

# $^{18}\text{F}$ -Labeled Positron Emission Tomographic Radiopharmaceuticals in Oncology: An Overview of Radiochemistry and Mechanisms of Tumor Localization

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Molecular imaging is the visualization, characterization, and measurement of biological processes at the molecular and cellular levels in a living system. At present, positron emission tomography/computed tomography (PET/CT) is one of the most rapidly growing areas of medical imaging, with many applications in the clinical management of patients with cancer. Although [ $^{18}\text{F}$ ]fluorodeoxyglucose (FDG)-PET/CT imaging provides high specificity and sensitivity in several kinds of cancer and has many applications, it is important to recognize that FDG is not a "specific" radiotracer for imaging malignant disease. Highly "tumor-specific" and "tumor cell signal-specific" PET radiopharmaceuticals are essential to meet the growing demand of radioisotope-based molecular imaging technology. In the last 15 years, many alternative PET tracers have been proposed and evaluated in preclinical and clinical studies to characterize the tumor biology more appropriately. The potential clinical utility of several  $^{18}\text{F}$ -labeled radiotracers (eg, fluoride, FDOPA, FLT, FMISO, FES, and FCH) is being reviewed by several investigators in this issue. An overview of design and development of  $^{18}\text{F}$ -labeled PET radiopharmaceuticals, radiochemistry, and mechanism(s) of tumor cell uptake and localization of radiotracers are presented here. The approval of clinical indications for FDG-PET in the year 2000 by the Food and Drug Administration, based on a review of literature, was a major breakthrough to the rapid incorporation of PET into nuclear medicine practice, particularly in oncology. Approval of a radiopharmaceutical typically involves submission of a "New Drug Application" by a manufacturer or a company clearly documenting 2 major aspects of the drug: (1) manufacturing of PET drug using current good manufacturing practices and (2) the safety and effectiveness of a drug with specific indications. The potential routine clinical utility of  $^{18}\text{F}$ -labeled PET radiopharmaceuticals depends also on regulatory compliance in addition to documentation of potential safety and efficacy by various investigators.

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Cancer is a systemic disease resulting from alterations in the interactions between oncogenes and tumor suppressor genes, which under normal physiological conditions control cell maturation, division, and migration.<sup>1</sup> Mutations in genomic DNA precede the development of overt neoplasia. A variety of both genetic and tumor microenvironmental fac-

tors determine the behavior of cancer. As a result of alterations in genotype, phenotypic functional changes (Table 1) such as altered metabolism, proliferation protein synthesis, and angiogenesis occur in cancer tissue, which ultimately lead to the development of discrete mass lesions.<sup>1</sup> Traditional noninvasive imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) are used primarily for imaging anatomical and morphological changes associated with an undying pathology. Historically, CT has been the modality of choice for the diagnosis and staging of malignant disease and for monitoring the response to treatments. These screening techniques, however, often lack the necessary sensitivity and specificity for early diagnoses of many cancers and for the detection of subcentimeter neo-

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**Table 1** Molecular and Functional Alterations in Cancer

Function	Increased	Decreased
Glucose metabolism	X	
Amino acid transport	X	
Protein synthesis	X	
DNA synthesis	X	
Membrane or lipid synthesis	X	
Receptor expression	X	
Angiogenesis, vascular density	X	
Vascular permeability	X	
Oncogene products	X	
Signal transduction	X	
Hypoxia	X	
Oxygen tension		X
Blood flow	X	X
Apoptosis	X	X
Many other genetic markers	X	X

Modified from Wahl.<sup>10</sup>

plasms and preneoplastic disease. To develop effective treatment modalities, especially, patient specific treatments, a more sensitive and specific detection of early malignancies is essential.

Molecular imaging is the visualization, characterization, and measurement of biological processes at the molecular and cellular levels in a living system. Radioisotope based molecular imaging techniques such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT) capture functional or phenotypic changes associated with pathology. It is an emerging field that aims to integrate patient-specific and disease-specific molecular information with traditional anatomical or structural imaging readouts. The hybrid or fusion-imaging of PET/CT is improving the sensitivity and specificity of clinical PET imaging technique. At present, PET/CT is one of the most rapidly growing areas of medical imaging with many applications that can be classified according to the generic use as summarized below:

1. Diagnosing malignancy: Differentiating malignant from benign disease
2. Identifying the site(s) of disease: To plan biopsy or surgery especially when cancer is suspected based on clinical biomarkers
3. Detecting the primary tumor: In patients with metastatic disease with an unknown or small primary tumor
4. Grading malignancy: Based on quantifying the amount of radiotracer uptake

5. Staging disease: Whole-body scans would provide the relative uptake of tracer through out the body
6. Identifying residual disease: Identification of residual viable cell mass after treatment
7. Detecting recurrences: Confirming the sites of recurrent (new) disease
8. Measuring the response to therapy: Objectively assessing the efficacy of specific treatment modalities.
9. Guiding radiation therapy: Identifying regions of tumor tissue with different radiosensitivities for effective radiation treatment.

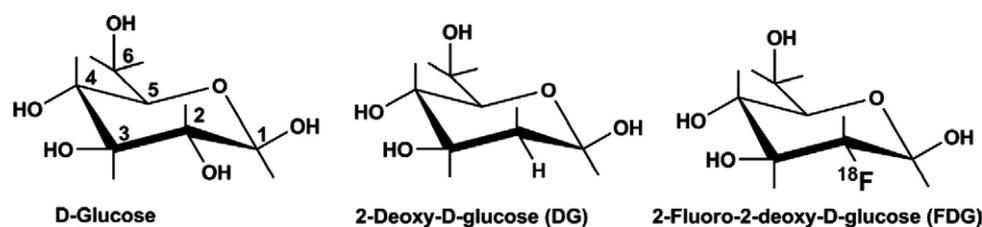
## Clinical FDG-PET

Accelerated glucose metabolism is one of the phenotypic or functional changes observed in cancer tissue and was first recognized by Warburg more than 80 years ago.<sup>2,3</sup> The phosphorylation of glucose, an initial and important step in cellular metabolism, is catalyzed by the enzyme hexokinase (HK), which converts glucose to glucose-6-phosphate, and helps to maintain the downhill gradient that results in the transport of glucose into cells through the facilitative glucose transporters.<sup>4</sup> Tumor cells are known to be highly glycolytic because of increased expression of glycolytic enzymes, especially hexokinase activity.

2-Deoxy-D-glucose (DG) (Fig. 1) was first developed in 1960 as a chemotherapeutic agent to inhibit glucose use by cancer cells.<sup>5,6</sup> After conversion to 2-DG-6-phosphate by hexokinase, further metabolism of DG is inhibited and DG is trapped in the cell. In 1976, Dr. Wolf and his colleagues at Brookhaven National Laboratory developed the synthesis of [<sup>18</sup>F]fluorodeoxyglucose (FDG) to study the cerebral glucose metabolism based on PET.<sup>7</sup> The first FDG-PET brain imaging studies were performed in 1977 at UCLA.<sup>8</sup>

The development of FDG as a PET radiopharmaceutical for imaging tumor metabolism was not as rapid as its use in brain and cardiac imaging. It took almost 10 years to optimize the FDG synthesis for routine clinical production. The tumor targeting properties of FDG were investigated in animal models and in patients with different malignancies for almost 20 years.

Several PET radiopharmaceuticals approved by Food and Drug Administration (FDA) for routine clinical PET imaging studies are listed in Table 2. FDG is currently the only PET radiopharmaceutical used for routine cancer imaging. FDG-PET was a research device until 1999 because there was no reimbursement by Medicare or any other payer worldwide. To get PET procedures reimbursed by Medicare, it was necessary to get



**Figure 1** Glucose and its analogs; 2-deoxyglucose (DG) and fluorodeoxyglucose (FDG).

**Table 2** FDA-Approved PET Radiopharmaceuticals

Radiopharmaceutical	FDA Approval of Clinical Utility		NDA Approval		
	Year	Indication	Year	Number	Manufacturer/Applicant
[ <sup>18</sup> F]Fluoride*	1972	Bone imaging	1972	17-042	New England Nuclear
<sup>82</sup> Rb-generator	1992	Myocardial perfusion	1989	19-414	Squibb Diagnostics
[ <sup>18</sup> F]FDG	1994	Epileptic foci	1994	20-306	Methodist Medical and CTI, Peoria, IL
[ <sup>18</sup> F]Fluoride	2000	Bone Imaging			
[ <sup>13</sup> N]NH <sub>4</sub> <sup>+</sup>	2000	Myocardial perfusion			
[ <sup>18</sup> F]FDG	2000	Epileptic foci in brain	2004	70-638	WMCCU, New York
		Myocardial glucose metabolism	2005	21-870	NSMC, New York
		Tumor glucose metabolism			
[ <sup>18</sup> F]FDG	2005	Alzheimer's disease (AD) and Frontotemporal dementia (FTD)			

NSMC, North Shore Medical Center, Manhasset, Long Island, NY; WMCCU, Weill Medical College of Cornell University, New York, NY.

\*The NDA holder ceased marketing this drug product in 1975. FDA currently lists sodium Fluoride F-18 injection in the Orange Book's "Discontinued Drug Product List."

FDA approval.<sup>9</sup> The approval of FDG-PET by the FDA in 2000 was a major breakthrough to the rapid incorporation of PET into nuclear medicine practice, particularly in oncology.

## Limitations of FDG-PET

Although FDG-PET/CT imaging provides high specificity and sensitivity in several kinds of cancer with many applications (Table 1) in the clinical management of an oncologic patient, it is important to recognize that FDG is not a "specific" radiotracer for imaging malignant disease. A number of factors (Table 3) can affect the FDG uptake in tumors and may explain the causes of false-positive and false-negative imaging data.<sup>10</sup> Various tissues and processes in the body use glucose to generate adenosine triphosphate (ATP) to meet the increased energy demands. The normal brain depends exclusively on glucose metabolism. Inflammatory cells and macrophages have greater glucose metabolic rates and accumulate higher amounts of FDG than tumor cells. Because FDG competes with glucose, the net uptake of FDG by tumor tissue depends on plasma glucose levels. In any given patient, the absolute tumor uptake of FDG depends on many factors and may not necessarily reflect tumor aggressiveness and the rate of tumor proliferation.<sup>10</sup>

## <sup>18</sup>F-Labeled PET Radiopharmaceuticals Excluding FDG

Highly "tumor-specific" and "tumor cell signal-specific" PET radiopharmaceuticals are essential to meet the growing demand of radioisotope-based molecular imaging technology for various applications (Table 4) to manage the highly complex patient-specific tumor biology. Molecular imaging has made rapid strides that go beyond the clinical applications of FDG-PET, to probe multiple aspects of tumor biology. The real power of molecular imaging goes beyond diagnosis by

identifying different biologic processes in a tumor using tracers that characterize both genotypic and phenotypic signatures.<sup>11</sup> Future clinical trials, with appropriate study design and regulatory guidance, will need to examine prospectively the use of imaging to help select cancer treatment. This is an important paradigm shift for PET, moving beyond detection in the direction of treatment selection.<sup>11</sup>

In the last 15 years, many alternative PET tracers have been proposed and were evaluated in preclinical and clinical studies to characterize the tumor biology more appropriately for clinical management of patients with cancer. Several publications have extensively reviewed the potential advantages and limitations of many PET radiopharmaceuticals.<sup>12-16</sup> <sup>18</sup>F-labeled radiotracers appear to be the most attractive option, mainly because of the wide availability of <sup>18</sup>F and the possibility of automated routine synthesis of these agents. Some of these tracers have been evaluated extensively in the clinic. The current issue of *Seminars in Nuclear*

