

Molecular imaging agents for clinical positron emission tomography in oncology other than fluorodeoxyglucose (FDG): applications, limitations and potential

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Dedicated to the memory of Dr. Antoine Noujaim

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ABSTRACT – Purpose: Recent efforts in radiopharmaceutical design for positron emission tomography (PET) imaging in clinical oncology have provided a library of tracers that have the potential to contribute to individualizing cancer patient management. These tracers can provide PET images that reveal aspects of the fundamental underlying biochemistry in the tumor before and during treatment. For a number of these PET tracers the cellular processing has been well described and they are now generally referred to as molecular imaging agents. Despite their recognized value in clinical oncology these tracers have not yet obtained widespread acceptance and are not generally available at centers performing PET scans. There are a number of barriers and challenges to the widespread use of these PET tracers that include; a limited clinical demand, challenges presented by the chemistry and formulation for clinical acceptability, the short physical half life of the PET radionuclides, regulatory issues and the overall costs associated with PET radiopharmaceutical production. In addition the interpretation of the PET images requires a clear understanding of the biochemical processes involved at the cellular level. A concerted effort is required among stakeholders including clinicians, scientists, industry and governmental agencies if the potential of these agents in clinical oncology is to be realized.

INTRODUCTION

2-Deoxy-2-¹⁸F-fluorodeoxyglucose (¹⁸F-FDG) is by far the most widely used positron emitting tomography (PET) radiopharmaceutical in clinical oncology. Over the last two decades this agent has proven to be an almost universally applicable

imaging agent for human cancers (1) in addition to its clinical applications in cardiology and neurology. The growth of ¹⁸F-FDG in clinical oncology has resulted from a combination of clinical demand, based on its accepted value in the detection and assessment of cancer, and an increasing availability fostered by regional ¹⁸F-FDG production sites and commercial sales of the radiotracer. Although it can not be said to be globally available it is now in use throughout North America, Western Europe and Japan with extensive availability in a number of other regions. Its expansion is likely to increase into additional jurisdictions.

The advent of clinical instruments that combine PET imaging with x-ray tomography scans (PET-CT) has added additional impetus for the growth of ¹⁸F-FDG PET imaging. Modern PET-CT instrumentation allows fused images that provide an exquisite combination of anatomical and functional information.

The widespread use of ¹⁸F-FDG and its accepted utility in tumor imaging has not however slowed the research efforts to identify additional PET tracers for oncology. With the power of ¹⁸F-FDG PET in oncology we might ask why we would need to expend effort to find additional agents. We can identify two incentives that encourage the search for novel PET tracers. Firstly ¹⁸F-FDG is recognized to have a number of limitations in tumor imaging which include;

- i) Normal uptake of ¹⁸F-FDG in metabolically active tissues including the brain makes tumors in or near these structures difficult to evaluate.
- ii) Tumors that are slow growing or highly differentiated often do not take up ¹⁸F-FDG adequately to permit definitive imaging. This has been noted with prostate tumors and with a number of neuroendocrine tumors (2-4).
- iii) ¹⁸F-FDG is excreted through the kidneys and urinary bladder making tumors in these organs hard to assess. This also complicates attempts to image prostate tumors.

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- iv) A number of hepatocellular carcinomas show relatively low uptake of ^{18}F -FDG related to the levels of cellular transport of the sugar by the GLUT transport system (5) or elimination of ^{18}F -FDG through up regulation of the intracellular glucose-6-phosphatase enzyme (6).
- v) ^{18}F -FDG is relatively non-specific and also accumulates at sites of inflammation and infection. Although in the proper context this increases the imaging indications for this radiopharmaceutical (7) it can be a significant complication in oncologic imaging in a number of settings including in post surgery assessment of residual tumor where inflammation and/or infection are likely to be present (8).

The second limitation of ^{18}F -FDG relates to its restricted applications in the growing field of molecular medicine. Molecular medicine is defined by the National Library of Medicine (9) as “A science that seeks to comprehend disease causes and mechanisms at the molecular level, and to apply

this basic research to the prevention, diagnosis and treatment of diseases and disorders.” Molecular medicine seeks to individualize patient treatment by creating a genetic and molecular profile specific to individual pathological processes in the body at a cellular level which can then be used to tailor disease therapy. Molecular imaging using PET is acknowledged to have a valuable role to play in this patient oriented science. In this context ^{18}F -FDG provides some very specific information about cellular dynamics related to energy requirements. The processes of cellular transport and metabolism of glucose and ^{18}F -FDG (Figure 1) have been extensively studied. ^{18}F -FDG uptake is a measure of tissue and cell glucose utilization (glycolysis). More specifically it can be regarded as a molecular imaging probe of hexose entry into cells mediated by the GLUT facilitative transporters as well as an indicator of cellular hexokinase enzyme activity. Gene mediated regulation of GLUT transporters and hexokinase activity contributes to the increased accumulation of ^{18}F -FDG in tumor cells relative to normal tissues.

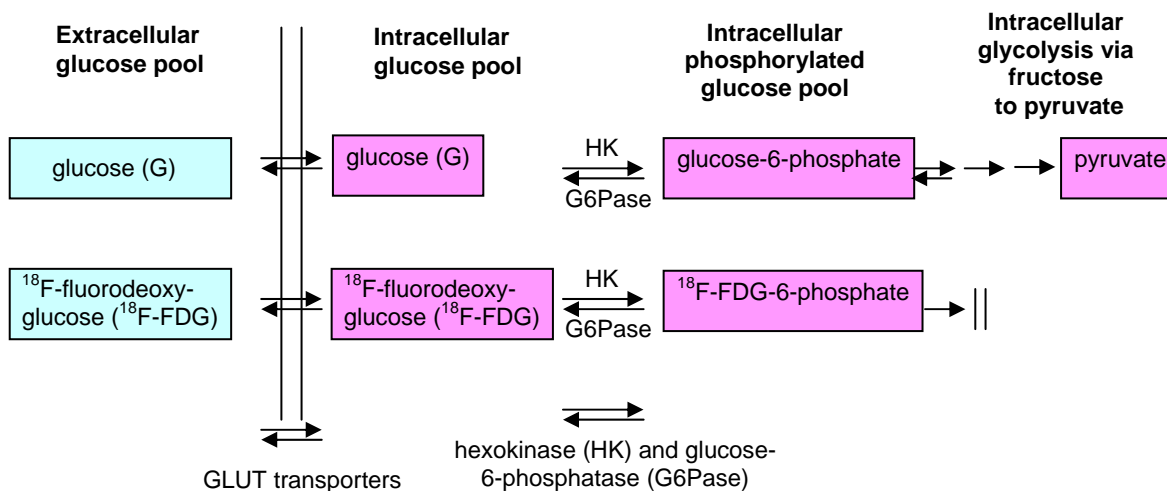


Figure 1. The transport and metabolism of glucose and 2-deoxy-2- ^{18}F -fluoro-D-glucose.

