Technologies for Translational Imaging Using Generators in Oncology Short Running Title: Imaging and Oncology

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Abstract: Improvement of scintigraphic tumor imaging is extensively determined by the development of more tumor specific radiopharmaceuticals. Thus, to improve the differential diagnosis, prognosis, planning and monitoring of cancer treatment, several functional pharmaceuticals have been developed. The application of molecular targets for cancer imaging, therapy and prevention using generator-produced isotopes is the major focus of many ongoing research projects. Radionuclide imaging modalities (single photon emission computed tomography, SPECT; positron emission tomography, PET) are diagnostic cross-sectional imaging techniques that map the location and concentration of radionuclide-labeled radiotracers. Generator produced isotopes, such as ^{99m}Tc and ^{68}Ga, are readily available and affordable. ^{99m}Tc ($t_{1/2}$=6 hr; 140 keV) is used for SPECT and ^{68}Ga ($t_{1/2}$=68 min; 511 keV, 89%) is used for PET. ^{99m}Tc- and ^{68}Ga-labeled agents using various chelators have been synthesized and their potential uses to assess tumor targets have been evaluated. Molecular targets labeled with ^{99m}Tc and ^{68}Ga can be utilized for the prediction of therapeutic response, monitoring tumor response to treatment and aiding in the differential diagnosis of tumor versus non-tumor tissue. Molecular targets for oncological research in (1) cell apoptosis, (2) gene and nucleic acid-based approach, (3) angiogenesis (4) tumor hypoxia, and (5) metabolic imaging are discussed. Numerous imaging ligands in these categories have been developed and evaluated in animals and humans. Molecular targets were imaged and their potential to redirect optimal cancer diagnosis and therapeutics was demonstrated.

Keywords: PET, SPECT, molecular imaging.

THE ROLE OF PET AND SPECT IN MOLECULAR IMAGING

Computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound are limited as prognostic tools because they do not provide cellular target information, thus, assessment of the effectiveness of cancer therapy is not optimal. The development of radiolabeled biochemical compounds and imaging devices to detect the radioactivity by external imaging has expanded the use of nuclear medicine studies in drug development. Drug discovery and development are accelerating due to rapid synthesis of potential drugs and development of high-throughput *in vitro* tests. Since molecular imaging plays a major role in drug development because of its ability to quantify drug properties *in vivo*, it is beginning to be used in all phases of drug discovery and development.

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) agents can aid in the determination of disease status. The reading of anatomic studies such as CT can be confounded by potential alterations due to cancer therapy, such as scarring. In such cases PET and SPECT agents can provide important information concerning the characterization of various aspects of a tumor (such as vascular angiogenesis, hypoxia, apoptosis, cellular signaling and transcriptional activity) [1-8]. In addition, PET and SPECT agents may assist in the determination of: optimal therapeutic dosing, the differential diagnosis between inflammation/infection and tumor recurrence, the sensitivity or resistance of a tumor to therapy, the tumor grade, expectations for treatment response, and the selection of patients who may best respond to a particular therapy. PET and SPECT agents show high specific activities because they are made through a nuclear transformation and use carrier free forms of isotopes; the PET and SPECT agents do not themselves have detectable pharmacologic effects.

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DEVELOPMENT OF RADIOPHARMACEUTICALS BEYOND [^{18}F] FLUORODEOXYGLUCOSE

[^{18}F]Fluorodeoxyglucose (FDG), a gold standard for PET, is complementary to CT and MRI and allows for the detection of unsuspected distant metastases. Although PET FDG has been concordant with the findings of CT and MRI in diagnosing various tumors, FDG also has its drawbacks. For example, a significant amount (>95%) of FDG concentrates in the cytosolic fraction, which results in false-positive lesions, misdiagnosing inflammation and/or infection as tumor recurrence [9]. Additionally, FDG does not provide accurate prediction of therapeutic response. Due to its high brain uptake [10], FDG radiation dosimetry makes it unsuitable for internal radiotherapy. In addition to the drug distribution, ^{18}F is inappropriate for internal radiotherapy due to its decay scheme, e.g. all positron, no beta emission. Because it is a cyclotron-produced radiopharmaceutical, there are the additional constraints of high cost and availability of a local cyclotron. On the other hand, radionuclide generator systems that can be produced in a local well-controlled facility can make the cyclotron-produced radiopharmaceutical agents much more accessible and affordable. Such systems are embraced by current FDA procedures and have a long history of successful clinical application.