**Table 3** Factors Affecting FDG uptake in Tumors

Factor	Increased	Decreased
Viable cancer cell number	X	
Tumor perfusion	X	
Hypoxia	X	
Glucose transporter expression	X	
Hexokinase activity	X	
Inflammation or infection	X	
Receptor agonists	X	
Chemotherapy acute	X	
Radiation therapy acute	X	
Receptor blockade		X
Chemotherapy effective		X
Radiation therapy chronic		X
Hyperglycemia		X
Insulin		X
Necrosis		X

Modified from Wahl.<sup>10</sup>

**Table 4** PET Radiopharmaceuticals: Mechanisms of Uptake and Localization

Biochemical Process	Radiotracer	Mechanism of Uptake or Localization
Blood flow/perfusion	[ <sup>15</sup> O]Water	Freely diffusible across membranes
Glucose metabolism	[ <sup>18</sup> F]FDG	Facilitated diffusion via glucose transporters. Substrate for hexokinase in glucose metabolism
Bone metabolism	[ <sup>18</sup> F]Fluoride	Incorporation in the hydroxyapatite crystals in bone
Membrane synthesis	[ <sup>11</sup> C]Choline, [ <sup>18</sup> F]Fluorocholine (FCH)	Substrates for choline kinase in choline metabolism
Lipid synthesis	[ <sup>18</sup> F]Fluoroacetate	Acetate is activated to acetyl-CoA in both the cytosol and mitochondria by acetyl-CoA synthetase
DNA synthesis	[ <sup>11</sup> C]Thymidine, [ <sup>18</sup> F]Fluorothymidine (FLT)	Substrates for thymidine kinase (TK-1) in DNA synthesis and reflects tumor cell proliferation rate
Hypoxia	[ <sup>18</sup> F]FMISO	Intracellular reduction and binding
Receptor Binding	[ <sup>18</sup> F]FES <sup>68</sup> Ga-DOTATOC <sup>68</sup> Ga-DOTANOC	Specific binding to estrogen receptors in breast cancer Specific binding to somatostatin receptor (SSTR-II) Specific binding to somatostatin receptor (SSTR-II, III, V)
AA transport and protein synthesis	[ <sup>18</sup> F]FDOPA [ <sup>11</sup> C]-L-methionine, [ <sup>18</sup> F]FMT, [ <sup>18</sup> F]FCCA	Precursor for the synthesis of dopamine Transport into the cells involves amino acid carrier protein. Intracellular trapping involves protein synthesis or transmethylation
Binding to tumor antigens	<sup>124</sup> I-, <sup>64</sup> Cu-, <sup>86</sup> Y-Labeled Antibodies	Specific binding to tumor associated antigenic binding sites (such as CEA, PSMA, CD20 and CD22)
Apoptosis	<sup>124</sup> I-Annexin V, <sup>64</sup> Cu-Annexin V	Specific binding to Phosphatidylserine (PS) on cell membrane
Angiogenesis	RGD peptide, [ <sup>18</sup> F]-FB-Elc(RGDyK)2	Integrin receptors ( $\alpha_v\beta_3$ ) on endothelial cells of neovasculature
Gene expression	[ <sup>18</sup> F]Oligonucleotide [ <sup>18</sup> F]FHBG	In vivo hybridization with mRNA Substrate to herpes virus thymidine kinase

Medicine specifically emphasizes the importance of several <sup>18</sup>F radiopharmaceuticals other than FDG for molecular imaging studies in oncology.

### <sup>18</sup>F: Ideal Positron-Emitting Radionuclide

A number of positron-emitting radionuclides that can be used for the development of successful commercial PET radiopharmaceuticals are listed in Table 5. <sup>11</sup>C is an ideal nuclide for labeling molecules of biological interest. The physical half-life, however, is too short for distribution and commercialization. The metallic nuclides, <sup>68</sup>Ga, <sup>64</sup>Cu, and <sup>86</sup>Y are appropriate for developing radiotracers based on peptides and proteins. Also, the availability of <sup>68</sup>Ga generator

provides an opportunity to prepare PET radiopharmaceuticals in house as and when needed. <sup>124</sup>I is ideal for labeling peptides and proteins but has a longer half-life and relatively lower specific activity compared with other positron-emitting nuclides. To develop PET radiopharmaceuticals, <sup>18</sup>F appears to be an ideal radionuclide for the following reasons:

- Low positron energy (0.64 MeV) with a short range in tissue (Max. 2.4 mm), which helps provide high-resolution images;
- Can be produced in high specific activity;
- Can be produced in large amounts in a cyclotron (>10 Ci);
- Has relatively high labeling yields (20-40%) in the synthesis of <sup>18</sup>F-PET tracers;

**Table 5** Positron-Emitting Radionuclides for PET

Nuclide	Half-life (min)	SA (Ci/ $\mu$ mol)	Decay % $\beta^+$	$\beta^+$ Energy (MeV)		Range in Water (mm)	
				Max.	Mean	Max.	Mean
<sup>11</sup> C	20.4	9220	99.77	0.9601	0.3856	4.1	1.1
<sup>68</sup> Ga	68.3	2766	87.7	1.8991	0.836	8.2	2.9
<sup>18</sup> F	110	1710	96.7	0.6335	0.2498	2.4	0.6
<sup>64</sup> Cu	768	245	17.87	0.6529	0.2781	2.9	0.64
<sup>86</sup> Y	884	213	12.4	1.2535	0.55	5.2	1.8
<sup>124</sup> I	6048	31	5.6	1.578	0.696	6.5	2.9
			11.0	1.5323	0.6859	6.3	2.3
			12.0	2.1350	0.9736	8.7	3.5

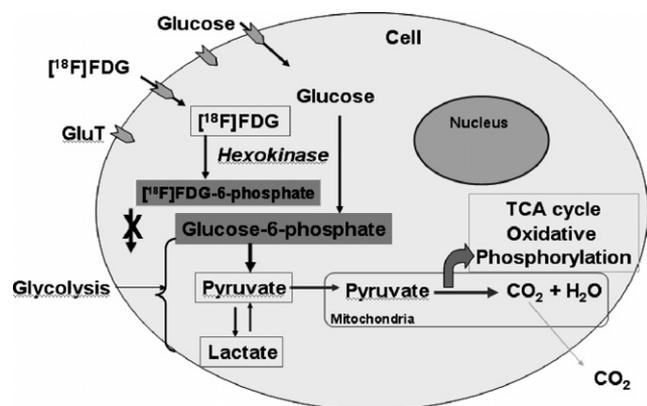
- Has acceptable radiation dosimetry for multiple studies in a patient; and
- The physical  $T_{1/2}$  (110 minute) allows for transport from the production site to the PET centers.

## Development of PET Radiopharmaceuticals: Biochemical Basis

PET radiopharmaceuticals can be generally classified based on their ability to image a specific biochemical process or based on their unique mechanism of localization in a specific organ/tissue of interest. A number of biochemical or functional changes present in cancer and the corresponding targeted radiopharmaceuticals are summarized in Table 4. It is an absolute requirement that these radiolabeled probes be designed in such a way that the administration of the radiolabeled probe does not disturb or perturb the biochemical process under investigation. Mechanism (s) of tumor uptake and localization of  $^{18}\text{F}$ -labeled radiopharmaceuticals discussed in this issue will be discussed below. It is important to understand that careful design and development of  $^{18}\text{F}$  radiopharmaceutical is necessary to preserve target specificity and to decrease in vivo metabolism. In addition, it is also important to appreciate that several alternative radiolabeled analogs are under development which may show even greater clinical utility in the future.

## Glucose Metabolism: [ $^{18}\text{F}$ ]FDG

FDG is a model PET radiopharmaceutical and is regarded as the “molecule of the century” in nuclear medicine. The simplified mechanism of uptake of FDG by tumor cells is now well understood. The most important molecule to provide energy for various biochemical reactions in the body is ATP, which is generated in mitochondria after metabolism of glucose in the mitochondria. Glucose is generally transported into the cell by facilitated diffusion through specific glucose transporters on the cell membrane. In the cytosol, glucose is phosphorylated by the enzyme hexokinase to glucose 6-phosphate, which subsequently is metabolized to carbon



**Figure 2** The cellular metabolism of D-glucose and [ $^{18}\text{F}$ ]FDG.

dioxide and water (Fig. 2). In 1950s it was shown that the hydroxyl group on carbon-2 of glucose molecule (Fig. 1) is not necessary for phosphorylation by hexokinase. DG enters the cell similar to glucose and is converted to deoxyglucose 6-phosphate which, however, does not undergo further metabolism and is trapped in the cell.<sup>17</sup> Therefore, the design of the FDG molecule is based on labeling carbon-2 atom in DG with  $^{18}\text{F}$ .<sup>7</sup> Incidentally the CF bond, which is more stable than CH bond, is chemically unrecognizable by hexokinase. As a glucose analog, FDG enters the cell membrane using the same transporters as glucose. It is then phosphorylated into [ $^{18}\text{F}$ ]FDG-6-phosphate. This metabolite is not a substrate for further enzymes and thus is trapped and accumulates inside the cell in proportion to the metabolism of glucose. Experimental studies in vitro and in vivo have clearly documented that the magnitude of FDG uptake in tumors, in general, relates quite directly to the number of viable cells.<sup>10</sup>

## Bone Metabolism: [ $^{18}\text{F}$ ]Fluoride

The normal bone undergoes constant remodeling, maintaining a balance between osteoclastic (resorptive) and osteoblastic activity. Bone involvement by cancer occurs most commonly when tumor cells are transported into the marrow. Most of the bone metastases are found in the red active marrow present mainly in the axial skeleton. As the lesion grows in the marrow, the surrounding bone undergoes osteoclastic and osteoblastic reactive changes.<sup>18,19</sup> The osteoblastic component of the metastasis represents reaction of normal bone to the metastatic process, and most sites of malignant bone involvement show reactive increased osteoblastic activity. In general, the radiographic appearance of a bone metastasis may be lytic, sclerotic (blastic), or mixed. Rapidly growing aggressive metastases tend to be lytic, whereas sclerosis is considered to indicate a slower tumor growth rate. Sclerosis may also be a sign of repair after treatment.<sup>19</sup> The incidence of lytic, blastic, and mixed types of bone metastases is different in various tumor types. In general, the purpose of bone imaging is to identify early bone involvement and to determine the full extent of the skeletal disease, to assess the presence of accompanying complications such as fractures and cord compression, and to monitor response to therapy.<sup>18</sup> Detection of bone involvement by various imaging modalities is based on either direct visualization of tumor infiltration or detection of the reaction of bone to the malignant process.