Table 1. Targets and agents for PET molecular imaging and their relevance in clinical oncology

Cellular Target	Relevance in Clinical Management of Cancer Patients	Potential, Clinically Relevant, PET Molecular Imaging Agent for Oncology
GLUT transporters, hexokinase activity	Tumor can be detected and graded through increased metabolism / energy utilization. Assessment of tumor response to therapy can be made.	¹⁸ F-DG, ¹¹ C-DG,
Amino acid transport and incorporation into polypeptides.	Tumors can be detected and graded through increased protein synthesis related to cell proliferation. Cellular growth kinetics and its alteration as a result of therapy can be measured.	¹¹ C-methionine, amino acid analogs of tyrosine, leucine, phenylalanine (¹⁸ F-fluoroethyltyrosine)
Nucleoside transport, nucleoside phosphorylation, DNA synthesis	Cell proliferation can be determined as a measure of tumor growth and growth kinetics. Cellular responses to therapy can be measured by changes in proliferative ability.	¹⁸ F-FLT, ¹¹ C-thymidine, ¹⁸ F-FMAU, ¹²⁴ I-UdR
Choline transport and choline kinase activity, phospholipid synthesis	Phospholipid synthesis for intracellular phospholipids and cell membranes relates to increased cell growth and division in proliferating tissue. Prostate tumors have high phospholipid synthesis rates. Measurement of tumor growth and response to therapy can be made.	¹¹ C-choline, ¹⁸ F-choline, ¹¹ C-acetate
Amine transport and decarboxylation	Amine precursor uptake and decarboxylation is a feature common to peptide producing endocrine tissue including neuroendocrine tumors. Tumors in this category can be detected and identified as having an endocrine origin.	¹⁸ F-FDOPA, ¹¹ C-hydroxytryptophan
Intracellular reductive environment, hypoxia	Hypoxic tumor tissue can be resistant to both chemotherapy and radiotherapy. Tumor response to oxygenation strategies can be measured.	⁶⁴ Cu-ATSM, ¹⁸ F-F-MISO, ¹⁸ F-FAZA
Cellular receptors / tumor markers	The presence or absence of cell cytosolic receptors can predict response to hormone therapy in breast and prostate tumors. Tumor heterogeneity and response to therapy can be measured. Tumor markers can be imaged.	¹⁸ F-FES, ¹⁸ F-dihydroxy-testosterone, ⁶⁸ Ga-DOTATOC, EGFR inhibitors
Apoptotic changes in cells	Cell death through apoptosis following therapy can be measured. Early assessment of imminent cell death can be made.	¹⁸ F-annexin V
Elevated or unique products from gene expression including enzymes and RNA.	Gene therapy can be monitored using a reporter gene strategy. mRNA can be targeted as a surrogate marker of gene expression using oligonucleotides.	¹⁸ F-gancyclovir/ HSV-1 tk reporter gene system, antisense oligonucleotides
Vascular endothelial factors related to angiogenesis	Angiogenesis accompanying tumor growth or therapy targeting microvascular development can be assessed.	Integrin/RDG peptide, VEGF/tk

Table 2. Physical properties of selected positron emitting radionuclides relevant in human clinical imaging.

Radionuclide	Mode of production	Half-life (Emissions)	β^+ energy (E_{\max}) MeV	Range of $E_{\max} \beta^+$ (mm in water)	Main chemistry used for radionuclide incorporation
Carbon-11	$^{14}\text{N}(p,\alpha)^{11}\text{C}$ (cyclotron)	20.4 min (β^+ only)	0.97	4	Methylation, covalent bond formation
Nitrogen-13	$^{16}\text{O}(p,\alpha)^{13}\text{N}$ (cyclotron)	9.9 min (β^+ only)	1.20	5	C-N covalent bonds, gas phase reactions
Oxygen-15	$^{14}\text{N}(d,n)^{15}\text{O}$ (cyclotron)	122 sec (β^+ only)	1.74	8	in target oxygen gases, water production
Fluorine-18	$^{18}\text{O}(p,n)^{18}\text{F}$ (cyclotron)	110 min (β^+ only)	0.64	2.5	Nucleophilic, electrophilic substitution
Copper-62	$^{63}\text{Cu}(p,2n)^{62}\text{Zn}$, $^{62}\text{Zn} \rightarrow ^{62}\text{Cu}$ (generator)	9.74 min (β^+ only)	2.9	13	Metal chelation
Copper-64	$^{64}\text{Ni}(p,n)^{64}\text{Cu}$ (cyclotron) $\text{Zn}(\text{various})^{64}\text{Cu}$ (cyclotron)	12.7 hr (β^+ , β^- , gamma)	0.66	2.6	Metal chelation
Gallium-68	$^{69}\text{Ga}(p,2n)^{68}\text{Ge}$, $^{68}\text{Ge} \rightarrow ^{68}\text{Ga}$ (generator)	68.3 min (β^+ , gamma)	1.9	9	Metal chelation
Bromine-76	$^{76}\text{Se}(p,n)^{76}\text{Br}$ (cyclotron)	16.2 hr (β^+ , gamma)	3.94	20	Halide addition of substitution
Iodine-124	$^{124}\text{Te}(p,n)^{124}\text{I}$ (cyclotron)	4.15 day (β^+ , gamma)	2.1	10	Halide addition or substitution

^{18}F -FDG's usefulness as a molecular imaging probe depends on the concept of bioisosterism in which the substitution of the fluorine group for the hydroxyl group produces a compound that has similar physicochemical and biological properties to the parent glucose. Efforts to produce a GLUT transportable glucose analog labeled with conventional single photon emitters such as $^{99\text{m}}\text{Tc}$, ^{123}I or ^{111}In have not met with success. The GLUT transporters will have relatively stringent requirements for the molecules they transport. Similarly the substrate specificity of enzymatic systems like hexokinase will not tolerate substantial alteration of the hexose structure. An important distinction must be made between the behavior of ^{18}F -FDG and glucose at the cellular level. ^{18}F -FDG is metabolically trapped after phosphorylation to ^{18}F -FDG-6-phosphate. Unlike glucose it is not a substrate for the subsequent enzymatic conversion to fructose-6-phosphate by phosphohexose

isomerase and thus does not further participate in the glycolytic pathway. Subtle differences between glucose and ^{18}F -FDG may also be expected when they are transported by GLUTs and phosphorylated by hexokinase. ^{18}F -FDG must be regarded as a surrogate marker for glucose although for some processes (transport and phosphorylation) the two molecules have very similar behavior.

^{18}F -FDG AND OTHER PET MOLECULAR IMAGING AGENTS IN CLINICAL ONCOLOGY

There are many molecular targets relevant to cancer that are not measured or assessed by ^{18}F -FDG. Several recent reviews have addressed this issue (10,11). Table 1 provides a list of currently assessable molecular targets in tumor cells and tissues along with a description of their relevance in clinical management of cancer patients and a list of

agents that have seen some significant clinical application as PET imaging agents or where clinical introduction of PET molecular imaging agents is imminent.

Table 2 provides a list of PET radionuclides that are discussed in this paper along with their physical properties and method of production. At present the library of PET clinical tracers in oncology include;

Agents Targeting GLUT Transporters and Hexokinase Enzyme Activity

Rapidly growing cancer cells will exhibit high rates of metabolism accompanied by a requirement for glucose to support cellular energy processes. Uptake of glucose is mediated by the high level of expression of both the facilitated hexose transporter GLUT1 and the enzyme hexokinase. In this regard ^{18}F -FDG represents a somewhat unique situation in that the transport and trapping mechanisms result in high concentrations of radioactivity in target tissues and consequently high tumor to background ratios which contribute to effective PET imaging. For many of the other molecular imaging agents there will be a much lower concentration of activity expected at the target site. In addition to ^{18}F -FDG as discussed above there is at present no other suitable PET radiopharmaceutical to assess glycolysis. There is some incentive to explore additional sugar analogs based on the recognized alteration in GLUT expression and substrate specificity for transport in certain tumor tissues (12). ^{11}C -glucose has been synthesized and examined in PET studies (13,14) but its application in oncology is hampered by the short physical half-life of the radionuclide which is not compatible with either the synthesis or biological disposition of ^{11}C -glucose. The analysis of ^{11}C glucose is further complicated by its conversion to labeled metabolites which show rapid efflux from tissues.

