosensors, SPR spectroscopy, and capillary electrophoresis may reduce electrophoresis and binding assays to a few seconds. These technologies are vital for spreading molecular imaging.

Technetium to the bedside

Compared with [^{18}F]FDG, molybdenum-99 and iodine-131, both by-products of uranium fission, were more rapidly converted to bedside products. Before satisfactory methods for imaging were developed, ^{131}I was already being used to treat thyroid cancer.[134] Technetium (^{99m}Tc), the most widely used radioisotope for diagnostics imaging (employed in 85% of all diagnostic imaging procedures),[135] is generated from ^{99}Mo. ^{99m}Tc has near ideal nuclear characteristics: 140 keV gamma ray emission for low-radiation doses to the patient and a 6-h half-life that allows same-day transport from radiopharmacies to hospitals. Technetium-based imaging was originally developed at Brookhaven National Laboratory in the 1960s.

It is unfortunate that lack of intellectual property protection covering the discovery of technetium-based imaging impeded investment in its development. Fortunately, this was compensated by the remarkably flexible chemistry of this transition metal. Technetium's facile chemistry allows preparation of small molecules for medical imaging of many organ-specific functions, including functions of metabolic bone, liver, kidney, heart, and brain. ^{99m}Tc has also been successfully used to 'tag' proteins, peptides, and mAb fragments.[136] Although the medical benefits of ^{99m}Tc agents were apparent to researchers early on,[137,138] widespread clinical usage did not occur until industry produced pharmaceutical grade ^{99m}Tc and packaged ^{99m}Tc reagents into practical 'kits.' Another reason ^{99m}Tc flourishes in clinical practice is that industry succeeded in reducing product costs to 10% or less of the total cost of the procedure (when one includes imaging and image interpretation).

Advancing clinical imaging technologies

In the 1970s, cyclotrons were explored for producing longer-lived SPECT (single photon emission tomography) isotopes such as ^{123}I, ^{201}Tl, and ^{111}In, now mainstays of nuclear medicine.[139–141] However, if these isotopes are prepared at a central production/manufacturing site with the intent of many days shelf-life, recently strengthened pharmaceutical regulations require that these radiopharmaceuticals be autoclaved, which greatly limits the type of products available. Radiopharmacies frequently finalize the preparation of SPECT agents, perform quality control, and provide unit doses to hospitals.

In the 1950s, imaging with rectilinear scanning improved with the introduction of multiple crystals and photomultiplier tubes.[142,143] The next great improvement was revolutionary: Multiple-head cameras and CT allowed tomography and the ability to visualize the localization of radioisotopes in a semi-quantitative manner. Today, researchers are optimistic that new solid-state materials from semi-conductor research

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such as cadmium telluride and zinc telluride will improve SPECT/CT images by significantly increasing detection sensitivity.[144]

Diagnostics imaging and therapeutics use and development

Image acquisition parameters remain critically important, and as imaging capabilities evolve the route to commercialization becomes more complex when it is intimately linked with the development of a radiopharmaceutical. An example is a recently approved automated analysis program that helps clinicians to detect abnormal brain activity. Every time improvements are made to the imaging software, regulatory approval is required. The program checks for abnormalities by comparing a patient's FDG PET brain scan to images in a database of age-matched normal subjects. To help the physician visualize patterns of abnormalities and their severity, the program presents its analysis using 3D stereotactic surface projection maps of the brain.[145] The technology may be particularly attractive for drug–disease combinations with other molecular tracers targeting neurodegenerative processes.[146]

Diagnostic imaging agents are sometimes integrated with a therapeutic development process. Two mAb regimens for relapsed or refractory non-Hodgkin lymphoma offer examples. In the first example, [^{131}I]Tositumomab (Bexxar; GlaxoSmithKline) is both the imaging and therapeutic agent. In the dosimetric step, the patient receives a relatively low dose of intravenous [^{131}I]Tositumomab. Following the dose, gamma camera and whole-body imaging are used to assess biodistribution of target cells (CD20-positive B lymphocytes) and calculate clearance kinetics. Provided that the biodistribution is acceptable, in the therapeutic step the patient receives an individualized dose of [^{131}I]Tositumomab. The dose is calculated based on the patient's total body clearance to provide a specified high level of ^{131}I total body irradiation.[147]

In the second example, Ibritumomab tiuxetan (Zevalin, Biogen Idec) also binds CD20 antigen. This mAb is radiolabeled with ^{111}In for imaging and ^{90}Y for cytotoxicity. Before the patient is administered [^{90}Y]Ibritumomab tiuxetan, the patient must first receive [^{111}In]Ibritumomab tiuxetan so that gamma imaging can be used to assess the biodistribution of the target cells. Only if the biodistribution meets defined criteria is it permissible to administer [^{90}Y]Ibritumomab tiuxetan, which kills cells by beta-emission and antibody-related cytotoxic mechanisms.[148]