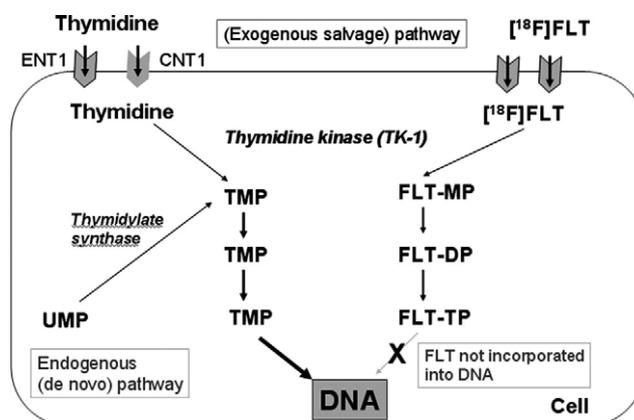
The absorption of fluorides by enamel, dentin, bone, and hydroxyapatite was reported in 1940 by Volker and coworkers.<sup>20</sup> [ $^{18}\text{F}$ ]Fluoride was first introduced as a bone-imaging agent by Blau et al. in 1962.<sup>21</sup> The FDA approved the New Drug Application (NDA) for bone imaging to define areas of altered osteogenic activity in 1972. In plasma, [ $^{18}\text{F}$ ]fluoride ions do not bind to plasma proteins and clear from circulation faster than  $^{99\text{m}}\text{Tc}$ -phosphonates. Fluoride ions diffuse through capillaries into bone extracellular fluid and are chemisorbed onto bone surface by exchanging with hydroxyl (OH) groups in hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) crystal of bone to form fluoroapatite.<sup>22</sup> Fluoride bone uptake mecha-

nism is similar to that of <sup>99m</sup>Tc-MDP. The uptake of both tracers in malignant bone lesions reflects the increased regional blood flow and bone turnover. However, because of faster blood clearance and higher capillary permeability, fluoride uptake in bone metastases is significantly higher compared with that of normal bone.<sup>22</sup> Although increased fluoride uptake has been reported in both sclerotic and lytic metastases,<sup>23,24</sup> the uptake of <sup>99m</sup>Tc-MDP in lytic lesions is relatively nonsignificant. Because both these tracers may also be seen in benign bone pathologies and nonmalignant orthopedic problems, bone agents are not considered as tumor-specific tracers. It is also important to recognize that FDG-PET is useful to detect bone metastases. Unlike fluoride and phosphonates, the uptake of FDG, however, is directly into tumor cells and not in the reactive bone.

### DNA Synthesis: 3'-deoxy-3'-<sup>18</sup>F]Fluorothymidine (FLT)

Increased cellular proliferation is a hallmark of the cancer phenotype.<sup>1</sup> Increased mitotic rate, cell proliferation, and lack of differentiation were regarded as the main factors responsible for accelerated growth of malignant tissue. Most benign tumors grow slowly over a period of years, but most malignant tumors grow rapidly, sometimes at an erratic pace. Cellular proliferation is specific to tumors, whereas increased glucose metabolism is a feature of tumors, but also associated with a variety of other processes, including inflammation and tissue healing.<sup>1</sup> Certain anticancer drugs were designed to stop the cell division but may not necessarily lead to cell death. As a result, tumor cellular proliferation drops without any significant change in the tumor energy metabolism. Therefore, molecular imaging probes designed specifically to measure proliferation are tumor-specific and may provide an impetus for PET imaging to be indicated as an appropriate technique, especially for measuring early response to treatment.

The DNA synthesis is a measure of proliferation. Because the number of cells in the S-phase of cell cycle is higher in tumor tissue compared with normal cells, there is also an increased requirement of substrates (nucleotides) for DNA synthesis in the tumor.<sup>25</sup> The 4 nucleotides required for DNA synthesis are cytosine, guanine, adenine, and thymidine. Thymidine is the only one incorporated exclusively into DNA and not RNA.<sup>25</sup> Intracellularly, thymidine is first phosphorylated in the cytoplasm by the enzyme thymidine kinase-1 (TK-1) to thymidine monophosphate (TMP), before incorporation into DNA. The level of TK-1 in a cell increases several-fold as it goes from a resting state to the proliferative phase and is destroyed at the end of S-phase.<sup>26</sup> TK-2 is a mitochondrial enzyme and not regulated by the cell cycle. Subsequently, TMP is phosphorylated to thymidine diphosphate (TDP) and then to thymidine triphosphate (TTP) before incorporation into DNA (Fig. 3). Therefore, radiolabeled thymidine analogs provide a measure of DNA synthesis and tumor cell proliferation.<sup>25</sup>



**Figure 3** DNA synthesis and the incorporation of thymidine into DNA. Thymidine is transported into the cell. It can also be made within the cell from uridine monophosphate (UMP). After transport into the cell, FLT is phosphorylated but not incorporated into DNA synthesis.

In the 1950s, [<sup>3</sup>H]thymidine was introduced to measure thymidine incorporation into DNA (thymidine labeling index) in tumor tissue.<sup>25,27</sup> Subsequently in 1972, [<sup>11</sup>C]thymidine was developed as a PET tracer and to measure proliferation rate.<sup>28</sup> However, because of rapid in vivo metabolism of this tracer,<sup>29</sup> [<sup>11</sup>C]thymidine is not optimal for routine PET imaging studies. In 1996, metabolically stable thymidine analogs, which are also substrates for the enzyme TK-1 were developed by Shields and coworkers.<sup>30,31</sup>

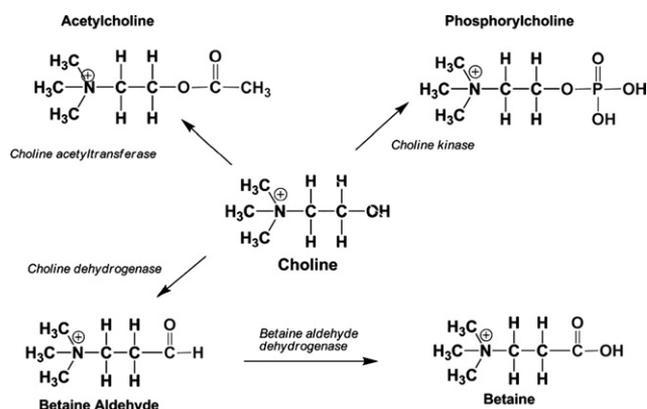
FLT is the most extensively investigated tracer to image cell proliferation. FLT is transported into cell similar to thymidine and then phosphorylated to [<sup>18</sup>F]FLT-5'-monophosphate by the enzyme TK-1. In vitro studies with tumor cell lines have demonstrated that FLT-MP is further phosphorylated to FLT-TP by the enzyme thymidylate kinase.<sup>32,33</sup> FLT phosphates, however, are impermeable to the cell membrane and resistant to degradation and are metabolically trapped inside the cells. The incorporation of FLT into DNA, however, was relatively insignificant (<1%).

Several radiolabeled molecular imaging probes (Fig. 4) were developed to measure DNA synthesis. [<sup>18</sup>F]Fluorouridine shows accumulation by proliferating cells, but the tracer is also incorporated both in DNA and RNA.<sup>34</sup> The pyrimidine analog, 2'-[<sup>18</sup>F]fluoro-5-methyl-1-β-D-arabinofuranosyluracil (FMAU) has been shown to be useful for imaging tumor cell proliferation.<sup>35</sup> FMAU can be taken up by cells and phosphorylated by TK-1 and TK-2 followed by DNA incorporation through DNA polymerase.<sup>36</sup> FMAU uses the same DNA synthetic pathway as thymidine and, therefore, has the potential to image DNA synthesis in tumors and normal proliferating tissues. It is important to recognize that FMAU is incorporated into DNA synthesis, but not FLT. This observation suggests that the hydroxyl group on Carbon-3 atom (Fig. 4) is essential for incorporation of thymidine analogs into DNA.

## Membrane Lipid Synthesis: [ $^{18}\text{F}$ ]Fluorocholeline (FCH) and $^{18}\text{F}$ -Acetate

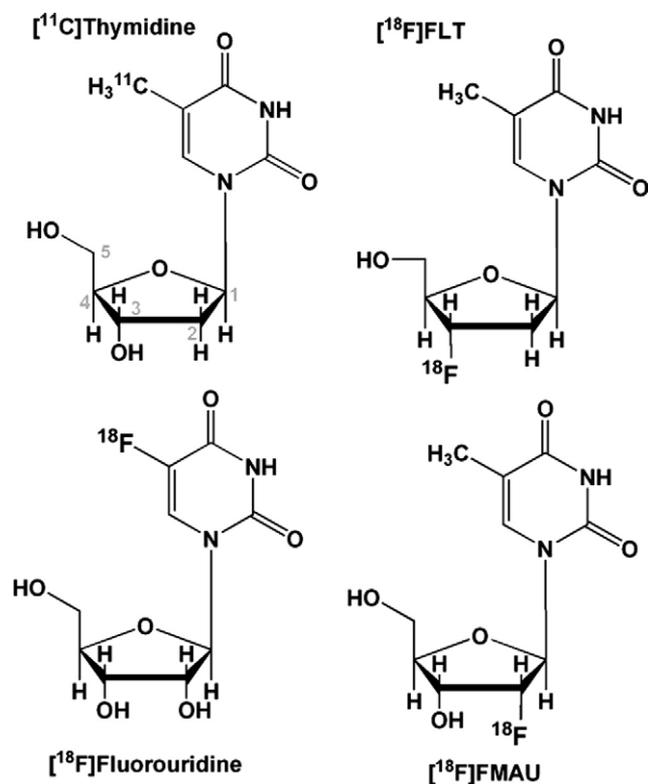
All cells use choline, a quaternary ammonium base as a precursor for the biosynthesis of phospholipids, which are essential components of all membranes.<sup>36</sup> In 1998, choline received the status of vitamin (group B) from the U.S. Food and Nutrition Board of the Institute of Medicine.<sup>37</sup> Choline enters most cells using specific low affinity, sodium-independent transporters. Within the cell, choline can be phosphorylated, acetylated or oxidized (Fig. 5). The phosphorylation of choline is catalyzed by the enzyme choline kinase.<sup>38</sup> Phosphorylcholine is an intracellular storage pool of choline and is further incorporated into phosphatidylcholine (lecithin), a major phospholipid of all membranes.<sup>36</sup> Choline is also a precursor for the synthesis of the neurotransmitter, acetylcholine. In addition, the metabolic pathway of choline also involves oxidation to betaine aldehyde then to betaine in blood, liver and kidney, the major sites for choline oxidation.<sup>39,40</sup>

It has been suggested that malignant transformation of cells is associated with the induction of *choline kinase* activity resulting in increased levels of phosphorylcholine. Furthermore, it is also known that rapidly proliferating tumors contain large amounts of phospholipids, particularly lecithin.<sup>41</sup> The formation and accumulation of membrane phospholipid-

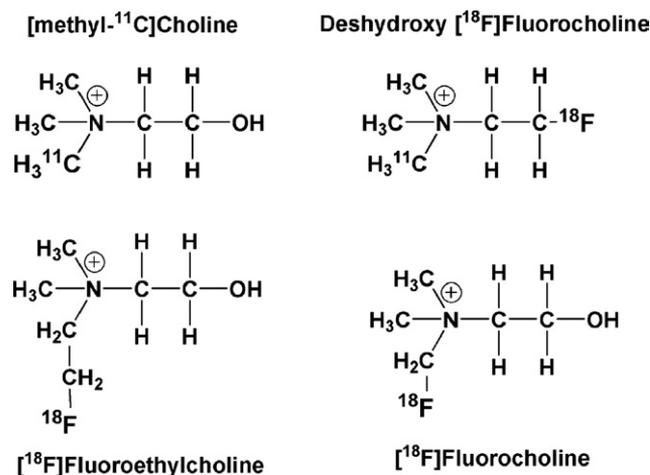


**Figure 5** The cellular metabolism of choline: It is phosphorylated, acetylated, and oxidized. Phosphorylcholine is further converted to phosphatidylcholine (lecithin), which is then incorporated into membrane synthesis.