Amino Acids for Transport and Incorporation into Polypeptides

A number of amino acid analogs have been used as PET imaging agents in oncology. Amino acids are the building blocks for cellular proteins and other polypeptides that are produced to accompany cell proliferation and tissue expansion during tumor growth. Tumor cells can exhibit increased amino acid transport and increased protein synthesis rates which can serve as molecular imaging targets for radiolabeled amino acid analogs.

There are many examples of the synthesis and in vivo investigation of PET amino acid analogs (15) but only a few have any substantial impact in clinical oncology. Methyl- ^{11}C -L-methionine (^{11}C -MET) has been used widely in PET imaging of brain tumors where it provides high contrast images. The very low level of amino acid utilization by normal brain permits the visualization of even relatively slow growing brain tumors (16). The short half life of the ^{11}C label has stimulated the development of longer lived PET amino acid tracers of which the most widely used clinical product is O-(2- ^{18}F fluoroethyl)-L-tyrosine (^{18}F -FET) first synthesized and used in humans in 1999 (17). A recent review explores the use of this tracer predominantly in brain tumors (18). FET is transported by amino acid transporters and accumulates in cells. However this amino acid analog does not appear to be involved in protein synthesis (18). Despite its inability to mimic amino acids and participate in macromolecule synthesis the uptake of ^{18}F -FET has been shown to correlate closely with ^{11}C -MET which shares the same transport system and which is readily incorporated into proteins (19).

Nucleoside Analogs for Measurement of Cellular Nucleic Acid Utilization and Cell Proliferation

Cell proliferation requires the expansion of the nucleic acid content in the cell prior to division. This is accomplished by the transport of nucleosides or their de novo synthesis as building blocks for DNA. Thymidine is a recognized marker for cell proliferation being moved into cells by the nucleoside transport system and being rapidly incorporated into DNA. Additionally thymidine, unlike the other three nucleoside building blocks is incorporated into DNA but not into RNA making it an accurate probe for DNA replication. ^{11}C -thymidine was first described as an in vivo marker of DNA synthesis in 1972 (20) and it has been used sporadically as a clinical measure of cell proliferation. Because it is modified only by the substitution of ^{11}C for ^{12}C it will behave identically to the native nucleoside (21). The major problems encountered with ^{11}C -thymidine relate to the short half-life of ^{11}C and to the catabolism of thymidine, mediated by the enzyme thymidine phosphorylase, leading to the production of ^{11}C -labeled metabolites (Figure 2).

A recently developed ^{18}F -fluorinated analog of thymidine, 3-deoxy-3- ^{18}F fluorothymidine (^{18}F -FLT), has eliminated some of the problems encountered with ^{11}C -labeled nucleoside analogs by having a longer radionuclide half-life and by not being a substrate for enzymatic catabolism (22-25). FLT, unlike thymidine, is not measurably incorporated into DNA. FLT uses the nucleoside transport system and is a substrate for thymidine kinase (TK1) but very little of the FLT-phosphate pool becomes incorporated into DNA (26). Despite the trapping of FLT at the phosphate stage a number of studies have shown that ^{18}F -FLT accumulation in tissue corresponds to the proliferation rate of the tissue (27,28). What is actually measured by ^{18}F -FLT uptake into tissues is cellular nucleoside transport and phosphorylation by thymidine kinase with a strong correlation with TK1 levels in cells (26,28).

Agents for the Measurement of Phospholipid Synthesis

Cell membrane phospholipid synthesis can be regarded as an indirect measure of cell proliferation. Choline is a precursor for phospholipids through its incorporation into phosphatidylcholine, a primary component of cell membrane construction. Tumor cells have also demonstrated elevated levels of choline kinase. Following on the detection of elevated levels of choline in prostate tumors by magnetic resonance spectroscopy (29) the PET molecular imaging agent ^{11}C -choline was developed (30) and has since had widespread application in the PET imaging of prostate tumors (31-33) in addition to bladder (34) and brain (35). The relationship between cell proliferation and ^{11}C -choline incorporation in prostate cancer is particularly complex involving phosphatidylcholine production,

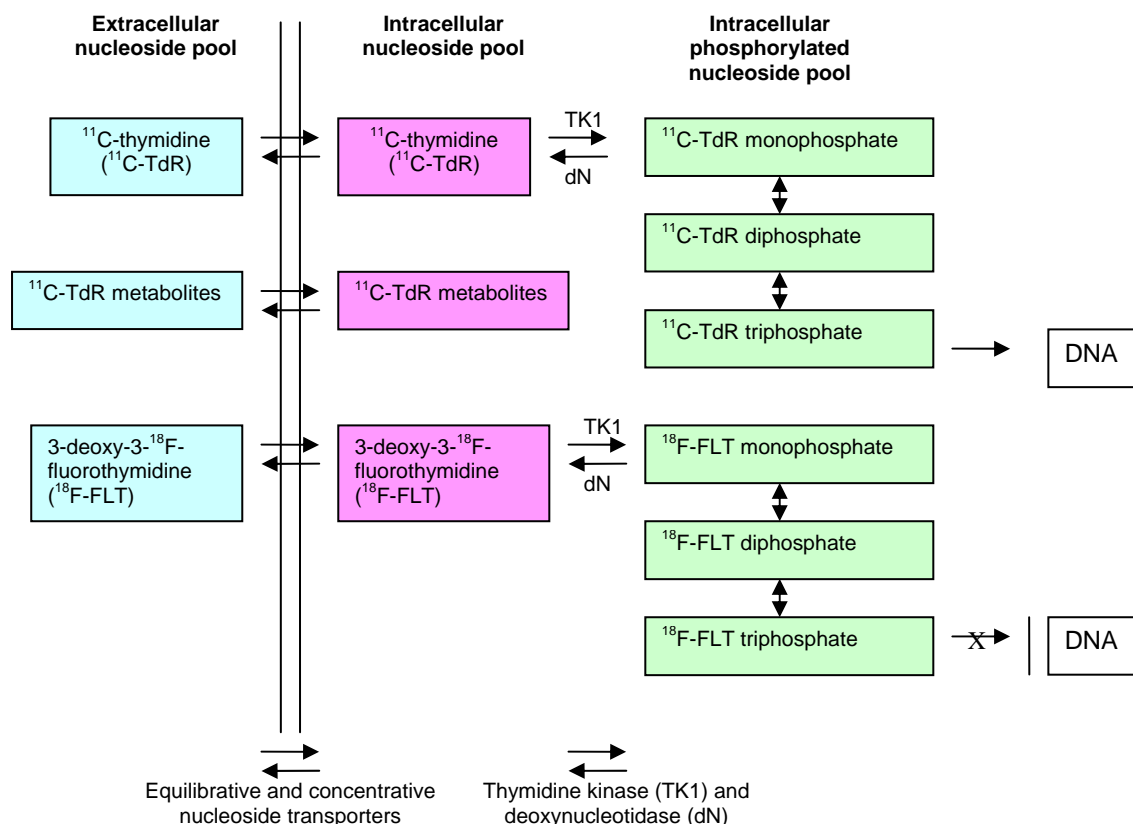


Figure 2. The transport and cellular utilization of thymidine and 3-deoxy-3- ^{18}F fluorothymidine (^{18}F -FLT).

phospholipid synthesis and upregulation of choline kinase and other enzymes. This has led to the conclusion that despite its recognized value in tumor visualization ^{11}C -choline uptake does not correlate directly with cell proliferation (36).