Trends for the future

With the new wave of biotherapeutics being developed by pharmaceutical and biotechnology companies, imaging biomarkers are increasingly in demand. The success of dual-energy X-ray absorptiometry in measuring bone mineral density in menopausal women and monitoring biphosphonate therapy for osteoporosis[149] suggests that imaging biomarkers for therapeutic response have huge potential. FDG PET/CT imaging has already contributed significantly in cancer radiation therapy planning and treatment monitoring.[150,151] Numerous imaging agents to detect proliferation, apoptosis, angiogenesis, hypoxia, and other diagnostic characteristics are being developed as biomarkers in cardiology, oncology, and neurology.[29,152–154] By enabling patient stratification and quantification of drug benefits, they will lead to more efficient clinical trials and quicker approval of innovative new drugs. Another benefit of using biomarker diagnostic agents in tandem with therapeutics is providing better guidance in choosing therapies. For example, a biomarker to stage tumors by angiogenesis might be used to decide between anticancer regimens. Detecting high-risk individuals earlier may lead to earlier interventions and better outcomes. The time, cost, distress, and unnecessary adverse drug events associated with inaccurate diagnoses would be lessened.

The hurdles for these new molecular imaging agents to gain widespread clinical use are significant. For example, radiolabeled Annexin V was considered one of the most promising agents for imaging apoptosis in many diseases (oncology, cardiology, autoimmune disease) where large numbers of cells die.[155] Theseus Imaging Corporation (a wholly owned subsidiary of North American Scientific, Inc., Chatsworth, CA) invested a significant amount of effort in developing a ^{99m}Tc-based apoptosis imaging agent for oncology applications. A Phase II study with the agent combined broad cytotoxic agents, steroids, and apoptosis imaging all within a 24-h period. In a very complex tumor environment, the imaging results were difficult to interpret, and further development was cancelled. In this instance, the cost of developing an imaging agent in combination with complex biology and pressures related to time-to-market and regulatory hurdles prevented this promising imaging candidate from forging ahead in the commercial arena.[156]

Using molecular imaging early in health care requires demonstrating better therapy and healthcare economic value. The recent report of imaging patients with amnestic mild cognitive impairment with [^{11}C]PIB, a beta-amyloid-specific tracer, provides just such evidence.[49] These patients convert to Alzheimer's disease

at the rate of 10–15% per year; a five-year delay of onset is estimated to decrease by 50% the prevalence of the disease.[157] As treatment options increase, with multiple therapeutics targeting different molecules involved in Alzheimer's pathophysiology, molecular imaging could guide physicians in which drugs to try first, based on their benefits and risks.

In the future we will have amassed numerous advanced technologies to combat the scourge of chronic disease. Advances in genomics and proteomics will be embedded in new *in vitro* diagnostics and combined with family history and other epidemiological data within our electronic patient records to stratify populations into 'risk groups'. Risk group stratification will help identify asymptomatic patients at high risk of disease. Molecular imaging may then be pursued for earlier diagnoses and earlier interventions, locating lesions, measuring their characteristics, and monitoring responses to treatment. Other imaging modalities such as optical imaging with fluorescent markers and MRI with hyperpolarized agents will also help, with each modality having specific advantages. We may also expect more multi-modality imaging; the convergence of PET/CT may be followed by Optical/MRI or PET/MRI convergence.[158,159] We may also see in the future an acceptance of reporter gene imaging in patients for cell tracking or monitoring stem cell treatment.

Summary

In 1988, Sir James Black won the Nobel Prize for Physiology and Medicine for realizing the pharmacotherapeutic potential of receptor-blocking drugs. He developed the first clinically useful beta-adrenergic receptor antagonist, propranolol, and the first clinically useful H_2-receptor antagonist, cimetidine. Sir James shared the award with Gertrude Elion and George Hitchings, who demonstrated differences in nucleic acid metabolism between normal human and cancer cells. On the basis of these differences a series of drugs were developed that blocked nucleic acid synthesis in cancer cells.

Molecular imaging builds on the work of these giants in our field. Molecular imaging enables us to measure in living humans receptor distribution and occupancy by exogenous therapeutics, and also downstream effects on molecular processes such as tumor metabolism and replication. Indeed, imaging has become the method of choice for longitudinal *in vivo* assessment of even the most novel therapeutic approaches, including gene, stem cell, and RNAi therapy. Tracers being used today not only characterize pathophysiology, but also inform choice of treatment and help in assessing the adequacy of therapy in terms of anatomic distribution, viability, replication, molecular effect, and systems biology. In other words, tracers can lead to optimized therapy on an individual basis. From basic research at the bench to biomarkers in applied pharmaceutical development to commercial diagnostics, imaging offers hope.