SELECTION OF ISOTOPES AND BIFUNCTIONAL CHELATORS FOR IMAGING

^{99m}Tc and ^{68}Ga are Useful Isotopes for Kit-Based Products Development

To develop novel or clinically used tracers, two types of chemistries are frequently used in the preparation of radiotracers: covalent and ionic. In covalent chemistry, either displacement or addition reactions are used to place an isotope in the molecule. The labeled product provides minimal structural alteration, however, the procedure may be lengthy, tedious, with low yield, and costly. Isotopes commonly used in covalent chemistry include ^{18}F, ^{123}I, ^{131}I, ^{75}Br, ^{77}Br and ^{11}C. In addition, PET radiosynthesis must be rapid because the radioisotope will decay during lengthy chemical synthesis and the radiosynthesis process poses a relatively high risk of radiation exposure. Cyclotron-produced tracers are constrained by the availability of local cyclotron and its high cost. Alterna-

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tively, radionuclide generator systems that can be produced in a well controlled facility have a long history of successful clinical application. A generator uses a parent-daughter nuclide pair wherein a relatively long-lived parent isotope decays to a short-lived daughter isotope that is used for imaging. The parent isotope, which is produced at a cyclotron facility, can be shipped to a clinical site and the daughter isotope eluted on site for clinical use. ^{68}Ga-based (68-minute half-life) PET agents have significant commercial potential because the isotope can be produced from a ^{68}Ge generator (275-day half-life) on site and be a convenient alternative to cyclotron-produced PET isotopes, such as ^{18}F- or ^{124}I-.

Although the maximum positron energy of ^{68}Ga (max=1.90 MeV, mean=0.89 MeV) is higher than that of ^{18}F (max=0.63 MeV, mean=0.25 MeV), a study using Monte Carlo analysis on spatial resolution revealed that under the assumption of 3 mm spatial resolution for PET detectors, the conventional full width at half maximum (FWHM) of ^{18}F and ^{68}Ga is indistinguishable in soft tissue (3.01 mm vs. 3.09 mm) [11]. This implies that with the spatial resolution at 5 to 7 mm of current clinical scanners, the imaging quality using ^{68}Ga-based tracers can be as good as that of ^{18}F-based agents and has enticed others to investigate the potential of ^{68}Ga-based imaging agents. For example, carcinoid tumors have been well imaged using ^{68}Ga-DOTATOC (a somatostatin receptor tracer) and PET [12].

Due to favorable physical characteristics (360 min half-life, 140keV), easy availability, and low price ($0.21/mCi vs. $50/mCi of ^{18}F), ^{99m}Tc is an agent of choice for labeling radiopharmaceuticals. ^{99m}Tc can be produced from a ^{99}Mo generator (2.75-day half-life) on site. Radiolabeling an imaging agent in kit form with ^{99m}Tc or ^{68}Ga can be easily achieved in high yield. This unique chelator-based imaging technique provides a novel route in a kit formulation, which may make it easier for patients to be evaluated and optimally treated.

Selection of Bifunctional Chelators for Imaging

Several chelators have been reported such as N_4 (e.g. Cyclam-14, DOTA), N_3S (e.g. MAG-3), N_2S_2 (e.g. ECD), NS_3, S_4 (e.g. sulfur colloid), diethylenetriamine pentaacetic acid (DTPA), O_2S_2 (e.g. DMSA), and hydrazinenicotinamide (HYNIC) [5, 13-16]. Among these chelators, DTPA has faster wash out and forms less stable complexes with ^{99m}Tc. ^{99m}Tc-HYNIC has been shown to be useful in imaging, but labeling HYNIC with ^{99m}Tc requires two chemicals, thiphenylphosphine and tricine, which are inconvenient for the kit preparation. The nitrogen and sulfur combination has been shown to be a stable chelator for ^{99m}Tc-bis-aminoethanethiol tetradentate ligands, also called diaminodithiol compounds, and are known to form very stable Tc(V)O-complexes on the basis of efficient binding of the oxotechnetium group to two thiosulfur and two amine nitrogen atoms. L,L-ethylenedicysteine (EC) is the most successful example of N_2S_2 chelates [14, 17-19]. EC can be labeled with both ^{68}Ga and ^{99m}Tc efficiently with high radiochemical purity and the preparation remains stable for several hours [20].