The accepted physical decay half-life advantage of ^{18}F over ^{11}C has resulted in the synthesis of several ^{18}F -choline analogs including ^{18}F -fluoroethylcholine (^{18}F -FEC) and ^{18}F -fluoromethylcholine (^{18}F -fluorocholine; ^{18}F -FCH) (37-39). ^{18}F -FCH has shown a similar uptake to ^{11}C -choline in animal studies and human prostate tumors (38) and has demonstrated its utility in prostate tumor imaging and staging in a number of clinical studies (40,41). Detailed studies of the relationship between cell proliferation and uptake of the fluorocholines have not been performed although their transport and interaction with choline kinase and other enzymes would account for the similarity to ^{11}C -choline in uptake in prostate tumors.

Amine Precursors Involved in Cell Uptake and Decarboxylation

Amine precursor uptake and decarboxylation is a feature common to peptide producing endocrine tissue including neuroendocrine tumors. Tumors in this category can be detected and identified as having an endocrine origin. The two main clinical PET tracers that have found some application in imaging studies in this category are ^{18}F -fluorodihydroxyphenylalanine (^{18}F -FDOPA) and ^{11}C -5-hydroxytryptophan. Endocrine tumors have the capacity to transport and accumulate these compounds as amino acid analogs following intercellular decarboxylation. Recent studies have explored the value of these agents in clinical studies of patients with neuroendocrine tumors (42-45). In clinical medical imaging of neuroendocrine lesions the PET agents will be compared with the more readily available single photon somatostatin receptor imaging agents such as ^{111}In -pentreotide. A clear advantage over the conventional imaging would have to be demonstrated to justify the PET imaging study. For example ^{18}F -FDOPA was shown to be a superior imaging agent for carcinoid tumors with a recommendation that it be used as the first-line scintigraphic imaging modality for these tumors while ^{111}In -pentreotide was superior as a scintigraphic imaging modality for noncarcinoid digestive tumors (42).

Agents to Assess Cellular Hypoxia

Rapidly growing tumors can develop oxygen deficiency (hypoxia) and regions of dead cells (necrosis) by outstripping the process of angiogenesis which strives to provide adequate blood flow and consequently oxygen and nutrients to support tissue growth and metabolism in neoplastic tissue. Hypoxic tumor tissues can be resistant to chemotherapy and to radiotherapy. A number of agents exploit the low oxygen tension in hypoxic tissues to permit intercellular trapping by reductive mechanisms. The trapping mechanism is well understood for the nitroimidazoles, which are the compounds most often exploited for imaging hypoxia. After entry into a viable cell the nitroimidazole molecule undergoes a one electron reduction. When oxygen is present the reduced form of the nitroimidazole is reoxidized but in the absence of oxygen the molecule may undergo further reductive processes and the reactive species thus formed become trapped in the cell through covalent binding with intercellular molecules (46). Another class of hypoxia imaging compounds (Cu-labeled diacetyl-bis(N^4 -methylthiosemicarbazone; Cu-ATSM) relies on reduction of a radioactive isotope of the chelated copper (eg. ^{64}Cu) and its subsequent trapping in the cell (47).

The main PET agents that have shown clinical utility to image hypoxia in tumors are ^{18}F -fluoromisonidazole (^{18}F -MISO), ^{64}Cu -ATSM and the recently introduced ^{18}F -fluoroazomycin-araboside (^{18}F -FAZA). The characteristics of binding and the dependency primarily on cellular oxygen levels have been examined in detail for these compounds (47-52). The target for these compounds is an enzymatically active reductive environment within viable cells that maintain the capacity to transport substrates. A number of clinical studies have been published for ^{18}F -FMISO (53,54) and those for ^{64}Cu -ATSM are imminent based on clinical studies with the non-PET agent ^{60}Cu -ATSM (55,56). Clinical studies with ^{18}F -FAZA are in progress at several centers but at present unpublished.

Cellular Receptor Imaging Agents

The presence of cellular receptors on the cell surface or in the cytoplasm that may be unique to tumors or to subpopulations of cells within tumors presents a number of opportunities to exploit PET imaging agents for detection and as a guide to

designing patient specific treatment. This is a very active area of research in imaging science and we can expect many clinical agents to be developed over the next few years. There are several examples where clinical PET imaging has already exploited cell receptors. One example is the cytoplasmic estrogen receptor which when activated by estrogen binding translocates to the nucleus. In the nucleus the activated receptor binds hormone specific DNA sequences resulting in the regulation of growth and differentiation of cells of the female and male reproductive systems and the growth and proliferation of breast cancer cells. Primary and metastatic breast cancer cells that express the estrogen receptor have been shown to respond to antiestrogen therapy with Tamoxifen. The PET imaging agent 16- α -[^{18}F]-fluoroestradiol (^{18}F -FES) was designed as an estrogen analog that recognizes and binds to the cytoplasmic estrogen receptor. The first patient studies with ^{18}F -FES were performed in 1988 (57) and the agent continues to be investigated for human breast cancer patients (58,59). There appears to be a clear application for ^{18}F -FES in identifying tumors that express estrogen receptors as a guide to selecting patients that will benefit from chemotherapy induced estrogen blockade. A recent study has also shown that quantitative PET studies with ^{18}F -FES can predict patient responses to hormonal therapy and may thus assist in the selection of appropriate treatment (59).

A second cell receptor molecular imaging agent, 16 β - ^{18}F -fluoro-5 α -dihydrotestosterone (^{18}F -dihydrotestosterone; ^{18}F -FDHT) has been synthesized (60) and used to image androgen receptors in prostate tumor cells in animal models (61) and in human clinical studies (62-64). Antiandrogen therapy is an accepted treatment modality for primary and metastatic prostate tumors but the effectiveness of therapy seems to be compromised by the emergence of tumor sub-populations that have lost their androgen dependency. In one study a reduction in the FDHT uptake in prostate tumors was noted after patients had been treated with the antiandrogen drug flutamide suggesting that ^{18}F -FDHT uptake could stratify patients for androgen therapy (64).

Recently there has been much interest in the targeting of PET radiopharmaceuticals to the family of somatostatin receptors on tumor cells. These receptors have been found on a variety of

neuroendocrine tumors, such as paragangliomas, carcinoids and breast tumors. There is a long history of somatostatin receptor imaging using the non-PET agent ^{111}In -DTPA-octreotide and this agent has found widespread application in clinical nuclear medicine (65,66). PET imaging of the somatostatin receptor has been accomplished using ^{66}Ga - and ^{68}Ga -labeled [1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic-acid-D-Phe¹-Tyr³]-octreotide (^{66}Ga -, ^{68}Ga -DOTATOC) which primarily binds to somatostatin receptor 2 (SSTR2) (67-71). The first clinical PET studies in patients with neuroendocrine tumors were reported in 2003 using ^{68}Ga -DOTATOC. Because the DOTATOC and related ligands also bind therapeutic radiometals including ^{90}Y (72) a number of clinical PET imaging studies with ^{68}Ga -DOTATOC have been performed in patients with metastatic neuroendocrine tumors scheduled for ^{90}Y -DOTATOC therapy (70,71).

The recognition of unique cell receptors or unique concentrations of receptors expressed by cancer cells is an area of intense research since these receptors provide opportunities for both detection and therapy of tumors. In this regard there have been exciting preclinical investigations with, for example, the family of epidermal growth factor receptors (73,74) including the Her2/neu receptor, a tyrosine kinase cell membrane receptor which is overexpressed in many breast cancers. It is relatively easy to develop antibody based imaging probes to cell surface receptors such as HER2/neu but the slow accumulation at the target and the persistent blood activity make antibodies generally unsuitable for PET imaging, especially with the shorter lived radionuclides. More promising PET probes are prepared from antibody fragments or peptide sequences that maintain the binding and specificity of intact antibodies. Recent work with diabodies and affibodies (75,76) serve to illustrate this approach. We should expect to see clinical PET imaging using these affinity molecules targeting HER2/neu and other cellular receptors in the near future.