Real-time studies of receptors and pathways in the brain, tumors, and other tissues enhance development and validation of pre-clinical *in vivo* and *in vitro* models of human disease. Molecular imaging is particularly well suited to these applications. It is non-invasive, and therefore can be used in longitudinal *in vivo* studies in similar disease models in both animals and humans. Furthermore, 'tracer' doses using 'organic' isotopes can quantitatively assess pathophysiology with minimal perturbation of systems being studied. Molecular imaging, and particularly PET-CT, has taken us to quantitation of sub-nanomolar molecular events in a clinically relevant milieu *in vivo*. We have come far since Hevesy's tracer principle of 1923 of a radioactive atom as 'representative of stable atoms of the same element,' and Michaelis–Menten concepts of enzyme velocity as a function of substrate concentration.

In cardiology, molecular imaging agents are responsible for major advances. The most important agents are based on ^{99m}Tc complexes, such as the cationic ligand [^{99m}Tc]Myoview, which after eight years in development by Amersham International (now owned by GE Healthcare) was approved by the FDA in 1996. With a photon energy peak optimal for gamma camera imaging and a short half-life favorable for patient dosimetry, ^{99m}Tc-labeled myocardial perfusion agents overcome limitations of earlier agents based on ^{43}K, ^{81}Rb, and ^{201}Tl. Coupled with SPECT imaging technologies, Myoview and Cardiolite ([^{99m}Tc]MIBI; Bristol-Myers Squibb Medical Imaging) make non-invasive evaluation of regional myocardial blood flow possible for millions of people each year. ^{99m}Tc-based molecular imaging has remarkably enhanced our ability to diagnose CAD, assess prognosis, detect viable myocardium, and evaluate therapies aimed at improving myocardial blood flow.

Dramatic developments in labeling strategies have removed the major limitations to applications for PET tracers. The multiplicity of reactive intermediates for incorporation of ^{11}C into organic molecules allows almost any molecule to be labeled within the constraint of its short half-life. Development of low-energy cyclotron methods for generating a full range of positron emitters enables labeling strategies independent of ^{11}C, and with a wider range of half-lives. The feasibility of PET studies in clinical research and diagnostics is dramatically improved. In drug development, tracers enable the enrichment of patient populations with

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likely responders, confirmation of penetration to site of action, and quantitation of mechanistic effects. As diagnostics, the same tracers can identify candidates for therapy, confirm ADMET (absorption, distribution, metabolism, excretion, and transport), and define magnitude of treatment benefit as a basis for adjustment of regimen.

Information technology has synthesized molecular imaging information across platforms and species. Imaging technology enables co-registration of images of high spatial resolution (CT, MR) with images of high molecular information content (PET), and unbiased, automated, and quantitative algorithmic assessment of resulting images. Linking imaging data across disparate platforms, including gene expression profiling, will inform diagnoses and initial choices of therapy, and individualize treatment regimens via monitoring of the magnitude of benefit after therapy has begun; that is to say, converting information to insight. Using Electronic Medical Records for post-marketing studies of new drugs will incorporate the results of imaging studies of drug effect to link biomarkers in FDA-mandated efficacy studies to community-based effectiveness studies more relevant to the payor community, such as NICE and CMS.

Standing on the shoulders of the great thinkers and doers like Hevesy, Anger, Wolf, and Black, we are on the verge of great leaps forward in health care, if only we will allow ourselves to achieve it.