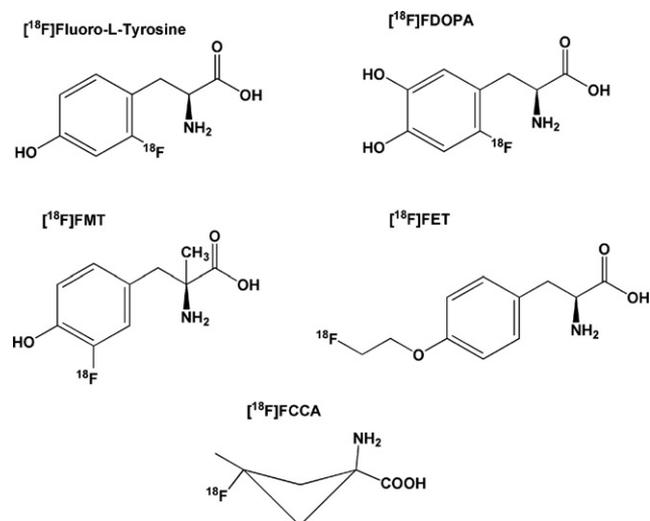
ids is coordinated with the cell cycle and occurs during the S phase.<sup>42</sup> The cells depleted with choline can not synthesize lecithin, resulting in the arrest in the G1 phase.<sup>43</sup> Thus, it was predicted that the uptake of radiolabeled choline would reflect the proliferative activity by estimating membrane lipid synthesis. Tumor cells with high proliferation rate, will have high uptake of choline to keep up with increased demands for the synthesis of phospholipids. In 1997, [ $^{11}\text{C}$ ]choline was introduced as a potential PET tracer to image brain and prostate cancer.<sup>44</sup> Since [ $^{11}\text{C}$ ]choline is rapidly oxidized in vivo,  $^{18}\text{F}$ -labeled choline analogs FCH and [ $^{18}\text{F}$ ]fluoroethylcholine (FECH; Fig. 6) were developed.<sup>45,46</sup> In vitro studies have clearly documented that these fluorinated choline analogs are good substrates for the enzyme choline kinase, but not for the enzymes involved in the oxidation of choline. As a result, no fluorinated derivatives of betaine have been observed.<sup>46</sup> The biodistribution of both FCH and FECH is very similar to that of choline, except for their very rapid urinary excretion. A nonmetabolizable fluorinated choline analog known as deshydroxy- $^{18}\text{F}$ fluorocholeline, (FDOC) was developed as a tracer to image the transport of choline into



**Figure 4** Radiolabeled thymidine analogs: In FLT, the  $^{18}\text{F}$  atom is attached to carbon-3 atom of the sugar whereas in FMAU, the  $^{18}\text{F}$  atom is attached to Carbon-2 atom. FMAU is incorporated into DNA but not FLT.



**Figure 6** Radiolabeled choline analogs.



**Figure 7** <sup>18</sup>F-labeled amino acid analogs.

tumor tissue.<sup>47</sup> FDOC, however, is not a substrate for the enzyme choline kinase and as a result, the tracer is not retained in tumor. Further studies have to clarify whether quantification of the transport capacity or the choline kinase activity may result in a better pathophysiological correlate and thus is the more useful process for tumor characterization.<sup>47</sup>

Acetate is readily taken up by cells and is activated to acetyl-CoA in both the cytosol and mitochondria by acetyl-CoA synthetase. Acetyl-CoA is a common metabolic intermediate for synthesis of cholesterol and fatty acids, which are then incorporated into membrane.<sup>48</sup> In normal cells and in myocardium, Acetyl-CoA is oxidized in mitochondria by the tricarboxylic acid cycle to carbon dioxide and water. But in tumor cells, most of the acetate is converted into fatty acids by a key enzyme fatty acid synthetase, which is overexpressed in cancer cells.<sup>49</sup> Acetyl-CoA is predominantly incorporated into intracellular phosphatidylcholine membrane microdomains that are important for tumor growth and metastasis. Therefore, [<sup>11</sup>C]acetate and <sup>18</sup>F-acetate were also proposed as tumor imaging agents.<sup>50-52</sup> In vitro studies have demonstrated that [<sup>14</sup>C]acetate was much better as a proliferation marker than [<sup>14</sup>C]choline.<sup>53</sup> The differences in sensitivity for tumor detection of these two tracers may be due to different enzymes and mechanisms involved in tumor uptake and retention of these two substrates.

## Amino Acid (AA) Transport and Protein Synthesis: [<sup>18</sup>F]FDOPA

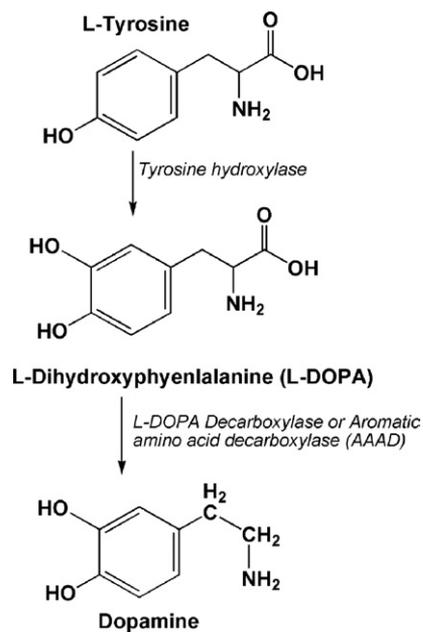
The tumor growth and development is characterized by an increase in the rate of protein synthesis. Because AAs are the building blocks for protein synthesis, carrier-mediated transport of AAs into cells is one of the most important and essential steps in protein synthesis. Subsequently, AAs are converted to the aminoacyl-t-RNA, which forms the polypeptide chain in the ribosome. AAs also undergo metabolism such as

transamination and decarboxylation. They are also precursors for many other biomolecules, such as hormones or neurotransmitters, and enter several metabolic cycles, for instance as methyl group donors.

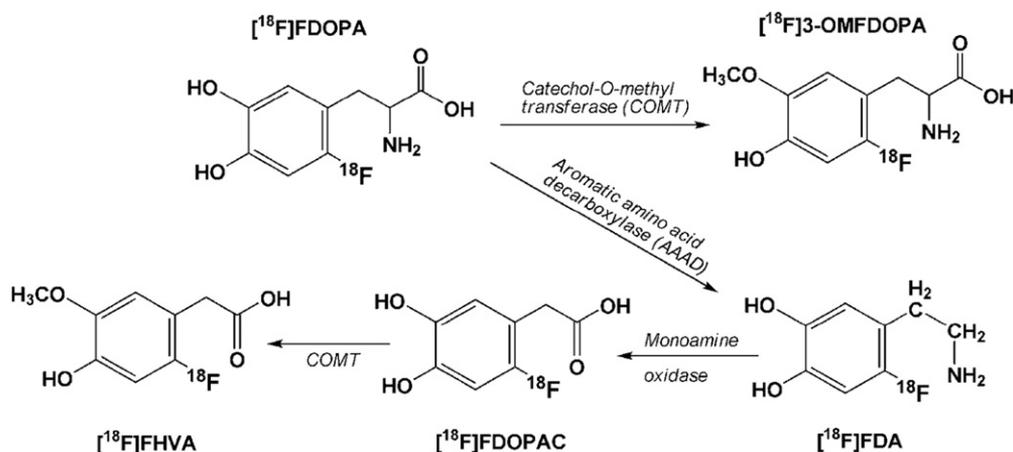
Although AAs may simply diffuse into the cells, their transport principally depends on more than 20 ubiquitous membrane transport systems. Most of the AAs are taken up by tumor cells not only through a Na<sup>+</sup> or an energy-independent L-type AA transporter system but also by Na<sup>+</sup>-dependent transporter systems A and B.<sup>54,55</sup> These transporters deal with aromatic AAs and ramified side-chain AAs, including leucine, valine, tyrosine and phenylalanine.<sup>15</sup> These amino acids are retained in tumor cells because of their higher metabolic activities than most normal cells. Malignant transformation increases the use of amino acids for energy, protein synthesis, and cell division. Tumor cells often overexpress transporter systems.<sup>56</sup> An overall increase in AA transport and/or an increase in protein synthesis rate by tumor cells may reflect proliferation.

[Carboxyl-<sup>11</sup>C]-L-leucine, [<sup>11</sup>C]-L-methionine, and [<sup>11</sup>C]-L-tyrosine are involved in protein synthesis and were introduced almost 30 years ago as tumor-imaging agents. Because they undergo metabolism in vivo, the exact position where <sup>11</sup>C is incorporated in the molecule is crucial for the measurement of protein synthesis. A number of <sup>18</sup>F-labeled tracers have been developed based on tyrosine and phenylalanine AAs (Fig. 7). The tumor uptake of all these <sup>18</sup>F-labeled AAs is mainly related to carrier-mediated active transport, and not to protein synthesis.

Dopamine, a neurotransmitter in the brain does not cross the blood-brain barrier and is synthesized from tyrosine (Fig. 8). The first step is the conversion of tyrosine to L-dihydroxyphenylalanine (L-DOPA). Subsequently, DOPA decarboxylase or aromatic amino acid decarboxylase (AAAD) converts L-DOPA to dopamine. Because L-DOPA is trans-



**Figure 8** Intracellular synthesis of dopamine from L-tyrosine.



**Figure 9** FDOPA metabolism: 3-O-methyl-6-fluoro-L-dopa (3-OMFDOPA), 6-fluorodopamine (FDA), L-3,4-dihydroxy-6-fluorophenylacetic acid (FDOPAC), and 6- $[^{18}\text{F}]$ fluorohomovanillic acid (FHVA).

ported into the brain via the large neutral amino acid carrier, L-3,4-dihydroxy-6- $[^{18}\text{F}]$ fluorophenylalanine (FDOPA) was developed to examine the transport of dopamine precursor from plasma.<sup>57,58</sup> FDOPA, however, undergoes extensive metabolism *in vivo*<sup>59</sup> as shown in Fig. 9. FDOPA is decarboxylated by AAAD to produce  $[^{18}\text{F}]$ fluorodopamine (FDA). FDOPA can be O-methylated by catechol-O-methyltransferase (COMT) to 3-O-methyl-6- $[^{18}\text{F}]$ fluoro-L-dopa (3-OMFDOPA), which is uniformly distributed throughout the brain. FDA can be oxidized by monoamine oxidase (MAO) to L-3,4-dihydroxy-6- $[^{18}\text{F}]$ fluorophenylacetic acid ( $[^{18}\text{F}]$ FDOPAC), which subsequently o-methylated by COMT to 6- $[^{18}\text{F}]$ fluorohomovanillic acid ( $[^{18}\text{F}]$ FHVA). AAAD and COMT are also present in peripheral tissues such as liver, kidneys, and lung. Both the decarboxylation of the compound and the release into blood of radiometabolites can be reduced with carbidopa, a decarboxylase inhibitor.