Agents Targeting Cells Undergoing Apoptosis

A number of groups are investigating PET labeled probes that can non-invasively image apoptosis. The induction of apoptosis is a very early indication of the effectiveness of chemotherapy that can be assessed long before there are changes in tumor size

or proliferative potential that can be assessed by other imaging modalities. At present preclinical PET investigations are proceeding with several analogs of annexin V labeled with either ^{18}F (77) or ^{124}I (78,79). Annexin V is one of a family of endogenous proteins that have a variety of functions including binding with calcium and phospholipids. Annexin V binds to cells with exposed surface phosphatidylserine (PS) groups. PS is normally restricted to the inner leaflet of cell membranes but becomes externalized at a very early stage in the apoptotic process presenting a target for annexin V binding. Although PET imaging in humans with annexin V analogs has not yet been published there have been several studies with cancer patients using $^{99\text{m}}\text{Tc}$ labeled annexin V in conventional imaging (80-83). The clinical value of the PET apoptotic imaging agents is yet to be established but the potential of obtaining a rapid assessment of the efficacy of anticancer treatment is a very strong motivation to continue to seek a PET tracer in this category.

Agents Targeting Gene Expression

With the modern understanding of the human genome and the genetic basis of cancer induction, progression and responses to therapy there is a growing interest in methods for imaging gene expression in vivo. This interest extends from endogenous genes that may be expressed in conjunction with the various phases of cancer progression or following therapy as well as to the imaging of genes transported into cells in gene therapy applications. Clinical PET has shown its potential for molecular imaging of gene therapy in oncology primarily in the area of advances in reporter gene detection as described in several recent reviews (84-86). The most widely used and successful reporter gene has been the herpes simplex virus 1 thymidine kinase gene (HSV1-tk). In this molecular imaging technique a therapeutic gene would be linked to the HSV1-tk gene using molecular biology methods and the combined gene would be delivered to target cells by a transfection methodology. Once inside the cell the HSV1-tk gene produces the herpes simplex virus 1 thymidine kinase enzyme (HSV1-TK) which allows for phosphorylation of nucleosides and nucleoside analogs. The essential feature of the HSV1-TK is that it has a much broader phosphorylation profile than endogenous mammalian thymidine kinase (TK). Thus nucleoside analogs that would not be

retained in cells expressing endogenous TK are trapped in cells possessing the mutant HSV1-TK enzyme due to selective phosphorylation (87).

The PET probes that have shown potential in this imaging method include 2'-fluoro-2'-deoxy-1- β -arabinofuranosyl-5- ^{124}I iodouracil (^{124}I -FIAU) (87,88), 2'- ^{18}F fluoro-2'-deoxy-5-methyl-1- β -arabinofuranosyluracil (^{18}F -FMAU) (89,90), 8- ^{18}F -fluorogancyclovir (91,92), 9-(4- ^{18}F -fluoro-3-[hydroxymethyl]butyl)guanine (^{18}F -FHBG) (93) and a number of others (84). These PET probes are not substrates for mammalian TK and after intravenous administration they would not be trapped by phosphorylation except in cells expressing the mutant HSV1-tk gene encoding for the less selective enzyme. Although the vast majority of reporter gene investigations have been carried out in vitro and in animal studies they have a great potential in human medicine and a number of clinical investigations with these imaging agents have been reported recently (94-98).

Gene expression can also be theoretically imaged by the use of PET radionuclide labeled antisense oligonucleotides which can bind with gene encoded mRNA within cells. This approach has the potential to detect gene activity relative to cancer or to responses to cancer therapy although it has not yet been exploited in a clinical setting (99-101).

Vascular Endothelial Factors Related to Angiogenesis

The increased angiogenesis present in tumors relative to normal tissues represents a potential target for molecular imaging. This presents the possibility not only of tumor detection but also the assessment of anti-angiogenesis therapies which are proving to be a powerful tool in cancer chemotherapy. One approach to PET molecular imaging of angiogenesis, presently in preclinical investigation, has been to label vascular endothelial growth factor (VEGF) by using ^{64}Cu -DOTA linked to VEGF (102). VEGF binding is specific to endothelial cells such as those present in rapidly growing microvasculature where it associates with two receptor tyrosine kinases Flt-1 and KDR (103). The receptors are greatly increased in the rapidly proliferating tumor endothelial cells when compared to the quiescent endothelial cells of normal vasculature.

A second approach to imaging angiogenesis has targeted the dimeric transmembrane glycoprotein integrin $\alpha\beta3$ which promotes angiogenesis and also plays a role in tumor cell growth and invasiveness (104). This integrin is virtually absent in mature vasculature and normal organs but is highly expressed in the endothelial cells of new vasculature produced during angiogenesis. The integrin $\alpha\beta3$ is a receptor for extracellular matrix proteins which have the peptide sequence arginine:glycine:aspartic acid (RGD) (104). A number of RGD peptides have been developed to explore both therapy and preclinical imaging of active endothelial growth (105) and a PET agent, ^{18}F -galacto-RGD, has exploited the selective binding between the integrin and the RGD tripeptide to perform imaging of angiogenesis in human cancer patients (106-108). A clear correlation has been noted between the accumulation of ^{18}F -galacto-RGD in tissue and the degree of integrin $\alpha\beta3$ expression (106).

THE REALITY: Few agents can be said to be “routine” in clinical PET imaging in oncology

With the exception of ^{18}F -FDG there is no general supply of PET tracers through commercial sources. The great demand for ^{18}F -FDG has encouraged commercial development through collaboration of existing companies such as the Schering – IBA venture in Western Europe or the development of commercial nuclear pharmacies such as Cardinal Health, Eastern Isotopes and PETnet in the United States. These companies supply only ^{18}F -FDG as a PET imaging agent in oncology. Some simple molecule, gas phase PET tracers to measure perfusion, oxygen utilization and blood flow including ^{11}C -CO, ^{11}C -CO₂, ^{15}O -O₂ and ^{15}O -H₂O are in general use but only at sites with in-house cyclotron facilities. We can additionally identify a handful of oncology tracers that are in widespread use and are produced routinely by PET centers for local or regional use. The list includes ^{11}C -methionine, ^{11}C -choline, ^{18}F -FLT, ^{18}F -FES, ^{18}F -FDOPA, ^{18}F -F-MISO and a few others. Notwithstanding the facile production of these PET agents they are not available to the majority of sites that have PET imaging capability but remain restricted to sites with in-house cyclotrons. The challenges to the widespread routine utilization of available PET tracers include the following:

- ii) Chemistry challenges;
- iii) Clinical acceptability of PET radiopharmaceuticals;
- iv) Physical half-life and other radionuclide properties;
- v) Regulatory issues;
- vi) Understanding of the molecular dynamics and pharmacokinetics;
- vii) Cost.

These items are explored in more detail below.

Limited Clinical or Research Demand

The demand for new tracers is attenuated by the wide applicability of ^{18}F -FDG in clinical oncology. In routine PET scanning, where the aim will be to determine the extent of cancer progression (staging) or the evaluation of residual or recurrent disease in patients who have had cancer surgery or therapy, ^{18}F -FDG is almost always effective. Possible exceptions would relate to the list of limitations presented earlier relating to specific anatomical sites or unusual biochemical situations in tumor cells. In much of the clinical imaging literature the terms PET and FDG-PET are synonymous. The clinical and research demand for new tracers also suffers because many physicians may be unaware of the specialized tracers that are available and the research literature that supports their utility in oncologic imaging. PET nuclear medicine physicians will have had specific training in the interpretation of PET images using ^{18}F -FDG but will usually have no background in the interpretation of scans with other PET agents. It is not surprising that they might be unable or unwilling to incorporate new tracers into their practice.