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REFERENCES

1. Frank R, Hargreaves R. *Nat Rev Drug Discov* 2003; **2**: 566–580.
2. Taine HA. Histoire de la France 1891; **7**: 28.
3. Torstenson R, Tedroff J, Hartvig P, Fasth KJ, Långström B. *J Cereb Blood Flow* 1999; **19**: 1142–1149.
4. Långström B, Bergson G. *Radiochem Radioanal Lett* 1980; **43**: 47–54.
5. Långström B, Obenius U, Sjöberg S, Bergson G. *J Radioanal Chem* 1981; **64**: 273–280.
6. Langstrom B, Kihlberg T, Bergstrom M, Antoni G, Bjorkman M, Forngren BH, Forngren T, Hartvig P, Markides K, Yngve U, Ogren M. *Acta Chem Scand* 1999; **53**: 651–669.
7. De Hevesy G. *Biochim J* 1923; **17**: 439–445.
8. Frost JJ, Wagner Jr HN, Dannals RF, Ravert HT, Links JM, Wilson AA, Burns HD, Wong DF, McPherson RW, Rosenbaum AE. *J Comput Assist Tomogr* 1985; **9**: 231–36.
9. Larsen P, Ulin J, Dahlström K, Jensen M. *Appl Radiat Isot* 1997; **48**: 153–157.
10. Kihlberg T, Karimi F, Långström B. *J Org Chem* 2002; **67**: 3687–3692.
11. Christman DR, Finn RD, Karlstrom KI, Wolf AP. *Int J Appl Radiat Isot* 1975; **26**: 435–441.
12. Långström B, Lundqvist H. *Int J Appl Radiat Isot* 1976; **27**: 357–363.
13. Comar D, Carton JC, Maziere M, Marazano C. *Eur J Nucl Med* 1976; **1**: 11–14.
14. Christman DR, Crawford EJ, Friedkin M, Wolf AP. *Proc Natl Acad Sci USA* 1972; **69**: 988–992.
15. Roeda D, Crouzel C, Van Zanten B. *Radiochem Radioanal Lett* 1978; **33**: 175–178.
16. Langstrom B, Rahman O, Itsenko O. *J Lab Radiopharmaceut Label Comp* 2007.
17. Shah F, Hume SP, Pike VW, Ashworth S, McDermott J. *Nucl Med Biol* 1994; **21**: 573–581.
18. Berger G, Maziere M, Knipper R, Prenant C, Comar D. *Int J Appl Radiat Isot* 1979; **30**: 393–399.
19. Ehrin E, Gawell L, Högbwerg T, de Pauli T, Ström P. *J Label Compd Radiopharm* 1986; **24**: 931–940.
20. Wagner HN, Burns HD, Dannals RF, Wont DF, Långström B, Duelfers T, Frost JJ, Ravert HT, Links JM, Rosenbloom SB, Lukas SE, Kramer AV, Kuhar MU. *Science* 1983; **221**: 1264–1266.
21. Gefvert O, Lundberg T, Wieselgren IM, M B, Långstrom B. *Eur J Pharmacol* 2001; **11**: 105–110.
22. Björkman M, Andersson Y, Doi H, Kato K, Suzuki M, Noyori R, Watanabe Y, Långström B. *Acta Chem Scand* 1998; **52**: 635–640.
23. Andersson Y, Långström B. *J Label Compd Radiopharm* 1995; **37**: 84–87.
24. Bjurling ÅP, Watanabe Y, Tokushige M, Oda T, Långström B. *J Chem Soc Perkin Trans* 1989; 1331–1334.
25. Bjurling P, Watanabe Y, Oka S, Nagasawa T, Yamada H, Långström B. *Acta Chem Scand* 1990; **44**: 183–188.
26. Bjurling P, Antoni G, Watanabe Y, Långström B. *Acta Chem Scand* 1990; **44**: 172–182.
27. Eriksson B, Örlefors H, Öberg K, Sundin A, Bergström M, Långström B. *Best Pract Res Clin Endocrinol Metab* 2005; **19**: 311–324.
28. Örlefors H, Sundin A, Gaske U, Juhlin C, Öberg K, Skogseid B, Långström B, Bergström M, Eriksson. *J Clin Endocrinol Metab* 2005; **90**: 3392–3400.
29. Mankoff DA, Shields AF, Krohn KA. *Radiol Clin North Am* 2005; **43**: 153–167.