Added Value for Chelator-Based Molecular Imaging Agents

In this article we discuss several technologies for the development of new radiotracers using existing pharmaceuticals that alter target functions. Once established, data on the newly developed radiotracers can help in the development of new more effective pharmaceuticals. Once a chelator is conjugated to a "drug" molecule it is considered a new molecule. We use the existing data on the mechanism of action of the "cold" anti-cancer "drug" to support our new "hot" radiopharmaceutical on assessment of target function. In turn, the data we collect on the radiopharmaceutical can help in the design and development of a new, for example more potent, anti-cancer drug, help determine optimal dosage or dose scheduling, and/or assist with assessment of other important end points.

In addition to assessing molecular targets, ^{99m}Tc might be useful in planning internal targeted radionuclide therapy with ^{188}Re-labeled agents. ^{188}Re can be obtained from an ^{188}W/^{188}Re generator, which makes it very convenient for clinical use. ^{188}Re is carrier free and might offer higher specific activity as in the case of ^{99}Mo/^{99m}Tc (37,000–74,000 MBq/mg; 1,000–2,000 mCi/mg Tc). ^{188}Re has high ß energy (2.1 MeV), short physical half-life (16.9 hr) and 155 keV γ-ray emission for radiotherapeutic dosimetric determination and imaging purposes. For instance, ^{188}Re-hydroxyethylidene diphosphonate (^{188}Re-HEDP) has been demonstrated to be safe and successful in palliating pain [21]. A dose escalation study with ^{188}Re-HEDP in prostate cancer patients has shown that 3.3 GBq(~90 mCi) dose is the maximum tolerated [22]. Site-specific delivery of anticancer agents or radiation is beneficial for solid tumors and surgically unresectable tumors. ^{188}Re hydrogel has been demonstrated to be useful against breast tumor growth in animal models [23]. In our laboratory, loco-regional prostate cancer therapy with hydrogel loaded with ^{188}Re and cisplatin showed tumor cell apoptosis at 48 hrs (Fig. **1-2**).

RADIOPHARMACEUTICALS IN THE PREDICTION OF THERAPEUTIC RESPONSE

The agents that could provide therapeutic prediction are either labeled molecular biomarkers or radiolabeled drugs. For example, radiolabeled annexin, a biomarker for apoptosis, is useful to evaluate the baseline level of apoptosis, predict the efficacy of therapy based on the detection of treatment-related apoptosis and possibly predict disease progression and prognosis [6, 20, 24]. As another example, assessment of pancreatic beta cell activity by a radiolabeled sulfonylurea receptor agent could provide early diagnosis of pancreatic diseases and monitor drug treatment response on pancreatic beta cells. In the pancreas, the beta cell comprises 60% of all cell types. Glipizide and nateglinide, sulfonylurea receptor agents, stimulate insulin release from pancreatic beta cells, which improves overall glycemic control in type 2 diabetes [25]. The image of ^{99m}Tc-DTPA-glipizide is shown in Fig. **3**. DTPA-Glipizide showed better response in lowering glucose level compared to Glipizide (Fig. **4**).

RADIOPHARMACEUTICALS IN DIFFERENTIAL DIAGNOSIS

Hypoxia as a Biomarker

It has been well established that hypoxic tumors are known to be resistant to traditional radio- and chemotherapy, resulting in higher local recurrence rates. The ability to quantify *in vivo* tissue hypoxia via imaging could potentially allow physicians to select patients for additional or alternative treatment regimens that circumvent the ominous impact of hypoxia [4, 26-28]. For example, metronidazole (MN), a 5-nitroimidazole analogue, has been shown to sensitize only anoxic cells in a dose dependent manner. Although more studies need to be done in cancer patients, clinical studies have shown that ^{99m}Tc-EC-MN, a hypoxic biomarker, is able to differentiate hypoxic regions in stroke patients [29].