Chemistry Challenges

The routine availability of PET tracers requires the resources and personnel to perform the chemistry and radiochemistry. The routine nature of ^{18}F -FDG now allows this product to be produced with full automation and without the requirement for skilled radiochemistry personnel. Many of the other PET oncology tracers have not yet achieved this routine status. The aim with ^{18}F radiopharmaceuticals and to an even greater extent with the shorter half-life ^{11}C tracers is to perform the radiolabeling as the final step in the synthesis followed by a rapid simple purification procedure. With radiolabeling as the ultimate chemistry step there is the requirement

to prepare a reactive precursor which may involve a complex and synthetically challenging series of chemistry steps. This will certainly be a consideration with the development of novel PET tracers and will require highly trained chemists. For many of the more common PET tracers in oncology the precursors are now available as GMP quality products from commercial suppliers.

^{18}F and ^{11}C and carbon remain the most used PET radionuclides for clinical products but a number of other radionuclides have had some application in clinical or preclinical studies. Table 2 lists the more common radionuclides in this category. This is a diverse group of elements including carbon, oxygen, nitrogen, halides (^{18}F , ^{76}Br , ^{124}I) and a variety of radiometals (^{62}Cu , ^{64}Cu , ^{68}Ga). Table 2 also indicates the main class of chemistry that is involved in the preparation of radiopharmaceuticals from each of the radionuclides. Diverse chemistry skills and specializations are required in the preparation of radiopharmaceuticals from these radionuclides. ^{18}F radiopharmaceuticals are generally prepared by nucleophilic or electrophilic substitution reactions resulting in stable carbon fluorine covalent bonds. ^{11}C is often incorporated into PET radiopharmaceuticals by methylation reactions using ^{11}C -methyl iodide or triflate although a number of other reactive small molecule carbon compounds have been exploited to prepare PET products. The halides ^{76}Br and ^{124}I can be incorporated by electrophilic aromatic substitution or addition reactions. Radiometals will generally rely on chelation to appropriately functionalized molecules.

Clinically acceptable PET radiopharmaceuticals will generally be prepared by automated chemistry. The advantages of automation include rapid synthesis, remote operation, the ability to isolate the automated units for radiation protection and for microbiological control (sterility), and the capacity to provide security, process control and data logging that makes the PET product acceptable to regulatory bodies. Commercial automated synthesis units (ASUs) are now available that prepare ^{18}F -FDG as well as a number of the more common non-FDG PET tracers. The radiation containment will require specially constructed hot cells with appropriate radiation shielding and provision to maintain an appropriate internal environment suitable for the preparation of a pharmaceutical

product that will ultimately be injected into a patient to be imaged. Personnel issues must also be considered since the requirements for reagent preparation for the ASUs, the monitoring and adjustment of radiochemistry parameters and the final purification and quality control of the product will require a highly trained staff.

Recognizing the need for trained radiochemistry and radiopharmacy staff and allocating the resources to hire them will go a long way to overcoming the challenges of radiopharmaceutical preparation. Manpower issues are complicated at present by a global shortage of scientists with suitable training in the fields of radiochemistry and radiopharmacy. A number of initiatives are underway to provide suitable opportunities for training additional practitioners in these fields. (For example: Nuclear Education Online run by the University of Arkansas and University of New Mexico [www.nuclearonline.org/nuclear_education/default.asp] and the Radiopharmaceutical Chemistry / Radiopharmacy postgraduate diploma course (PDC), organized by the Universities of Frankfurt am Main, Leipzig and ETH Zurich [www.pharma.ethz.ch/nd/].)

Even with the availability of ASUs, hot cells and trained personnel it may not always be possible to perform the chemistry necessary to prepare a product routinely. There are instances where the complexity or sensitive nature of a synthetic process has been an impediment to its translation into a clinical oncology setting outside of a few selected laboratories. For example the elegant production of ^{11}C -DOPA and ^{11}C -5-hydroxytryptophan using a multienzymatic synthesis developed by Langstrom and co-workers (109) (Figure 3) does not appear to have been adopted by other laboratories despite the application of these tracers in imaging neuroendocrine malignancies (43-45).

Clinical Acceptability of PET Radiopharmaceuticals

PET radiopharmaceuticals will generally be administered by intravenous injection. Any new radiopharmaceutical will therefore require testing for its suitability for human administration by this route. Most radiopharmaceuticals will be provided at high specific activity, and as tracers will be administered in chemical doses that will not elicit

physiological responses in the patients. Issues of chemical toxicity are of less significance than standard therapeutic drug products but nonetheless there will be many quality control issues that will need to be addressed. Each new product will require a formalized series of quality control tests that will fall into the general categories of a) immediate release quality control tests (performed on each batch of product before release); b) retrospective quality control tests (performed on each batch of product after release) and; c) supporting characterization tests (performed periodically on selected batches after release). The actual tests performed will be product and institution specific but are designed to ensure both the quality and the safety of the product. As an example the three categories of tests for FDG as performed at the Edmonton PET Center are presented in Tables 3-5.

When PET radiopharmaceuticals are purchased from a commercial supplier the responsibility for product testing and certification will be assumed by the manufacturer. The likely scenario for many of the new PET radiopharmaceuticals and certainly the ^{11}C products is that they will continue to be produced in-house. Thus the responsibility for the quality control testing will be undertaken by personnel and using equipment that are part of the local PET program. In many instances the cost and complexity of assuring a quality product will be a significant barrier to the adoption of additional PET oncology tracers beyond FDG.

Physical Half-life and Other Radionuclide Properties

The physical half-life of some PET radionuclides severely restricts their availability and their elaboration into routine clinical tracers in oncology. ^{13}N ($t_{1/2} = 9.9$ m) and ^{15}O ($t_{1/2} = 122$ s) can only be used where an in-house cyclotron is present and where the local chemistry is in place to provide suitable PET tracers (e.g. ^{13}N -ammonia, ^{15}O -water). ^{11}C ($t_{1/2} = 20.4$ min) tracers can only be used locally for distribution to sites within a delivery radius achievable within a few half lives. ^{18}F ($t_{1/2} = 110$ m) radiopharmaceuticals can be widely distributed where transportation options are available and this is well illustrated by the ability of commercial suppliers to provide ^{18}F -FDG to almost any location in the United States and Western Europe. A number of longer lived PET radionuclides are being used in the development of oncology imaging agents including ^{64}Cu (110) ($t_{1/2} = 12.7$ h), ^{76}Br (111) ($t_{1/2} = 16$ h) and ^{124}I (111) ($t_{1/2} = 4.15$ d) and several generator products mainly ^{68}Ga (112) and ^{62}Cu (113) can be widely distributed (Table 2). These radionuclides and their radiopharmaceuticals can not yet be considered to be routinely available and their impact on clinical imaging has to date been slight although their longer half-life continues to stimulate interest in the development of clinical imaging products.