30. DiMasi JA. *Clin Pharm Ther* 2001; **69**: 297–307.
31. Galluppi GR, Rogge MC, Roskos LK, Lesko LJ, Green MD, Feigal Jr DW, Peck CC. *Clin Pharmacol Ther* 2001; **69**: 387–399.
32. Grahnen A, Karlsson MO. *Clin Pharmacokinet* 2001; **40**: 317–325.
33. Lesko LJ, Rowland M, Peck CC, Blaschke TF. *J Clin Pharmacol* 2000; **40**: 803–814.
34. Reigner BG, Williams PE, Patel IH, Steimer JL, Peck C, van Brummelen P. *Clin Pharmacokinet* 1997; **33**: 142–152.
35. Sheiner LB. *Clin Pharm Ther* 1997; **61**: 275–291.
36. Barker J, Garner RC. *Rapid Commun Mass Spectrom* 1999; **13**: 285–293.
37. Garner RC, Barker J, Flavell C, Garner JV, Whattam M, Young GC, Cussans N, Jezequel S, Leong D. *J Pharm Biomed Anal* 2000; **24**: 197–209.
38. Garner RC. *Curr Drug Metab* 2000; **1**: 205–213.
39. Bergström M, Grahne'n A, Långstrom B. *Eur J Pharmacol* 2003; **59**: 357–366.
40. Axelsson S, Engdahl KÅ, Långström B, Matsson O. *J Am Chem Soc* 1990; **112**: 1156–1161.
41. Axelsson S, Matsson O, Långström B. *J Phys Org Chem* 1991; **4**: 77–86.
42. Matsson O, Persson J, Axelsson S, Långström B. *J Am Chem Soc* 1993; **115**: 5288–5289.
43. Fowler JS, Wolf AP, MacGregor RR, Dewey SL, Logan J, Schlyer DJ, Langstrom B. *J Neurochem* 1988; **51**: 1524–1534.
44. Schou M, Halldin C, Sóvágó J, Pike VW, Hall H, Gulyas B, Mozley DP, Dobson D, Schukin E, Innis RB, Farde L. *Synapse* 2004; **53**: 57–67.
45. Bergström M, Långström B. Pharmacokinetic studies with PET. In *Progress in Drug Research*, Rhudin M (ed.). 2005; **62**.
46. Engler H, Forsberg A, Almkvist O, Blomquist G, Larsson E, Savitcheva I, Wall A, Ringheim A, Langstrom B, Nordberg A. *Brain* 2006; **129**: 2856–2866.
47. Bleicher KH, Bohm HJ, Muller K, Alanine AI. *Nat Rev Drug Discov* 2003; **2**: 369–378.
48. Pritchard JF, Jurima-Romet M, Reimer ML, Mortimer E, Rolfe B, Cayen MN. *Nat Rev Drug Discov* 2003; **2**: 542–553.
49. Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, Bergstrom M, Savitcheva I, Huang GF, Estrada S, Ausen B, Debnath ML, Barletta J, Price JC, Sandell J, Lopresti BJ, Wall A, Koivisto P, Antoni G, Mathis CA, Langstrom B. *Ann Neurol* 2004; **55**: 306–319.
50. Sirol M, Aguinaldo JG, Graham PB, Weisskoff R, Lauffer R, Mizsei G, Chereshnev I, Fallon JT, Reis E, Fuster V, Toussaint JF, Fayad ZA. *Atherosclerosis* 2005; **182**: 79–85.
51. Bach-Gansmo T, Danielsson R, Saracco A, Wilczek B, Bogsrud TV, Fangberget A, Tangerud A, Tobin D. *J Nucl Med* 2006; **47**: 1434–1439.
52. Vanderheyden JL, Liu G, He J, Patel B, Tait JF, Hnatowich DJ. *Nucl Med Biol* 2006; **33**: 135–144.
53. Udenfriend S, Gerber LD, Brink L, Spector S. *Proc Natl Acad Sci USA* 1985; **82**: 8672–8676.
54. Wu S, Liu B. *BioDrugs* 2005; **19**: 383–392.
55. Eckelman WC, Gibson RE. The design of site-directed radiopharmaceuticals for use in drug discovery. In *Nuclear Imaging in Drug Discovery, Development and Approval*, Burns HD, Gibson RE (eds). Birkhauser: Boston, 1992; 113–134,
56. Rich RL, Myszka DG. *Curr Opin Biotechnol* 2000; **11**: 54–61.
57. Lofas S. *Assay Drug Dev Technol* 2004; **2**: 407–415.
58. Myszka DG, Rich RL. *Pharm Sci Technol Today* 2000; **3**: 310–317.
59. Montalto MC, Collard CD, Buras JA, Reenstra WR, McClaine R, Gies DR, Rother RP, Stahl GL. *J Immunol* 2001; **166**: 4148–4153.
60. Day YS, Baird CL, Rich RL, Myszka DG. *Protein Sci* 2002; **11**: 1017–1025.
61. Shoghi-Jadid K, Small GW, Agdeppa ED, Kepe V, Ercoli LM, Siddarth P, Read S, Satyamurthy N, Petric A, Huang SC Barrio JR. *Am J Geriatr Psychiatry* 2002; **10**: 24–35.
62. Lockhart A, Ye L, Judd DB, Merritt AT, Lowe PN, Morgenstern JL, Hong G, Gee AD, Brown J. *J Biol Chem* 2005; **280**: 7677–7684.
63. Gandy SE, Buxbaum JD, Suzuki T, Ramabhadran TV, Caporaso GL, Nairn AC, Greengard P. *Neurobiol Aging* 1992; **13**: 601–603.
64. Gandy S, Petanceska S. *Biochim Biophys Acta* 2000; **1502**: 44–52.
65. Shoghi-Jadid K, Barrio JR, Kepe V, Huang SC. *Mol Imaging Biol* 2006; **8**: 151–162.
66. Shoghi-Jadid K, Barrio JR, Kepe V, Wu HM, Small GW, Phelps ME, Huang SC. *Nucl Med Biol* 2005; **32**: 337–351.
67. Simmons MK, Manjeshwar R, Agdeppa ED, Mattheyses RM, Kiehl TR, Montalto MC. *Mol Imaging Biol* 2005; **7**: 69–77.
68. Zhang X, Xiong Z, Wu Y, Cai W, Tseng JR, Gambhir SS, Chen X. *J Nucl Med* 2006; **47**: 113–121.
69. Pozner-Moulis S, Pappas DJ, Rimm DL. *Cancer Res* 2006; **66**: 7976–7982.