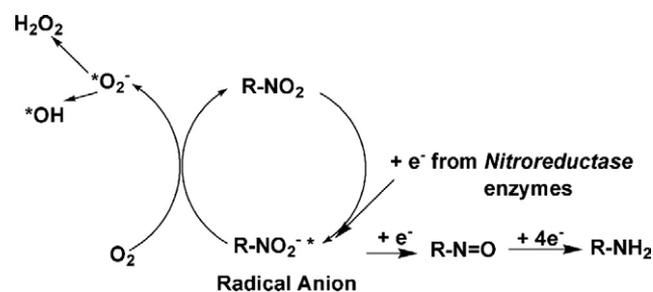
In oncology, FDOPA has been proposed initially as a biomarker for melanomas. Recently, very encouraging results have been reported in infrequent tumors such as neuroendocrine tumors (NETs), medullary thyroid carcinomas, pheochromocytomas.<sup>60-62</sup> The ability of NETs to accumulate and decarboxylate L-DOPA is well known. Increased activity of L-DOPA decarboxylase was found to be a hallmark of NETs.<sup>63</sup>

In addition to FDOPA, several important  $^{18}\text{F}$ -labeled amino acids (Fig. 7) are under extensive clinical investigation. Among them, O-(2- $[^{18}\text{F}]$ fluoroethyl)-L-tyrosine (FET) and L-3- $[^{18}\text{F}]$ fluoro- $\alpha$ -methyltyrosine (FMT) have shown significant diagnostic potential to image brain tumors.<sup>64</sup> Recent clinical studies have shown potential clinical utility of a synthetic, nonmetabolizable amino acid analog, known as 1-amino-3- $[^{18}\text{F}]$ fluorocyclobutane-1-carboxylic acid (FCCA).<sup>65</sup> With most of the radiolabeled amino acids, however, the cellular uptake and retention appear to be mainly influenced by the amino acid transport process.

## Tumor Hypoxia: $[^{18}\text{F}]$ Fluoromisonidazole (FMISO)

With increasing tumor size, there is a reduced ability of the local vasculature to supply sufficient oxygen to rapidly dividing tumor cells.<sup>66</sup> The resulting hypoxia may inhibit new cell division or even lead to cell death, but it may also lead to adaptive responses that will help cells survive and progress. The presence of hypoxia in tumors has long been established as a key factor in tumor progression and in the resistance of tumors to therapy.<sup>67</sup> Well-oxygenated cells are more sensitive to the cytotoxic effects of ionizing radiation compared with poorly oxygenated cells. Therefore, hypoxia in tumor tissue seems to be an important prognostic indicator of response to either chemotherapy or radiation therapy.<sup>67</sup>

2-Nitroimidazole (azomycin) was developed in the 1950s as an antibiotic targeted against anaerobic germs. In 1979, Chapman and coworkers were the first to propose nitroimidazoles as bioreducible markers of hypoxia and as a sensitizing factor for radiation therapy of hypoxic tumors.<sup>68</sup> It was observed that nitroimidazoles enter cells by passive diffusion



**Figure 10** The mechanism of intracellular trapping of nitroimidazoles. Under hypoxic conditions, the nitro group undergoes electron reduction to form reactive radicals which on further reduction form covalent bonds with intracellular macromolecules.

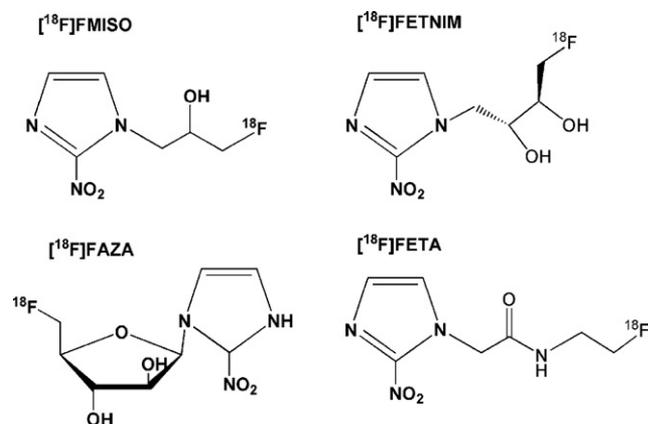


Figure 11 <sup>18</sup>F-labeled analogs of nitroimidazole.

and undergo a single electron reduction to form a potentially reactive species.<sup>68,69</sup> When oxygen is abundant, the molecule is immediately reoxidized. However, under hypoxic conditions, further reduction of the nitroimidazole molecule occurs that forms covalent bonds to intracellular macromolecules, in a process of metabolic trapping within the hypoxic cell (Fig. 10).

In 1984, [<sup>18</sup>F]fluoromisonidazole (FMISO) was proposed as a tracer for determining tumor hypoxia.<sup>70,71</sup> It binds selectively to hypoxic cells both *in vitro* and *in vivo*. Since then, FMISO-PET has been used to quantitatively assess tumor hypoxia in lung, brain, and head-and-neck cancer patients and in the hearts of patients with myocardial ischemia. FMISO is relatively hydrophilic and diffuses across cell membranes, showing a passive distribution in normal tissues. Because of high lipophilicity, slow clearance kinetics, reaction mechanisms, low uptake in hypoxic cells and the absence of active transport of this radiotracer, the identification and

quantification of hypoxic tumor areas necessitate imaging for longer periods of time post injection. It is the most extensively studied hypoxic PET radiopharmaceutical. Several other analogs (Fig. 11) such as [<sup>18</sup>F]fluoroerythronitroimidazole (FETNIM), [<sup>18</sup>F]fluoroetanidazole (FETA), and 1-(5-[<sup>18</sup>F]Fluoro-5-deoxy-α-D-arabinofuranosyl)-2-nitroimidazole (FAZA) have been developed with more favorable pharmacokinetics.<sup>72-75</sup> FETNIM is more hydrophilic than FMISO and shows promise for hypoxia imaging in humans. FETA demonstrates oxygen-dependent binding and retention in tumors that are very similar to that for FMISO, but less rapidly metabolized. Recently, it has been reported that FAZA displayed a hypoxia-specific uptake mechanism and provided tumor-to-background ratios (T/B ratios) superior to that of the standard hypoxia tracer FMISO.<sup>74</sup>

## Estrogen Receptor Binding: [<sup>18</sup>F]Fluoroestradiol (FES)

Estrogen, a steroidal hormone, produces many physiological effects primarily by regulating gene expression by binding to specific estrogen receptors (ERs). Estradiol (Fig. 12), the most potent form of estrogen in the body binds to ERs found in the cell nucleus of the female reproductive tract, breast, pituitary, hypothalamus, bone, liver, and other tissues, and also in various tissues in men.<sup>76</sup> For decades, it was assumed that there was only one type of ER known as αER. However, a second ER form, βER, found to bind estradiol with a comparable *K<sub>d</sub>* to αER, was recently described.<sup>77</sup> Like alpha, beta is a nuclear receptor and looks nearly identical to alpha in its so-called DNA-binding domain, the segment of the receptor that acts as a

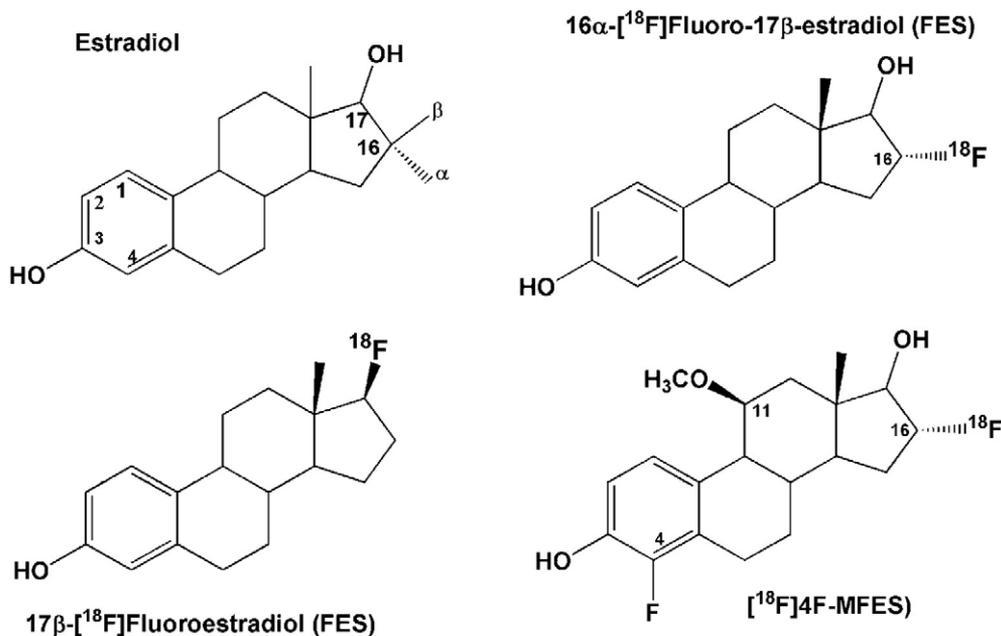


Figure 12 Estradiol and <sup>18</sup>F-labeled estradiol analogs.

genetic switch, flicking some genes on and some genes off. While the alpha receptor predominates in the uterus and mammary gland, the beta is mostly in the ovaries, the testes and also in the osteoblasts.

The growth of breast epithelial cells is an estrogen-mediated process that depends on estrogen acting through an ER and results in the induction of progesterone receptor. The ER status is an important prognostic factor in breast cancer because ER+ tumors have a slower rate of growth and are likely to respond to hormonal therapy.<sup>76</sup> The main mechanism of action of antiestrogen tamoxifen is believed to be a block of division of estrogen-dependent tumor cells with arrest in the G0 or G1 phase of the cycle. However, 30-40% of all breast cancers do not express estrogen receptors (ER-), and of the tumors with estrogen receptors (ER+), up to 50% will not respond to endocrine treatment.<sup>76,78</sup> Therefore, a noninvasive method which would accurately evaluate and quantify the presence of ER on the tumor and its metastases could help better select patients for treatment and predict the therapeutic response.

In the last 25 years, more than 20 fluorinated estrogen derivatives have been proposed for imaging studies. Retention of radiolabeled estradiol in estrogen target tissues through specific binding on the  $\alpha$ ER target was first reported by Jensen and coworkers.<sup>79</sup> Estradiol and its derivatives are lipophilic and transported in the bloodstream bound either to sex hormone binding protein (SBP) or to albumin. SBP binding protects steroids against liver metabolism and ensures their transport to the target tissues. Moreover, their affinity for SBP also contributes to their cell uptake, through membrane receptors for SBP.<sup>76</sup> SBP receptors are present in a higher percentage of ER+ tumors (75%) than in ER- tumors (37%). Thus membrane sequestration of estradiol is likely to occur preferentially in hormone responsive tumors.

The most promising radiolabeled estrogen analog identified to date is 16 $\alpha$ -[<sup>18</sup>F]fluoro-17 $\beta$ -estradiol (FES), which has good ER binding affinity and can be prepared in high effective specific activity.<sup>80,81</sup> A number of clinical trials have documented the potential utility of FES-PET to identify ER+ breast tumors. But it is not necessarily an optimal tracer due to its rapid conversion to circulating radiometabolites, which prevent optimal localization of the tracer at ER binding sites.<sup>82</sup> A number of estradiol analogs (Fig. 12) with substitutions at various positions have been advanced to improve target selectivity. Among these analogs, two important agents may have significant potential for clinical evaluation. Replacing hydroxyl group with <sup>18</sup>F at C17 position would result in [<sup>18</sup>F]fluoro-17 $\beta$ -estradiol, which has high affinity for the ER, and an excellent correlation between the tumor uptake in vivo and the tumor ER concentration measured in vitro.<sup>81</sup> Addition of both a 4-fluoro and 11 $\beta$ -methoxy group onto FES yields 11 $\beta$ -methoxy-4,16 $\alpha$ -[16 $\alpha$ -<sup>18</sup>F]difluoroestradiol (4F-MFES) with potential improved properties for estrogen receptor imaging studies.<sup>83</sup> This tracer recently showed increased in vivo stability and the highest target-to-nontarget ratios in an animal model.<sup>84</sup>

## Radiochemistry of <sup>18</sup>F-Labeled Radiopharmaceuticals

### Production of <sup>18</sup>F

Basically there are 2 kinds of targets used in a cyclotron to produce two different chemical forms of <sup>18</sup>F. A liquid target for the production of <sup>18</sup>F as nucleophilic fluoride ion (<sup>18</sup>F<sup>-</sup>) and a gas target for the production of <sup>18</sup>F as an electrophilic fluorine gas ([<sup>18</sup>F]F<sub>2</sub>). The most common nuclear reaction used to produce <sup>18</sup>F as fluoride ion is based on proton bombardment of <sup>18</sup>O atoms using highly enriched <sup>18</sup>O water as the target material.<sup>85,86</sup> A typical target body is made of silver, tungsten or titanium and holds 0.3-3.0 mL of target water. Several curies of <sup>18</sup>F can easily be made in 1 to 2 hours using 10 to 19 MeV protons with a beam current of 20 to 35  $\mu$ A. While the theoretical SA of <sup>18</sup>F is 1700Ci/ $\mu$ moles, the NCA <sup>18</sup>F produced is generally <10 Ci/ $\mu$ moles.