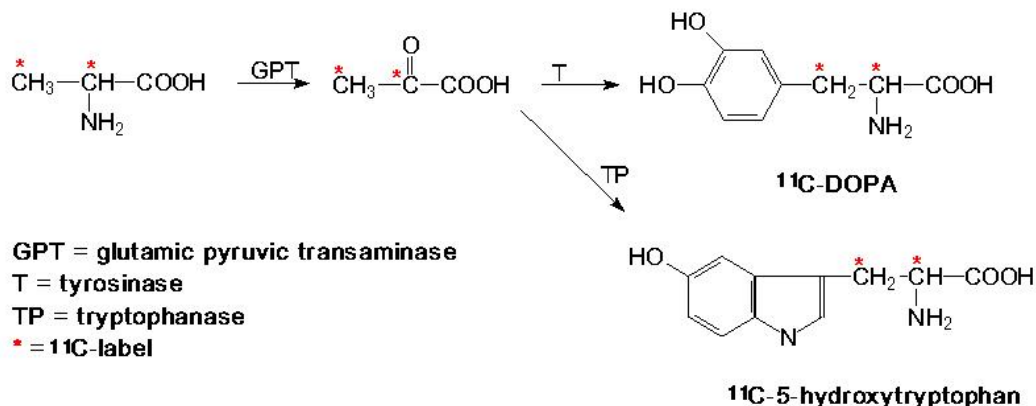


Figure 3. A synthetic scheme for the production of the PET radiotracers ^{11}C -DOPA and ^{11}C -5-hydroxy-tryptophan involving multiple enzymatic steps (109).

Table 3. Summary of typical immediate release quality control tests for ^{18}F -FDG.

Test	Method	Preliminary Specification
Appearance	Visual Inspection	Clear, colourless, no visible matter
Radioactivity Assay	Dose Calibrator	> 150 MBq / mL
Radiochemical Purity	TLC (area integration)	> 90% ^{18}F -FDG < 4% ^{18}F -Fluoride
Radiochemical Identity	TLC (Rf)	Rf: +/- 0.05 Rf of FDG Std
pH	pH paper	pH: 4.5 – 8.5
Terminal Filter Integrity Test	Bubble Point	Passes Test
Radionuclidic Identity	Half-life Determination	105 – 115 minutes
Chemical Purity: Residual Kryptofix	TLC (spot test)	< 50 μg / mL

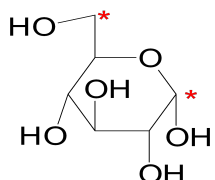
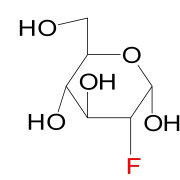
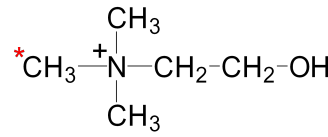
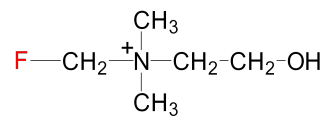
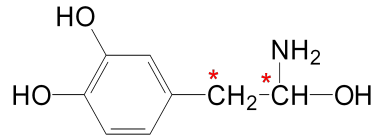
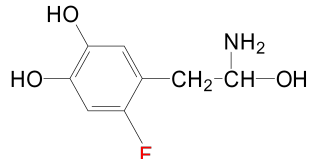
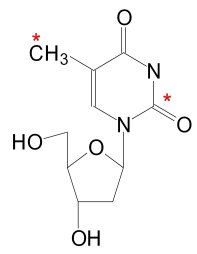
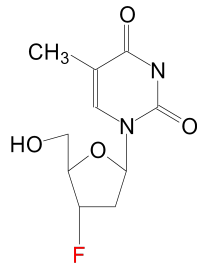
Table 4. Summary of typical retrospective quality control tests for ^{18}F -FDG.

Test	Method	Specification
Endotoxin Test	Bacterial Endotoxin Test USP on Pool	< 175 EU / dose
Sterility	Sterility Test USP on Pool	Sterile

Table 5. Summary of typical supporting characterization tests for ^{18}F -FDG.

Test	Method	Preliminary Specification
Radionuclidic Purity	Gamma spectrometry	> 99.5% 0.511 MeV +1.022 MeV + Compton scatter
Chemical Purity:		
Acetonitrile	GC	< 0.04% (w/v)
Ethanol	GC	< 0.5% (w/v)
Chlorodeoxyglucose	HPLC	< 0.5 mg / dose
FDG	HPLC	< 10 mg / dose
Particulates	Particulate Test USP	Small volume parenteral USP
Osmolality	Osmometer	260 – 300 mOs/kg

Table 6. Structures of matched ^{11}C and ^{18}F radiopharmaceuticals recognizing equivalent molecular

Molecular target	^{11}C product	^{18}F product
Metabolism / energy utilization	 ^{11}C -glucose	 ^{18}F -fluorodeoxyglucose
Phospholipid synthesis	 ^{11}C -choline	 ^{18}F -fluoromethylcholine
Amine decarboxylation	 ^{11}C -DOPA	 ^{18}F -FDOPA
Cell proliferation / DNA synthesis	 ^{11}C -thymidine	 ^{18}F -fluorothymidine

In addition to the issues of delivery of the radionuclides or the radiopharmaceuticals that are synthesized from them the half-life can impact on other aspects of tracer production and use. The half-life of some of the PET radionuclides severely restricts their elaboration into suitable radiopharmaceuticals due to the time requirements of chemical processing, purification and quality control. The short physical half-life radionuclides will not be compatible with slow biological targeting or slow blood clearance. In a number of molecular imaging situations both ^{11}C and ^{18}F products have been developed. Where these products have the same biological behavior and

ability to assess similar molecular targets the longer lived radiopharmaceutical may be preferred. Table 6 gives a number of examples where both an ^{11}C and an ^{18}F PET oncology molecular imaging agent have been developed.

Half-life and availability are the most important factors affecting the clinical acceptability of radionuclides and the radiopharmaceuticals derived from them. In addition the quality of PET images will be affected by the positron energy and by the presence of single photon emissions where the decay mode is not purely by positron emission. As Table 2 indicates the positrons from different

radionuclides are emitted over a wide range of E_{\max} values and the range of an E_{\max} particle can be quite substantial. Positrons do not annihilate until they have lost most of their kinetic energy and thus the position of photon production will be different than the position of the decaying radionuclide. For a number of reasons the E_{\max} particle range does not give an accurate indication of the actual magnitude of this separation. Firstly the average energy of positrons is much less than the E_{\max} value and the mean positron range will be consequently much shorter (114). In addition the positron does not travel in a straight line but has a tortuous path that shortens the overall distance between radionuclide and annihilation event. For energies up to E_{\max} of 1.5 MeV the influence of positron travel will be negligible (115,116). A recent analysis has discussed the influence of positron energy on spatial resolution in PET including calculations in various tissues (117).

The positron energy is less significant than factors such as the non-collinearity of the coincident positrons and PET camera factors in determining the overall spatial resolution. PET images will also be degraded in the situation where single photons are produced in the decay process in addition to positrons. For example ^{76}Br emits gamma rays at 0.559 and 0.657 MeV and ^{124}I at 0.602 MeV that will fall within the normal energy detection window of PET scanners. Consequently, without correction for these prompt gamma rays, the image resolution for these two isotopes will be poorer than a pure beta emitter such as ^{18}F (114).

Regulatory Issues

The use of PET tracers in clinical oncology will necessarily fall under the scrutiny of local and national regulatory agencies. Local committees will usually be required to assess human ethics for a new imaging agent. Approvals will depend on evidence of safety and efficacy of the radiopharmaceutical. In many countries new PET radiopharmaceuticals will be regarded as new drug entities and will be subjected to the same regulatory process as is used for the approval of therapeutic drug products. This regulatory process can be a severe impediment to bringing PET tracers into the clinical setting since the rigorous drug assessment is expensive and time consuming. Drug companies have shown little interest in working through the regulatory issues with novel PET tracers

recognizing that the small market for the PET tracers other than ^{18}F -FDG means that these products are unlikely to be financially viable. Most countries will permit clinical trials to be carried out where a novel PET imaging agent is to be assessed or where an established PET tracer is to be used in a new patient population. Clinical trials will require regulatory approvals but are generally less burdensome than approvals for new drugs. Clinical trials will allow the assessment of a PET tracer to be carried out in a limited number of patients but will help to evaluate the drug as a candidate for a more extensive testing. A number of jurisdictions have now acknowledged the distinction between tracer radiopharmaceuticals and therapeutic drugs and will permit small trial studies to be performed with very minimal regulatory burden for the researchers. The regulatory process will also require strict adherence to quality control issues during radiopharmaceutical preparation. The specific requirements for radiopharmaceutical manufacture will vary with jurisdiction but in many cases they provide a significant commitment in terms of space and personnel and can discourage the in-house preparation and evaluation of novel tracers.