Metabolic Imaging Biomarkers

Glucosamine is a glucose analogue similar to FDG and its cellular uptake is via a glucose transporter process [30]. However, its regulatory products of glucosamine-6-phosphate mediate insulin activation, downstream signaling and translocation, which up-regulate mRNA expression and tumor growth [31]. For instance, Sp-1 is one of the known transcription factors whose activity may be post-translationally targeted by glucosamine [32, 33]. Sp-1 binding sites may be present in the promoters of potent angiogenic growth factors such as VEGF and IL-8 and regulate oncogenic

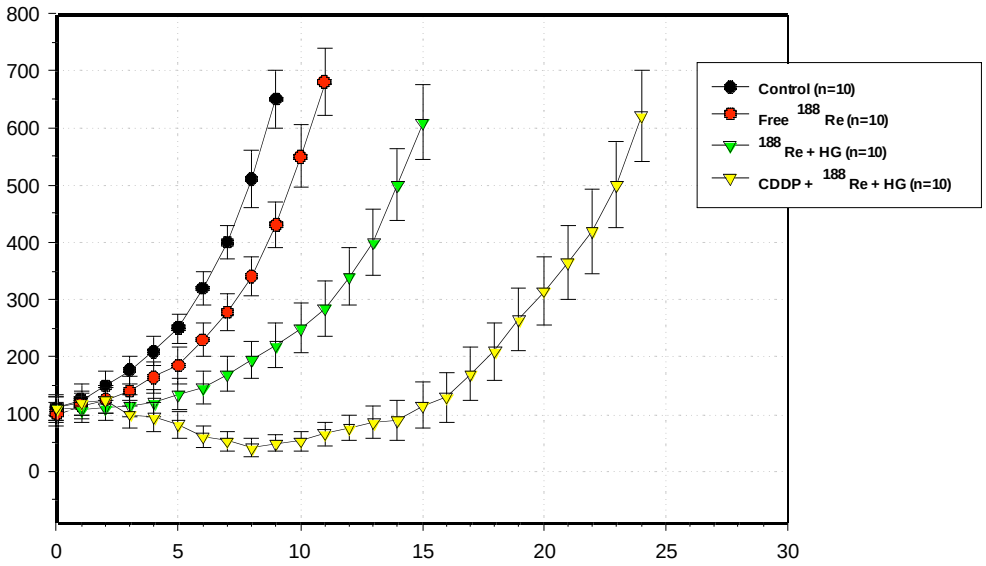
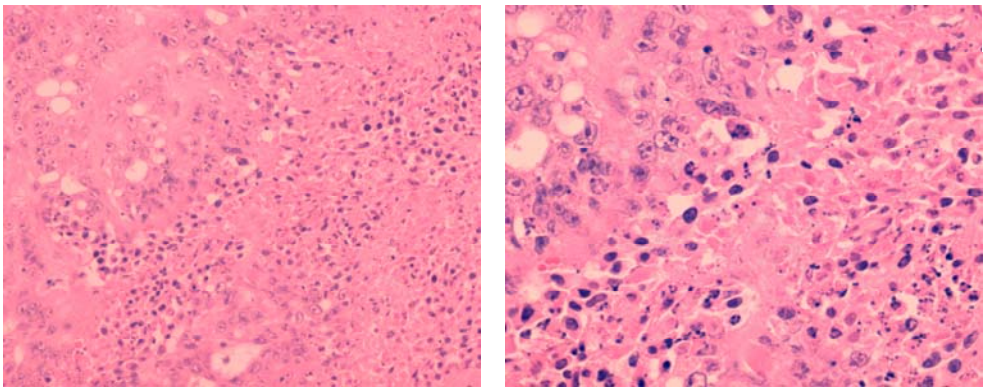


Fig. (1). Effect of ^{188}Re-Hydrogel on Nude Mice Bearing Human Prostate Tumors.



20X magnification **40X magnification**

Fig. (2). H&E staining of prostate tumor tissue 48hrs post-^{188}Re-hydrogel and cisplatin therapy.