The most common nuclear reaction to produce [<sup>18</sup>F]Fluorine is based on deuteron bombardment of <sup>20</sup>Ne atoms using natural neon gas.<sup>86</sup> A passivated nickel target (NiF) is loaded with neon gas with 0.1% of natural fluorine gas. Following bombardment for 1 to 2 hours with 8 to 9 MeV deuterons, <1.0 Ci of [<sup>18</sup>F]F<sub>2</sub> is generated with very low SA (10-20 mCi/ $\mu$ moles). A "double shoot" method was developed to produce [<sup>18</sup>F]fluorine gas based on proton bombardment of <sup>18</sup>O atoms using <sup>18</sup>O oxygen gas loaded into a gas target.<sup>87,88</sup> After irradiation, <sup>18</sup>F species stick to the walls of the target. <sup>18</sup>O target gas is removed from the target and loaded with argon gas mixed with cold 1% fluorine gas. A second short irradiation for <10 minute will generate [<sup>18</sup>F]F<sub>2</sub> gas. This method is very useful to make electrophilic <sup>18</sup>F using cyclotrons generating proton beams only. Because [<sup>18</sup>F]F<sub>2</sub> is always diluted with carrier (cold) fluorine gas, the SA of electrophilic [<sup>18</sup>F]fluorine is very low and not optimal for synthesizing high specific activity <sup>18</sup>F-labeled radiopharmaceuticals.

### Sodium Fluoride <sup>18</sup>F Injection

This is a no-carrier added sodium [<sup>18</sup>F]fluoride that is used for diagnostic purposes in conjunction with PET imaging. The clinical indication of fluoride for bone imaging has been approved by FDA in 2002. As a PET radiopharmaceutical, however, NDA was not approved by FDA for routine manufacturing of sodium fluoride F-18 injection. The active ingredient [<sup>18</sup>F]fluoride can be produced by proton bombardment using either <sup>20</sup>Ne or <sup>18</sup>O as a target. Sodium fluoride F-18 injection is provided as a ready to use isotonic, sterile, pyrogen-free, clear and colorless solution in 0.9% aqueous sodium chloride. The pH of the solution is 6 to 8 and should not contain any preservatives.

### <sup>18</sup>F Radiochemistry

Fluorine is the most electronegative of all the elements and can react with many organic and inorganic chemicals. Being a powerful oxidizing agent, it may bind to quartz and glassware. It can react as an electrophile (positively charged) or a neutrophile (negatively charged) chemical

species. Electrophilic reactions involve carrier-added (CA) or low SA [<sup>18</sup>F]F<sub>2</sub> gas, whereas nucleophilic reactions involve no carrier-added (NCA) or high SA <sup>18</sup>F<sup>-</sup> ion. In addition, to these two primary <sup>18</sup>F precursors, several other secondary precursors (Fig. 13) have been developed to radiolabel a number of organic molecules. Among them, <sup>18</sup>F-CH<sub>3</sub>COOF (acetyl hypofluorite), metal fluorides (such as K<sup>18</sup>F, Cs<sup>18</sup>F), and tetra-*n*-butyl ammonium fluoride (nBu<sub>4</sub>N<sup>18</sup>F) are the most widely used <sup>18</sup>F precursors in fluorination reactions.<sup>89</sup>

### Electrophilic Fluorination Reactions

The [<sup>18</sup>F]F<sub>2</sub> precursor has only one of the atoms as <sup>18</sup>F whereas the other is stable <sup>19</sup>F atom. Therefore the labeling yield are always <50%. The first synthesis of FDG reported in 1976 was based on [<sup>18</sup>F]F<sub>2</sub> gas. The other reactive precursor is <sup>18</sup>F-acetyl hypofluorite. With these precursors, direct electrophilic fluorinations are not regioselective and the <sup>18</sup>F atom can attack any of the C-C double bond in the molecule. Therefore, these precursors are used only in rare situations where nucleophilic reactions are not appropriate. However, regioselective electrophilic fluorodemetalation reactions were developed to take advantage of reactive electrophiles in the preparation of <sup>18</sup>F radiotracers. FDOPA synthesis is the most common PET radiopharmaceutical that is routinely prepared using [<sup>18</sup>F]F<sub>2</sub> gas.

### Nucleophilic Fluorination Reactions

The most successful approach for preparing high SA <sup>18</sup>F radiotracers is based on nucleophilic fluorination reactions since [<sup>18</sup>F]fluoride can be produced in high SA (2-10 Ci/μmoles). Synthesis of <sup>18</sup>F radiopharmaceuticals using fluoride ion utilize 2 general categories or types of chemical reactions; aliphatic nucleophilic substitution, also known as substitution nucleophilic bimolecular (S<sub>N</sub>2), and aromatic nucleophilic substitution (S<sub>N</sub>Ar).

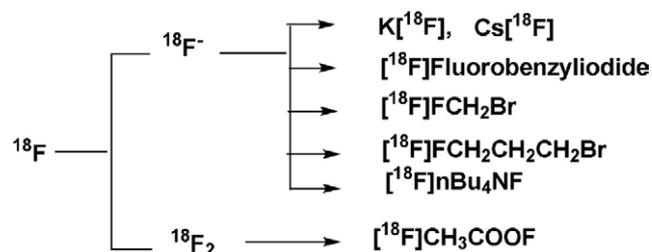
In S<sub>N</sub>2 reactions, the fluoride ion attacks and binds to the carbon atom of the substrate at 180° opposite to a leaving group such as a weak base (iodide, bromide, triflate, tosylate, nosylate, mesylate, etc). These reactions generally take place in basic or neutral conditions in the presence of an appropriate aprotic solvent (such as acetonitrile) in which the reactants show good solubility. [<sup>18</sup>F]Fluoride ion is generally complexed with a metal or positively charged ion. When alkali metal halides (K<sup>18</sup>F, Cs<sup>18</sup>F, Rb<sup>18</sup>F) are used, it is essen-

tial to have the metal to be coordinated by cryptands and polyaminoethers (such as Kryptofix 2.2.2) so that the relatively free fluoride can show very good reactivity. Instead of alkali metal halides, a variety of tetraalkylammonium [<sup>18</sup>F]fluorides have also been used. Sometimes, [<sup>18</sup>F]fluoride ion is first converted to another reactive species such as alkyl halides ([<sup>18</sup>F]fluorobromoethane, [<sup>18</sup>F]fluorobromopropane) and benzylic halides.<sup>89</sup> S<sub>N</sub>Ar nucleophilic reactions were designed to incorporate <sup>18</sup>F atom directly into the aromatic ring as well as into prosthetic groups containing aromatic ring of a molecule. In these reactions, the leaving group is activated by the electron-withdrawing groups ortho and or para to the leaving group.

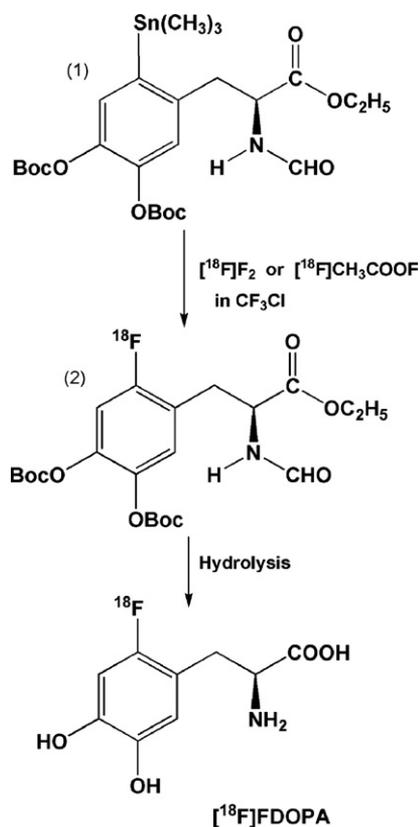
In many of these nucleophilic reactions, preparation of the reactive [<sup>18</sup>F]fluoride species involves Kryptofix as a phase transfer catalyst and drying with acetonitrile to produce anhydrous [<sup>18</sup>F]fluoride ion. To introduce <sup>18</sup>F atom using [<sup>18</sup>F]fluoride, the molecule of interest must have an alkyl alcohol group, which can be activated by forming a corresponding alkyl sulfonate ester derivative such as triflate, tosylate, etc. Using this approach, several organic precursors have been synthesized for routine synthesis of <sup>18</sup>F radiopharmaceuticals. A number of radiotracers of clinical interest such as FDG, FLT, FMISO, and FES can all be prepared (described below) using commercially available precursors. In the preparation of certain radiotracers, however, the <sup>18</sup>F labeled alkyl halides are prepared first using aliphatic nucleophilic reaction and then the <sup>18</sup>F-alkyl halide (also known as “synthon”) is subsequently used to prepare the radiotracer of interest.<sup>90</sup> For example, [<sup>18</sup>F]fluorobromomethane is the synthon used in the preparation of FCH.

### FDOPA Synthesis

Firnau and coworkers in 1973 reported the synthesis of 5-[<sup>18</sup>F]fluoro-DOPA.<sup>91</sup> The metabolism of this tracer, however, was accelerated because the fluorine atom was close to the hydroxyl groups of FDOPA. Consequently, 6-[<sup>18</sup>F]fluoro-L-DOPA was developed and the first FDOPA-PET brain imaging was performed in 1984 to visualize the dopaminergic neurons in basal ganglia.<sup>57,92</sup> A number of methods have been developed to synthesize FDOPA. The electrophilic radiofluorination by destannylation of FDOPA precursor, has emerged as the preparation method of choice and has been used by many groups employing a custom-built apparatus or a commercial PC-controlled automated synthesis module.<sup>93-95</sup> The FDOPA precursor (Fig. 14) is an aryl substituted trialkyl tin derivative, known as TriBoc-L-Dopa ethyl ester. Fluorination of the precursor in freon solvent can be performed using [<sup>18</sup>F]F<sub>2</sub> gas directly or acetyl hypofluorite reagent, <sup>18</sup>F-CH<sub>3</sub>COOF. The trimethylstannyl group (Sn(CH<sub>3</sub>)<sub>3</sub>) is replaced by the <sup>18</sup>F atom. The alkyl (tertiary butyl and ethyl) groups in the precursor protect the hydroxyl and carboxyl groups during fluorination. Finally, following acidic removal (deprotection) of the protective groups using hydrogen bromide (HBr), FDOPA can be generated. Purification with high-performance liquid chromatography (HPLC) is required to achieve high chemical and ra-



**Figure 13** <sup>18</sup>F primary and secondary precursors used in nucleophilic and electrophilic fluorination reactions.