70. Camp RL, Chung GG, Rimm DL. *Nat Med* 2002; **8**: 1323–1327.
71. McCabe A, Dolled-Filhart M, Camp RL, Rimm DL. *J Natl Cancer Inst* 2005; **97**: 1808–1815.
72. Balimane PV, Han YH, Chong S. *AAPS J* 2006; **8**: E1–E13.
73. Patel S, Ndubizu O, Hamill T, Chaudhary A, Burns HD, Hargreaves R, Gibson RE. *Mol Imaging Biol* 2005; **7**: 314–323.
74. Caro LG. *J Biophys Biochem Cytol* 1961; **9**: 539–553.
75. Caro LG, Forro Jr F. *J Biophys Biochem Cytol* 1961; **9**: 555–565.
76. Caro LG, Van Tubergen RP, Kolb JA. *J Cell Biol* 1962; **15**: 173–188.
77. Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M. *J Neurochem* 1977; **28**: 897–916.
78. Caviness Jr VS. *Brain Res* 1982; **256**: 293–302.
79. Bayer SA, Altman J, Russo RJ, Dai XF, Simmons JA. *J Comp Neurol* 1991; **307**: 499–516.
80. Ignacio MP, Kimm EJ, Kageyama GH, Yu J, Robertson RT. *Anat Embryol* (*Berl*) 1995; **191**: 89–100.
81. Phelps ME, Huang SC, Hoffman EJ, Selin C, Sokoloff L, Kuhl DE. *Ann Neurol* 1979; **6**: 371–388.
82. Huang SC, Phelps ME, Hoffman EJ, Sideris K, Selin CJ, Kuhl DE. *Am J Physiol* 1980; **238**: E69–E82.
83. Solon EG, Balani SK, Lee FW. *Curr Drug Metab* 2002; **3**: 451–462.
84. Potchoiba MJ, Nocerini MR. *Drug Metab Dispos* 2004; **32**: 1190–1198.
85. Melega WP, Luxen A, Perlmutter MM, Nissenson CH, Phelps ME, Barrio JR. *Biochem Pharmacol* 1990; **39**: 1853–1860.
86. Gambhir SS, Barrio JR, Phelps ME, Iyer M, Namavari M, Satyamurthy N, Wu L, Green LA, Bauer E, MacLaren DC, Nguyen K, Berk AJ, Cherry SR, Herschman HR. *Proc Natl Acad Sci USA* 1999; **6**: 2333–2338.
87. Varnas K, Halldin C, Hall H. *Hum Brain Mapp* 2004; **22**: 246–260.
88. Bergstrom M, Awad R, Estrada S, Malman J, Lu L, Lendvai G, Bergstrom-Pettermann E, Langstrom B. *Mol Imaging Biol* 2003; **5**: 390–396.
89. Kung MP, Kung HF. *Nucl Med Biol* 2005; **32**: 673–678.
90. Gibson RE, Burns HD, Hamill TG, Eng WS, Francis BE, Ryan C. *Curr Pharm Des* 2000; **6**: 973–989.
91. Hoffman EJ, Huang SC, Phelps ME. *J Comput Assist Tomogr* 1979; **3**: 299–308.
92. Burns RS, Chiueh CC, Markey SP, Ebert MH, Jacobowitz DM, Kopin IJ. *Proc Natl Acad Sci USA* 1983; **80**: 4546–4550.
93. Albanese A, Granata R, Gregori B, Piccardi MP, Colosimo C, Tonali P. *Neuroscience* 1993; **55**: 823–832.
94. Tanner CM. *Trends Neurosci* 1989; **12**: 49–54.
95. Scheller D, Chan P, Li Q, Wu T, Zhang R, Guan L, Ravenscroft P, Guigoni C, Crossman AR, Hill M, Bezard E. *Exp Neurol* 2006; 11 [Epub ahead of print].
96. Palm S, Enmon Jr RM, Matei C, Kolbert KS, Xu S, Zanzonico PB, Finn RL, Koutcher JA, Larson SM, Sgouros G. *J Nucl Med* 2003; **44:** 1148–1155.
97. Gambhir SS, Barrio JR, Wu L, Iyer M, Namavari M, Satyamurthy N, Bauer E, Parrish C, MacLaren DC, Borghei AR, Green LA, Sharfstein S, Berk AJ, Cherry SR, Phelps ME, Herschman HR. *J Nucl Med* 1998; **39**: 2003–2011.
98. Alauddin MM, Conti PS, Mazza SM, Hamzeh FM, Lever JR. *Nucl Med Biol* 1996; **23**: 787–792.
99. Morin KW, Atrazheva ED, Knaus EE, Wiebe LI. *J Med Chem* 1997; **40**: 2184–2190.
100. Tjuvajev JG, Stockhammer G, Desai R, Uehara H, Watanabe K, Gansbacher B, Blasberg RG. *Cancer Res* 1995; **55**: 6126–6132.
101. Tjuvajev JG, Finn R, Watanabe K, Joshi R, Oku T, Kennedy J, Beattie B, Koutcher J, Larson S, Blasberg RG. *Cancer Res* 1996; **56**: 4087–4095.
102. Tjuvajev JG, Avril N, Oku T, Sasajima T, Miyagawa T, Joshi R, Safer M, Beattie B, DiResta G, Daghighian F, Augensen F, Koutcher J, Zweit J, Humm J, Larson SM, Finn R, Blasberg R. *Cancer Res* 1998; **58**: 4333–4341.
103. Tjuvajev JG, Chen SH, Joshi A, Joshi R, Guo ZS, Balatoni J, Ballon D, Koutcher J, Finn R, Woo SL, Blasberg RG. *Cancer Res* 1999, **59**: 5186–5193.
104. Serganova I, Doubrovin M, Vider J, Ponomarev V, Soghomonyan S, Beresten T, Ageyeva L, Serganov A, Cai S, Balatoni J, Blasberg R, Gelovani J. *Cancer Res* 2004; **64**: 6101–6108.
105. Yaghoubi S, Barrio JR, Dahlbom M, Iyer M, Namavari M, Satyamurthy N, Goldman R, Herschman HR, Phelps ME, Gambhir SS. *J Nucl Med* 2001; **42**: 1225–1234.
106. Balogh L, Andócs G, Thuróczy J, Németh T, Láng J, Bodó K, Jánoki GA. *Acta Veterinaria Brno* 1999; **68**: 231–239.
107. Zaret BL, Strauss HW, Martin ND, Wells Jr HP, Flamm Jr MD. *N Engl J Med* 1973; **288**: 809–812.