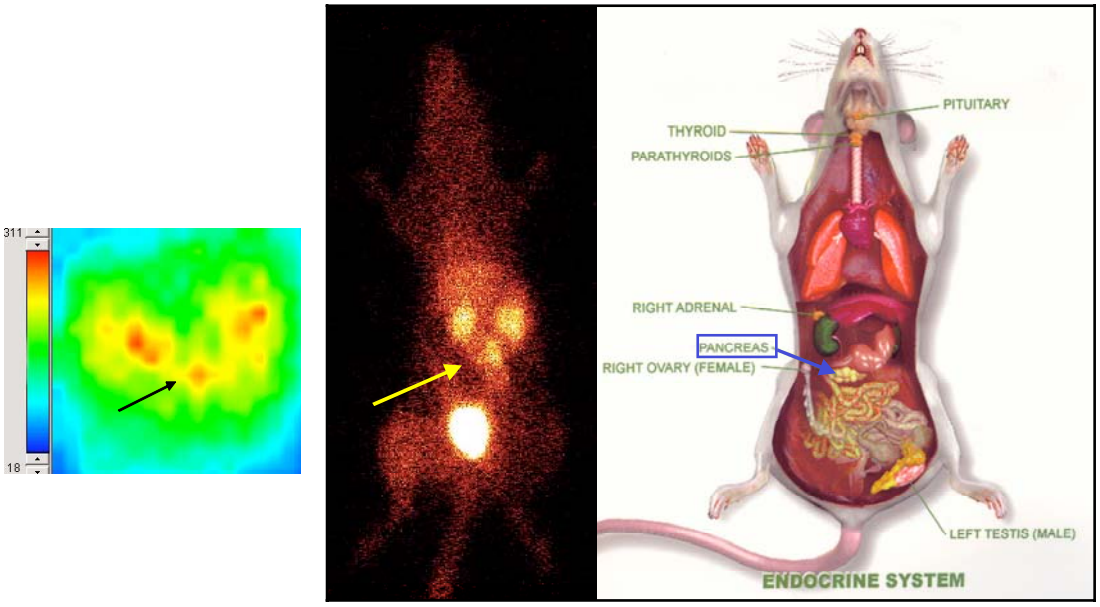


Fig. (3). Female F-344 rats were imaged with ^{99m}Tc-DTPA-Glipizide (300 μCi, i.v.). Selectedplanar images of ^{99m}Tc-DTPA-Glipizide are presented at 15 min post-injection. eZ scope (Anzai Medicial Inc, Japan) also showed that pancreas could be imaged. Arrow designates pancreas.