**Figure 14** FDOPA synthesis. The precursor (1) is an aryl substituted trialkyl tin derivative, known as Triboc-L-Dopa ethyl ester. The fluorinated intermediate (2) on hydrolysis will produce FDOPA.

radiochemical purity. The final decay corrected yields are typically 20 to 30% and the specific activity is about  $37 \text{ GBq}/\mu\text{mol}$  ( $1.0 \text{ Ci}/\mu\text{mol}$ ) at the EOS. Nucleophilic methods using the  $[^{18}\text{F}]$ fluoride ion are being developed and may have the potential to provide a higher yield and a higher specific activity.

## FLT Synthesis

The synthesis of FLT was first reported in 1991, but the low radiochemical yield prevented routine clinical use of the compound.<sup>96</sup> Subsequently, Grierson and Shields reported improvements in the FLT synthesis in 1998.<sup>97</sup> Since then, a number of precursors and different labeling protocols have been evaluated to improve labeling yields and the radiochemical purity.<sup>98-100</sup> The most reliable radiosynthesis of  $[^{18}\text{F}]$ FLT involves a simple 3-step procedure based on a protected nosylate precursor known as 3-N-Boc-5'-O-dimethoxytrityl-3'-O-nosyl-thymidine. Fluorination reactions involve displacement of 3-O-nosyl group with  $[^{18}\text{F}]$ fluoride and yield an  $^{18}\text{F}$ -labeled protected intermediate, which after hydrolysis generates  $[^{18}\text{F}]$ FLT (Fig. 15). The final radiochemical yields of FLT vary (10-40%) and depend on the mass amount of precursor and kryptofix used. Increased amounts of precursor may yield higher labeling yields, but may also increase chemical impurities in the final product. We found that the kryptofix plays a very important role and that by carefully adjusting the precursor/kryptofix molar ratio, higher labeling yields with minimal

chemical impurities can be obtained.<sup>101</sup> HPLC purification is essential to reduce chemical impurities. Recently, Oh and coworkers<sup>102</sup> reported a new, fully automated method for the synthesis of FLT with a 50% radiochemical yield, by modifying a commercial FDG synthesizer and its disposable fluid pathway.

## FMISO Synthesis

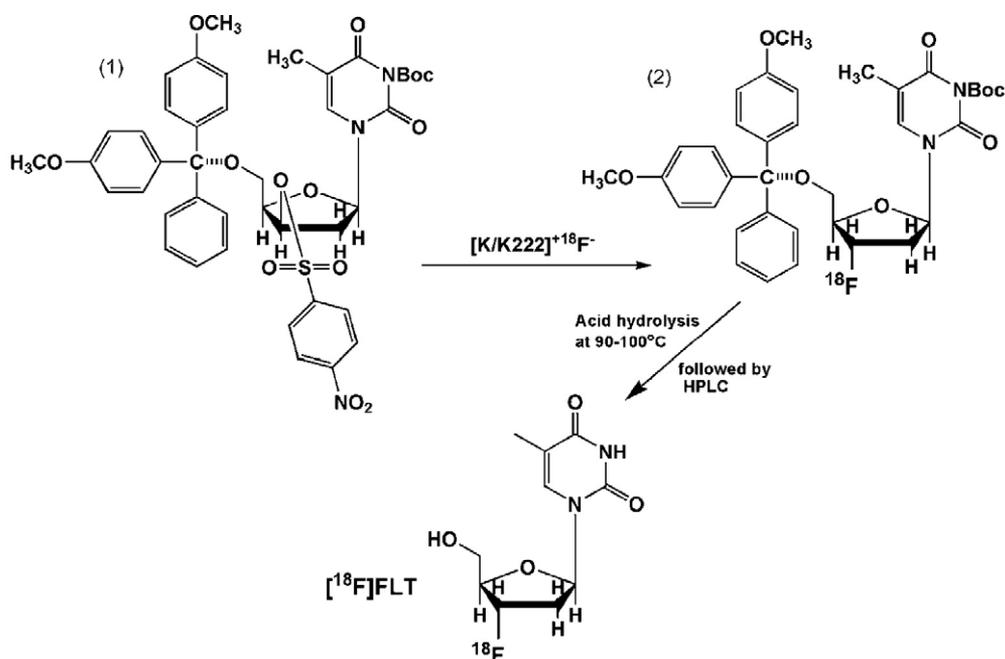
In 1986, Jerabeck and coworkers first reported the synthesis of fluoronitroimidazoles.<sup>103</sup> Subsequently, Grierson and coworkers in 1989<sup>104</sup> proposed a 2-step synthesis of FMISO with a high yield (40% at EOB) and high purity with a specific activity of  $37 \text{ TBq}/\text{mmol}$ . The first step involves synthesis of fluoroalkylating agent  $^{18}\text{F}$ -epifluorohydrin, which subsequently reacts with 2-nitroimidazole to yield FMISO. HPLC purification was needed to obtain high radiochemical purity. Further modifications were reported with increasing labeling yields.<sup>105-108</sup> The most practical method is based on nucleophilic substitution of the tosylate leaving group by  $^{18}\text{F}$ -fluoride on the tetrahydropyranyl-protected precursor 1-(2'-nitro-1'-imidazolyl)-2-O-tetrahydropyranyl-3-O-toluenesulfonylpropanediol (NITTP), followed by hydrolysis of the protecting group (Fig. 16). An automated synthesis of  $[^{18}\text{F}]$ FMISO by this method using either HPLC or Sep-Paks for the purification of the radiotracer was recently reported.<sup>109</sup> The whole synthesis time was  $<50$  minute. The radiochemical yield obtained when using NITTP was found to be  $\leq 40\%$  and reproducible, with a radiochemical purity  $\geq 97\%$ , and a specific activity of about  $34 \text{ TBq}/\text{mmol}$ .

## FES Synthesis

The first synthesis of FES was reported in 1984 by Kiesewetter and coworkers.<sup>110</sup> FES is synthesized by nucleophilic displacement of the aliphatic triflate of 3,16 $\beta$ -bis (trifluoromethanesulfonyloxy)-estrone using tetrabutylammonium  $[^{18}\text{F}]$ fluoride, followed by hydrolysis and ketone reduction. HPLC purification provided a 30% radiochemical yield of FES in about 90 minute. In 1983, Tewson developed a new procedure in which the nucleophilic substitution of  $[^{19}\text{F}]$ fluoride led directly to  $[^{19}\text{F}]$ FES using 3-O-Methoxymethyl-16, 17-O-sulfonyl-16-epiestriol (MMSE) as a precursor.<sup>111</sup> This procedure worked well with non radioactive fluoride but not with no carrier added  $[^{18}\text{F}]$ fluoride. The first successful preparation with  $[^{18}\text{F}]$ fluoride with further modifications, however, was published subsequently.<sup>112</sup> A one-pot synthesis of FES (Fig. 17) based on the MMSE precursor was developed subsequently to provide radiochemical yields of 30 to 45% with a specific activity of about  $37 \text{ GBq}/\mu\text{mol}$  ( $1.0 \text{ Ci}/\mu\text{mol}$ ) in 60 to 120 minutes.<sup>5</sup> An automated module was developed for the synthesis of FES using the same method to give about 50% radiochemical yields in 50 minute.<sup>113</sup>

## FCH Synthesis

In 1997, Hara and coworkers<sup>114</sup> developed and synthesized the first  $^{18}\text{F}$  labeled choline analog, 2- $[^{18}\text{F}]$ fluoroethylcholine (FEC). On the basis of structural similarity, DeGrado and



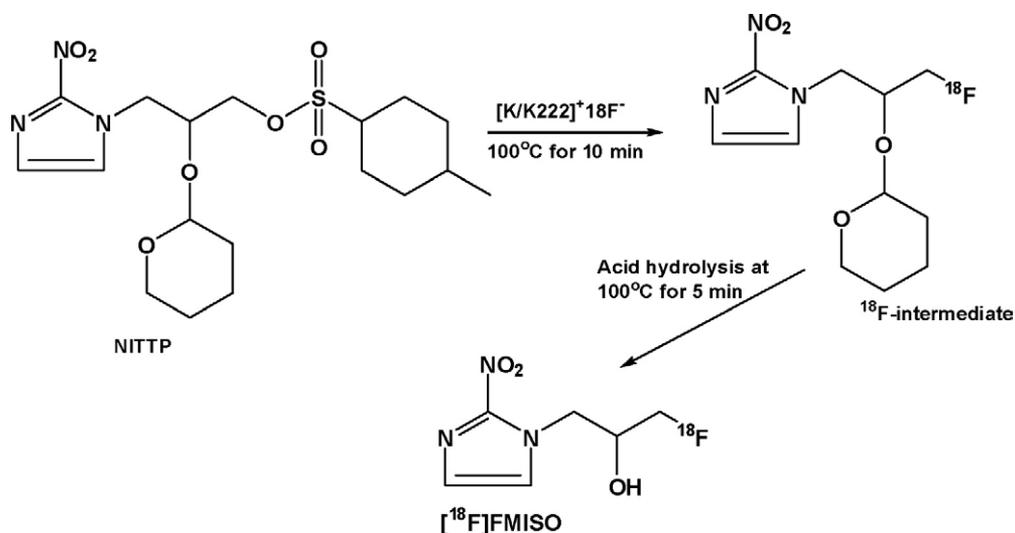
**Figure 15** FLT synthesis. The protected nosylate precursor (1) is known as 3-N-Boc-5'-O-dimethoxytrityl-3'-O-nosyl-thymidine. The fluorinated intermediate (2) on hydrolysis will produce FLT.

coworkers<sup>115</sup> speculated that [<sup>18</sup>F]fluoromethylated choline (FCH) would mimic choline transport and metabolism more closely than that of the FEC. In 2001, DeGrado and coworkers<sup>116</sup> reported the first synthesis of FCH based on nucleophilic fluorination method. The <sup>18</sup>F precursor, [<sup>18</sup>F]fluorobromomethane (FBM) was first prepared using [<sup>18</sup>F]fluoride and dibromomethane (DBM). Subsequently, FBM was reacted with dimethylethanolamine to yield FCH with a radiochemical purity greater than 98% and an uncorrected radiochemical yield of 20 to 40%). This method was modified to automate the synthesise using commercial FDG synthesis module.<sup>117,118</sup> An automated method of FCH synthesis was

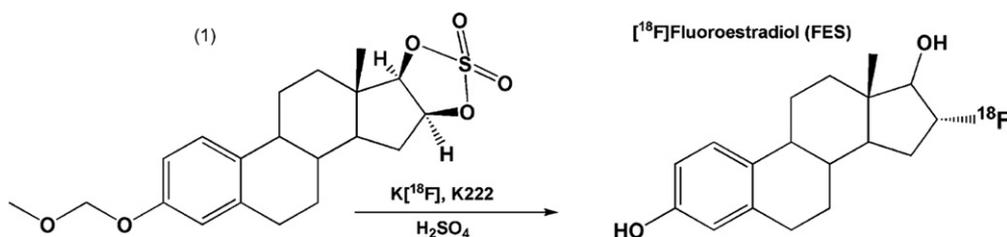
also achieved by the reaction of [<sup>18</sup>F]fluoromethyl triflate (FMT) with dimethylethanolamine on a Sep-Pak column.<sup>119</sup> The total time required for obtaining the finished chemical was 30 minute. The radiochemical yield (decay corrected) was 80% with the radiochemical purity and chemical purity of > 98%. The synthesis procedure using both FBM and FMT is shown in Fig. 18.