Understanding of the Molecular Dynamics and Pharmacokinetics

In order to use the full potential of PET molecular imaging agents in oncology there must be a thorough understanding of the molecular dynamics and tissue pharmacokinetics of the tracers. Unfortunately issues related to cellular delivery, transport, metabolism, trapping and intracellular localization of many PET tracers is poorly understood. Where PET tracers are to be used as markers of molecular processes there must be a clear understanding of the cellular biochemistry. An advantage with some of the ^{11}C -labeled PET radiopharmaceuticals is that they are identical to native biological molecules for which the biochemical pathways for metabolism and catabolism have been thoroughly elucidated. This claim of equivalency for the PET tracers requires that the isotopic substitution of ^{11}C for ^{12}C causes negligible perturbation to biochemical processes. When analogs of naturally occurring biological molecules are used some care must be exercised to define the actual molecular target and cellular processes involved.

As an example we can consider the use of ^{11}C -thymidine and its analog 3-deoxy-3- ^{18}F fluorothymidine (^{18}F -FLT) to assess cell proliferation. While both of these PET radiotracers are generally accepted as probes to assess cell proliferation, only ^{11}C -thymidine is actually incorporated into the DNA of replicating cells (Figure 2). Because ^{11}C -thymidine also undergoes extensive metabolism its distribution in tissues is complex and the analysis of tissue proliferation based on radioactivity measurement requires a multicompartamental mathematical analysis (118,119). ^{18}F -FLT would seem to be an improvement over ^{11}C -thymidine not only because of the longer physical half-life of ^{18}F but also because of a simpler biological profile in the cell. ^{18}F -FLT is a substrate for the nucleoside transporters, undergoes minimal degradation to metabolic products and is phosphorylated by thymidine kinase (TK-1). However ^{18}F -FLT is not incorporated into DNA. The uptake of ^{18}F -FLT in tissue may correlate with cell proliferation but strictly speaking the PET image obtained in proliferating tissue reflects increased nucleoside transport and increased activity of TK-1 in converting the nucleoside analog into the phosphate forms (27,28,120). Additional discussion of the appropriate interpretation of the *in vivo* distribution and PET images obtained with molecular imaging tracers has been published recently for the radiopharmaceutical pairings of ^{11}C -glucose with glucose as well as ^{11}C -thymidine with ^{18}F -FLT (120).

The biochemical complexity of cellular processing for PET radiopharmaceuticals does not have to be an impediment to their routine use. In many situations the simple interpretation of radiotracer distribution will be adequate to provide clinically useful and relevant information. Therefore for example if we consider ^{18}F -FLT as a marker of tissue proliferation this will almost always be accurate and appropriate. It will only be in the unusual circumstance where transport and phosphorylation of ^{18}F -FLT very poorly correlate with actual cell proliferation that the image interpretation may provide misleading information relevant to disease management.

Cost

PET imaging requires a substantial commitment of healthcare dollars. The basic infrastructure to use

^{18}F -FDG will consist of a PET camera or more appropriately for modern imaging a combined PET-CT camera. Most PET imaging centers will use commercially supplied ^{18}F -FDG making this the only additional cost without considering the clinical costs for performing and interpreting the study and the cost of specialized training for the physicians and nuclear medicine technologists. If a center wishes to use a molecular imaging tracer other than ^{18}F -FDG there are substantial additional costs involved. Included in these costs can be: i) cyclotron, targetry and operating personnel; ii) chemistry costs including automated synthesis units, hot cells and trained radiochemists; iii) quality assurance costs including analytical instrumentation, construction of appropriate facilities for GMP radiopharmaceutical production and the provision of trained staff to perform quality control testing; iv) preparation of regulatory documents to support new drug submissions or clinical trials applications to local and national medical oversight bodies; v) training of clinical staff in the appropriate use of the PET tracers and in the interpretation of PET images.

The substantial cost of PET studies in oncology has been the subject of intense scrutiny with the usual goal being to demonstrate the cost effectiveness of PET imaging. There are more than 130 publications listed by PubMed since 1994 that deal with cost effectiveness of PET in cancer management (121). PET with ^{18}F -FDG can provide images that guide clinical decision making and can prevent ineffective treatment thereby generating substantial cost savings (e.g. (122,123)). Perhaps more importantly PET imaging can decrease patient morbidity from inappropriate treatments and contribute to patient quality of life in a palliative setting and to more effective and potentially curative strategies in a treatment setting. An understanding of the cost effectiveness of PET in oncology has contributed to reimbursement for specified applications by health administration bodies in many countries although this is far from universal and has required intense lobbying. When additional PET molecular imaging agents are introduced into clinical practice it will certainly take time and effort before reimbursement is possible. Thus the initial costs must be assumed by the hospitals and clinics that wish to use a PET tracer other than ^{18}F -FDG.

The seven items discussed above have combined to slow the introduction of additional PET agents into clinical oncology and they continue to present a formidable challenge to the scientists and clinicians who work in this field.

CONCLUSION

Non-FDG PET tracers can play a valuable role in defining the molecular basis of human cancer and in individualizing patient treatment and care. A number of the newer molecular imaging tracers are relevant to modern drug and radiotherapy treatment strategies targeting such processes as angiogenesis, unique cell cytosolic and surface markers and cell proliferation biochemistry. Determining the molecular-biochemical profile of a specific tumor can be combined with immediate and effective treatment decisions. The value of the molecular imaging agents is increased if we have a thorough understanding of their *in vivo* distribution, their molecular targets and their biochemical behavior at the cellular level. At present most new PET tracers that have a specific rather than a general application in oncologic imaging have remained restricted to one or only a few PET centers. Other than ^{18}F -FDG only a handful of tracers can be regarded as having global acceptance and these will only be found where there are in-house or local resources for isotope production and PET radiochemistry. Clinical demand and commercial production have combined to make ^{18}F -FDG routinely available in many countries and it remains to be seen if this can be repeated with the more popular non-FDG tracers.

One potential stimulus for the development of novel PET tracers in oncology comes from the recognition that these agents can play a role in anticancer drug development. It is now accepted that molecular imaging can speed up drug development by providing direct evidence of drug action using both animal PET and human clinical trials. Drug companies are increasingly contracting with PET centers to carry out molecular imaging studies in conjunction with drug trials as a rapid assessment of efficacy. Although ^{18}F -FDG has taken a leading role in this endeavor (124) the value of non-FDG tracers is now widely recognized (125).

The development of PET molecular imaging probes is necessarily a multidisciplinary science using the

expertise of nuclear physicists for the production of novel radionuclides, radiochemists and radiopharmacists for the elaboration of the radiotracers, biologists and experimental oncologists to determine the details of targeting and molecular processing of the tracers and clinical scientists to effectively evaluate the human imaging. The future of this science will depend on collaborative efforts among PET centers and their supporting clinical colleagues, governmental agencies regulating the PET tracer development or providing research funding and companies with an interest in imaging instrumentation or drug development.

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