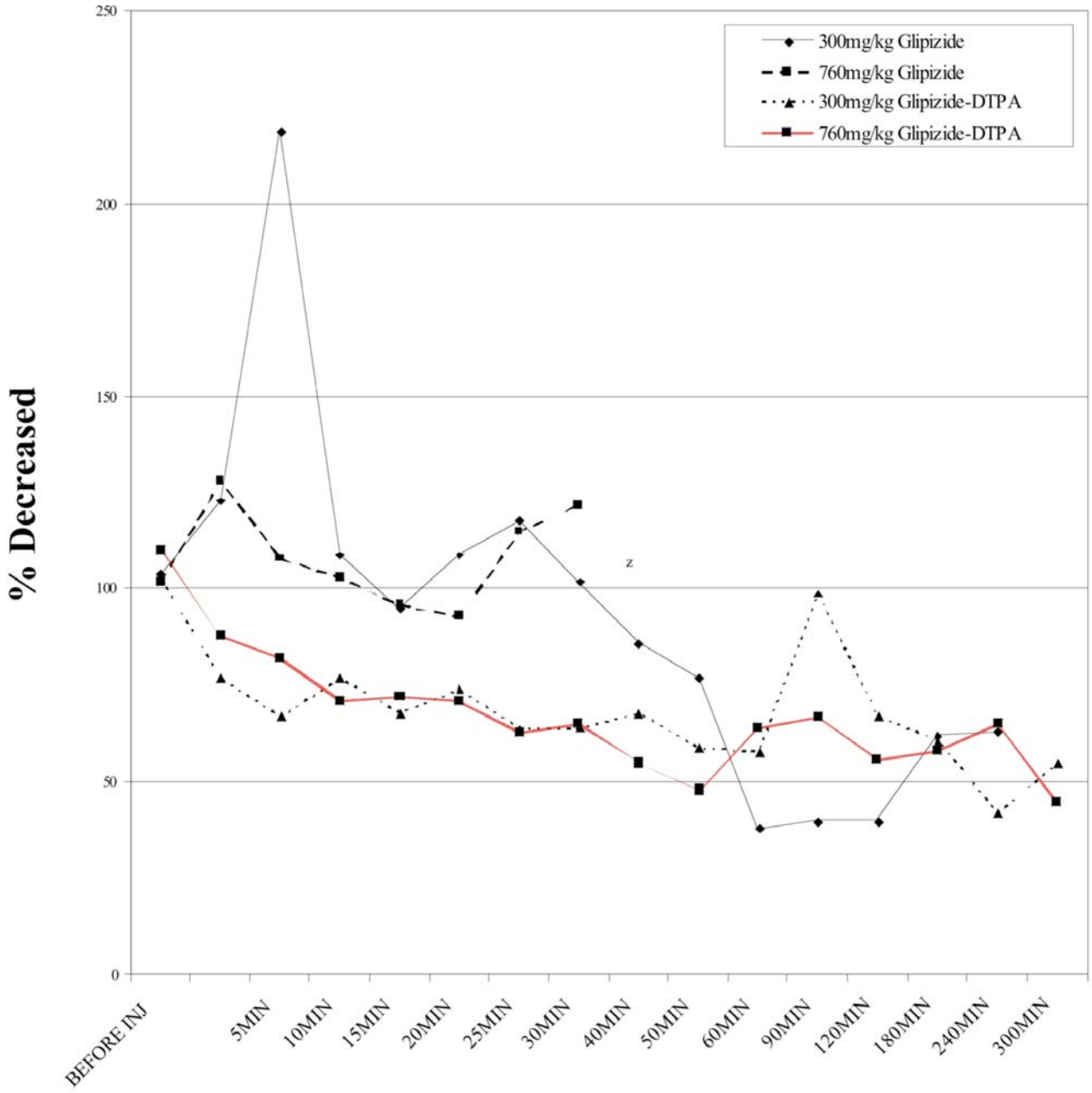


Fig. (4). Effect of Glipizide and DTPA-Glipizide on Blood glucose levels. Blood glucose levels were descreased by Glipizide and DTPA-Glipizide at the dosages tested. DTPA-Glipizide had faster onset and more steady than Glipizide.

factors with cell cycle factors at basal levels of transcription or repression. Preclinical and clinical studies have shown that ^{99m}Tc-EC-glucosamine is able to differentiate inflammation/infection and tumor growth in animal models and in humans [20, 34, 35].

Cell Proliferation Biomarkers

Synthesis and biological activity of labeled thymidine or uridine, which were incorporated into DNA/RNA, have been reported [36-39]. For instance, 3'-deoxy-3'-^{18}F-fluorothymidine (^{18}F-FLT) is a new tracer which images cellular proliferation by entering the salvage pathway of DNA synthesis. However, DNA incorporation rate of FLT is low and the related chemistry is complex [40, 41]. To continuously explore other purine-based analogues using chelation radiochemistry, we then synthesized ^{99m}Tc- and ^{68}Ga-EC-guanine (EC-Guan) for evaluation of cell proliferation [42]. ^{68}Ga-EC-Guan was able to differentiate inflammation versus tumor (Fig. **5**). *In vitro* cell confluence, cell cycle analysis, cellular uptake and *in vivo* imaging studies suggest that ^{99m}Tc- and ^{68}Ga-EC-Guan may be useful as tumor proliferation imaging agents.