108. Beller GA, Bergmann SR. *SPECT PET J Nucl Cardiology* 2004; **11**: 71–86.
109. Berman DS, Salel AF, DeNardo GL, Mason DT. *Circulation* 1975; **52**: 619–626.
110. Strauss HW, Pitt B. *Semin Nucl Med* 1977; **7**: 49–58.
111. Kailsanath P, Sinusas A. *Cardiol Rev* 2001; **9**: 160–172.
112. Deutsch E, Bushong W, Glavan KA. *Science* 1981; **214**: 85–86.
113. Deutsch E, Ketering AR, Libson K, Vanderheyden J-L, Hirth WJ. *Int J Rad Appl Instrum* 1989; **16**: 191–232.
114. Kelly JD, Higley B, Archer CM. *J Nucl Med* 1989: **30**: 733.
115. Holman LB, Jones AG, Lister-James J. *J Nucl Med* 1984; **25**: 1350–1355.
116. Strauss HW, Harrison K, Langan JK, Lebowitz E, Pitt B. *Circulation* 1975; **51**: 641–645.
117. Trampisch W, Sattler EL, Schraub A. *Biophysik* 1971; **7**: 355–357.
118. Tilbury RS, Dahl JR, Mamacos JP. *Int J Appl Radiat Isot* 1970; **21**: 277–281.
119. Billinghurst MW. *Studies of Cellular Function using Radiotracers*. Colombetti LG (ed.). CRC Press: Boca Raton, FL, USA, 1982; 250. Reviewed in E. Anthony Evans. *J Label Compd Radiopharm* 1983; **20**: 785–786.
120. Janbar M, Neta P. *Int J Appl Radiat Isot* 1963; **14**: 119–122.
121. Ter-Pogossian MM, Phelps ME, Hoffman EJ, Mullani NA. *Radiology* 1975; **114**: 89–98.
122. Hoffmann EJ, Phelps ME, Mullani NA, Higgins CS, Ter-Pogossian MM. *J Nucl Med* 1976; **17**: 493–502.
123. Budinger TF. *J Nucl Med* 1983; **24**: 73–78.
124. Nehmeh SA, Erdi YE, Ling CC, Rosenzweig KE, Schoder H, Larson SM, Macapinlac HA, Squire OD, Humm JL. *J Nucl Med* 2002; **43**: 876–881.
125. Nehmeh SA, Erdi YE, Pan T, Pevsner A, Rosenzweig KE, Yorke E, Mageras GS, Schoder H, Vernon P, Squire O, Mostafavi H, Larson SM, Humm JL. *Med Phys* 2004; **31**: 3179–3186.
126. Gallagher BM, Ansari A, Atkins H, Casella V, Christman DR, Fowler JS, Ido T, MacGregor RR, Som P, Wan CN, Wolf AP, Kuhl DE, Reivich M. *J Nucl Med* 1977; **18**: 990–996.
127. Som P, Atkins HL, Bandoypadhyay D, Fowler JS, MacGregor RR, Matsui K, Oster ZH, Sacker DF, Shiue CY, Turner H, Wan CN, Wolf AP, Zabinski SV. *J Nucl Med* 1980; **21**: 670–675.
128. Fowler JS, Wolf AP. The synthesis of carbon-II, fluorine-18 and nitrogen-13 labeled radiotracers for biomedical research. In *Pub. Technical Information Center U.S. Department of Energy, Oak Ridge, Tennessee, USA. Nuclear Science Series NAS-NS-3201* Sept. 1982; 124. Reviewed in E. Anthony Evans. *J Label Compd Radiopharm* 1983; **20**: 669–670.
129. Tewson TJ, Welch MJ, Raichle ME. *J Nucl Med* 1978; **19**: 1339–1345.
130. Siegel BA, Dehdashti F. *Eur Radiol* 2005; **15** (Suppl 4): D127–D132.
131. Barrio JR, MacDonald NS, Robinson Jr GD, Najafi A, Cook JS, Kuhl DE. *J Nucl Med* 1981; **22**: 372–375.
132. Tewson TJ, Krohn KA. *Semin Nucl Med* 1998; **28**: 221–234.
133. Lee CC, Sui G, Elizarov A, Shu CJ, Shin YS, Dooley AN, Huang J, Daridon A, Wyatt P, Stout D, Kolb HC, Witte ON, Satyamurthy N, Heath JR, Phelps ME, Quake SR, Tseng HR. *Science* 2005; **310**: 1793–1796.
134. Ner SC, Quimby EH, Schmidt C. *Am J Med* 1949; **7**: 731–740.
135. Richards P. *Int J Appl Radiat Isot* 1966; **17**: 427.
136. Deutsch E, Nicolini M, Wagner Jr HN. Technetium in chemistry and nuclear medicine. In *Proceedings of the International Symposium on Technetium in Chemistry and Nuclear Medicine*, Padua, Italy, 1982. Published by Cortina International: Verona, May 1984; 246. Distributed by Raven Press: New York City, USA. Reviewed in D. P. Nowotnik *J Label Compd Radiopharm* 1985; **22**: 867–868.
137. Pitt B, Strauss HW. *Am J Cardiol* 1976; **38**: 739–746.
138. Strauss HW, Betram P (eds). *Cardiovascular Nuclear Medicine* (2nd edn). C.V. Mosby Co: St. Louis, 1979; 429 Reviewed in Keith E. Britton. *J Label Compd Radiopharm* 1981; **18**: 1555–1556.
139. Thakur ML, Nunn AD. *Int J Appl Radiat Isot* 1972; **23**: 139–140.
140. Lambrecht RM, Wolf AP. *Radiat Res* 1972; **52**: 32–46.
141. Bradley-Moore PR, Lebowitz E, Greene MW, Atkins HL, Ansari AN. *J Nucl Med* 1975; **16**: 156–160.
142. Anger HO. Nature 1952; **170**: 200–201.
143. Anger HO. *Am J Roentgenol Radium Ther Nucl Med* 1953; **70**: 605–612.
144. Brzymialkiewicz CN, Tornai MP, McKinley RL, Bowsher JE. *IEEE Trans Med Imaging* 2005; **24**: 868–877.