## Automated Synthesis Modules

Computer-controlled automation of the synthesis of PET radiopharmaceuticals is desirable for routine commercial pro-



**Figure 16** FMISO synthesis. The precursor (1) is known as NITTP. The fluorinated intermediate (2) on hydrolysis will produce FMISO.



**Figure 17** The precursor (1) is known as 3-O-Methoxymethyl-16, 17-O-sulfuryl-16-epiestriol (MMSE). The fluorinated intermediate on hydrolysis will produce FES.

duction and to reduce radiation exposure for personnel involved in the production of these PET drugs. A number of automated synthesis modules (ASM) for routine production of  $^{18}\text{F}$  radiopharmaceuticals (such as FDG and FDOPA) are commercially available (Table 6). ASMs are based on the principle of unit operations, in which a complex synthetic procedure is reduced to a series of simple operations (or reactions) such as evaporation, fluorination, chromatography, hydrolysis, purification and sterilization, etc. These operations are controlled by PCs with software programs that are user friendly and flexible to change various reactions conditions. Some of these ASMs such as TracerLab FX are based on using sterile disposable kits with ready to use reagent vials for each batch production. At this time several commercial ASMs are used routinely for the synthesis of radiochemically pure, sterile and pyrogen free FDG for clinical studies. In the near future, such ASMs will be available for the production of FLT, FCH, and other PET radiopharmaceuticals.

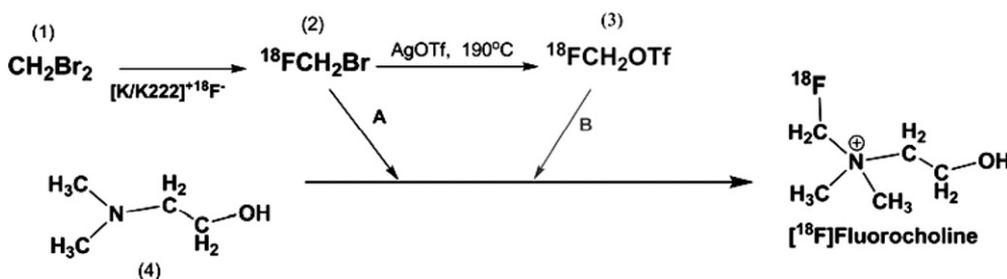
## Radiation Dosimetry

Based on biodistribution studies in human subjects, the radiation dosimetry estimates for several  $^{18}\text{F}$  radiopharmaceuticals have been reported.<sup>120-129</sup> A summary of the published estimates for several  $^{18}\text{F}$  agents in comparison to the currently accepted FDG radiation dosimetry estimates is shown in Table 7. The original radiation dose estimates for FDG were reported by Jones and coworkers<sup>120</sup> in 1982. Subsequently, several reports have made certain modifications to meet MIRD criteria.<sup>121-122</sup> With FDG, the whole body radiation dose (effective dose equivalent: EDE) is approximately 8 mSv

(800 millirem) for 370 MBq (10 mCi) of dose administered. Urinary bladder (critical organ) receives approximately 2.7 mSv. The dose to urinary bladder wall is based on 2 hour void interval. For all other radiopharmaceuticals listed in Table 7, the EDE is 0.48-1.4 mSv/mCi ( $0.55 \pm 0.32$ ) of dose administered. For most of the  $^{18}\text{F}$  radiotracers listed in Table 7 (Fluoride, FDG, FDOPA, FLT and FMISO), the critical organ is the urinary bladder receiving the highest dose. With  $^{18}\text{F}$  as fluoride for bone imaging, the dose to the bladder wall is 910 mrad/mCi. The critical organ, however, is the liver (470 mrad/mCi) with FES and the kidney with FCH (642 mrad/mCi).

## PET Radiopharmaceuticals: The Role of FDA

FDA approval of a drug or any radiopharmaceutical typically involves submission of a "New Drug Application" (NDA) by a manufacturer or a company clearly documenting 2 major aspects of the drug: (1) manufacturing of PET drug and 2) safety and effectiveness of a drug with specific indications. After extensive review of NDA application, FDA may approve the NDA for routine clinical use. The NDA was approved for  $^{18}\text{F}$ -fluoride in 1972 for bone imaging using conventional gamma cameras. The first PET radiopharmaceutical to get FDA approval in 1989 was  $^{82}\text{Sr} \rightarrow ^{82}\text{Rb}$  for myocardial perfusion imaging studies. Since that time, approval of NDAs for PET radiopharmaceuticals was complicated because most of the  $^{18}\text{F}$  and  $^{11}\text{C}$  radiotracers were prepared by cyclotron facilities in academic research facilities. In 1994, however, FDA approved the first NDA for the manufacture of FDG



**Figure 18** Fluorocholine (FCH) synthesis. Dibromomethane (1) is first fluorinated to generate fluorobromomethane (FBR; 2), which reacts with the precursor, dimethylethanolamine (4), to produce FCH. In a second method, FBR can also be converted to fluoromethyltriflate (3), which would react more efficiently with the precursor (4) to produce FCH.

**Table 6** Automated Synthesis Modules

Radiotracer/Precursor	Synthesis Module	Manufacturer	Comments
<sup>18</sup> F]FDG	TRACERlab MX <sub>FDG</sub>	GE	Disposable cartridge
	TRACERlab FX <sub>FDG</sub>	GE	Multiple use
	Metatrace FDG	CTIPETNET	Infinite run capability
	FDG-Plus Synthesizer	Bioscan	Disposable cassette
<sup>18</sup> F]FDOPA	Synthera	IBA Molecular	
	TRACERLab FX <sub>FDOPA</sub>	GE	FDOPA from [ <sup>18</sup> F]F <sub>2</sub>
<sup>18</sup> F-Tracers	TRACERlab FX <sub>F-N</sub>	GE	Nucleophilic fluorination by use of [ <sup>18</sup> F]F <sup>-</sup> (fluoride)
	Synthera	IBA Molecular	Modular multipurpose synthesizer

by The Methodist Medical Center (and CTI, Inc) in Peoria, IL and for 2 clinical indications: (1) to assess myocardial glucose metabolism and (2) to identify epileptic foci with altered glucose metabolism. That NDA (20-306) was not good for FDG manufactured at other centers. In the last 2 decades, FDG was used by many investigators as an Investigational New Drug (IND). Today, all the clinical FDG-PET imaging work in oncology in the United States operates under the Practice of Medicine and Pharmacy laws (that is, through prescription by a physician and dispensing by a registered pharmacist).

In 1997, President Clinton signed into law the FDA Modernization Act (FDAMA) to regulate PET drugs<sup>130</sup> mandating FDA to specifically provide guidelines to develop procedures for PET community to obtain NDA (or ANDAs) for PET radiopharmaceuticals. The FDAMA suspended the requirement that PET drugs be marketed under approved applications until 2 years after FDA established approval procedures and cGMP requirements for the manufacture of PET drugs.

Because certain PET drugs have been used clinically for 10 to 20 years under INDs, the FDA decided to conduct its own review of the published literature. The FDA's goal was to evaluate the safety and effectiveness of the PET drugs such as [<sup>18</sup>F]FDG, <sup>13</sup>N-ammonia and [<sup>18</sup>F]fluoride for certain indications and to facilitate the process of submitting NDAs for these products for the PET drug industry. Based on its literature review and findings and the recommendations of the Advisory Committee, the FDA has developed the basis for the approval of NDAs and ANDAs for 3 PET radiopharmaceuti-

cal. In a notice in the *Federal Register* in March 2000 (the PET Safety and Effectiveness Notice),<sup>9</sup> FDA presented its findings for three PET drugs for certain indications. It is important to appreciate that the success of FDG-PET in oncology was primarily attributable to the FDA's approval of indications by documenting the safety and effectiveness based on published literature (during a period of 20 years) and not based on data generated in multi-center Phase I, II, and III clinical studies sponsored by a manufacturer of FDG. Since FDA approved the indications in oncology and cardiology, the insurance companies were willing to reimburse for FDG-PET studies in these areas. Recently, in 2005 FDA also approved the use of FDG-PET brain scans in the differential diagnosis of Alzheimer's disease (AD) and Fronto-temporal dementia (FTD). It is important to understand that at this time more than 150 sites in this country manufacture FDG with out a formal approved NDA.

### PET Drugs: Current Good Manufacturing Practices (CGMPs)

CGMP is a minimum standard that ensures that a drug meets the requirements of safety and has the identity strength, quality, and purity characteristics it is represented to possess. In April 2002, FDA proposed a first draft version of CGMP guidance in conjunction with revised preliminary draft proposed regulations. In September 2005, the FDA formally proposed a rule and a guidance on CGMP regulation for the

**Table 7** Comparison of Radiation Dosimetry of <sup>18</sup>F-Labeled PET Radiopharmaceuticals

Radiopharmaceutical	Reference	EDE (mrem/mCi)	Radiation Dose (mrad/mCi of dose injected)					
			BM	Testes	Ovary	UB	Kidney	Liver
[ <sup>18</sup> F]FDG	120 to 122	81	40	41	41	270*	78	88
[ <sup>18</sup> F]Fluoride	123 to 124	100	10	29	39	910*	71	15
[ <sup>18</sup> F]FDOPA	125	74	39	55	52	556*	101	57
[ <sup>18</sup> F]Fluorothymidine†	126	112	106	54	77	654*	143	203
[ <sup>18</sup> F]FMISO	127	48	37	56	67	78*	59	67
[ <sup>18</sup> F]Fluoroestradiol	128	80	48	44	66	190	128	470*
[ <sup>18</sup> F]Fluorocholine	129	137	75	39	67	358	642*	257

\*Critical organ.

†Average for males and females based on a single 6-hour voiding.

production of PET drugs.<sup>131</sup> The guidance document clearly describes acceptable approaches that would enable PET drug producers to meet the requirements in the proposed regulation. In addition, the guidance addresses resources, procedures, and documentation for all PET drug production facilities, academic and commercial. It is important to recognize that the proposed regulations on CGMP requirements contain the minimum standards for quality production of PET drugs at all types of PET production facilities; not-for-profit, academically oriented institutions as well as commercial producers. These documents represent the outcome of many years of interaction between the FDA and the PET community. Many of the written responses and comments from the public meetings have been incorporated into the proposed rule and draft guidance documentation. The proposed regulations also incorporate principles from the United States Pharmacopeia (USP) general chapter on PET drug compounding. The USP contains standards that are of significant regulatory importance for PET drugs. Currently, under section 501(a)(2)(C) of the Federal Food, Drug, and Cosmetic Act (the Act), a compounded PET drug is adulterated unless it is produced in compliance with USP compounding standards and official monographs for PET drugs.

Neither the proposed rule nor the draft guidance is final at this time. This means the proposed rule is not binding or effective. After FDA evaluates the comments received on the rule and the guidance, the agency expects to publish the final versions of both documents in the near future. Two years after that final rule takes effect, PET producers will be required to submit NDAs before marketing a PET drug. In the mean time, various PET radiopharmaceuticals can be evaluated clinically under the Radioisotope drug research committee (RDRC) approval or using the formal "Investigational New Drug (IND) research protocols. For the next 2 to 3 years, the manufacture and distribution of PET drugs (discussed in this issue) in the United States can operate under the Practice of Medicine and Pharmacy laws. Routine commercial utility of various PET radiopharmaceuticals (reviewed in this issue), however, first would require approval of "clinical indication" by FDA. To achieve this goal, it is very important that investigators at academic research centers and radiopharmaceutical industries work closely with regulatory agencies such as FDA.

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