DOI: 10.1002.jlcr

145. Minoshima S. *Neuroimaging Clin N Am* 2003; **13**: 769–780.
146. Tang BN, Minoshima S, George J, Robert A, Swine C, Laloux P, Borght TV. *Eur J Nucl Med Mol Imaging* 2004; **31**: 1487–1494.
147. Davies AJ. *Expert Opin Biol Ther* 2005; **5**: 577–588.
148. Wiseman GA, Gordon LI, Multani PS, Witzig TE, Spies S, Bartlett NL, Schilder RJ, Murray JL, Saleh M, Allen RS, Grillo-Lopez AJ, White CA. *Blood* 2002; **99**: 4336–4342.
149. Bach-Mortensen P, Hyldstrup L, Appleyard M, Hindso K, Gebuhr P, Sonne-Holm S. *Calcif Tissue Int* 2006; **79**: 1–6.
150. Shields AF. *Mol Imaging Biol* 2006; **8**: 141–150.
151. Larson SM, Carrasquillo JA. *Semin Nucl Med* 1984; **14**: 268–276.
152. Rajendran JG, Krohn KA. *Radiol Clin North Am* 2005; **43**: 169–187.
153. Lahorte CM, Vanderheyden JL, Steinmetz N, Van de Wiele C, Dierckx RA, Slegers G. *Eur J Nucl Med Mol Imaging* 2004; **31**: 887–919.
154. Haubner R, Wester HJ. *Curr Pharm Des* 2004; **10**: 1439–1455.
155. Pomper MG. *Cancer Imaging* 2005; **5**: S16–S26.
156. Nunn AD. *Invest Radiol* 2006; **41**: 206–212.
157. Brookmeyer R, Gray S, Kawas C. *Am J Pub Health* 1998; **88**: 1337–1342.
158. Sosnovik D, Weissleder R. *Prog Drug Res* 2005; **62**: 83–115.
159. Seemann MD. *Technol Cancer Res Treat* 2005; **4**: 577–582.