RADIOPHARMACEUTICALS FOR MONITORING TREATMENT RESPONSE

Amino Acid Transport

The epidermal growth factor receptor (EGFR) is expressed in a variety of human solid tumors. When EGFR is triggered, tyrosine kinase (TK) is phosphorylated and leads to the initiation of receptor-mediated signal transduction, cell proliferation, survival, angiogenesis, and metastasis. TK inhibitors [such as gefitinib

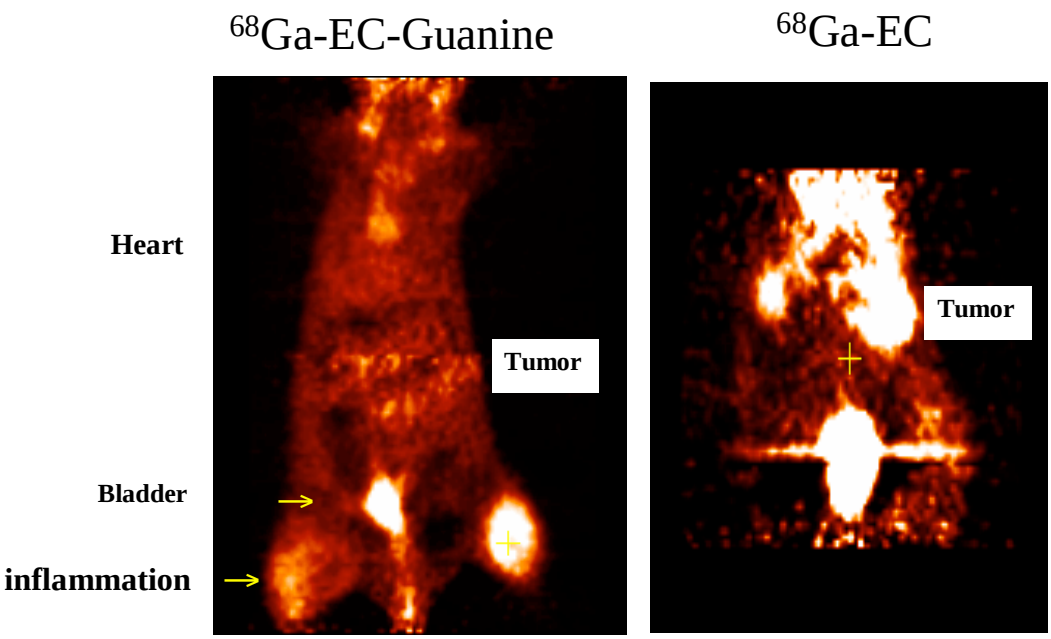


Fig. (5). PET images of ^{68}Ga-EC-Guanine and ^{68}Ga-EC (500 mCi /rat, iv) in inflamation (by turpentine) and breast tumor-bearing rats showed that ^{68}Ga-EC-Guanine could differentiate tumor vs. inflammation.

(marketed as Iressa), erlotinib (marketed as Tarceva), and imatinib mesylate (marketed as Gleevec)] have shown effectiveness against tumor growth [43]. Radiolabeled amino acids (such as tyrosine and lysine) are involved in protein synthesis via an amino acid transporter [44-46], which is suitable to assess the end-point of TK activity. Our results using PET/CT in animal models have demonstrated that tumors can be clearly visualized by ^{68}Ga-cyclam-tyrosine (Fig. **6**). Dynamic PET images have shown that tumor uptake reached a plateau at 3-5 min post-administration of ^{68}Ga-cyclam-tyrosine (Fig. **7**).

Development of Hybrid Imaging Agents

The ability to acquire functional and anatomic information in a single scheduled examination offers many clinical and workflow benefits. The successful marriage between PET and CT is making CT almost a standard component of any PET purchase. Despite the attention lavished on PET and PET/CT over the past few years, SPECT remains the bread and butter of the nuclear medicine workload worldwide. Planar imaging in the past with single photon emitting radionuclides has been a suboptimal technique with low diagnostic sensitivity. However, SPECT has improved its sensitivity for detecting deeply seated lesions while still maintaining a significantly lower cost than PET. Generator-produced isotopes such as ^{99m}Tc-, ^{188}Re and ^{68}Ga should be preferred in clinical practice over cyclotron-produced isotopes due to ease of access and lower cost. Nevertheless, SPECT images are inherently susceptible to attenuation artifacts. Differing scatter and absorption of photons between emission and detection can distort the final images, and failure to correct the attenuation along the photon's path can cause spatial misrepresentation. Therefore, nowadays CT-derived anatomical maps are being used to correct the attenuation and improve diagnostic accuracy [47].

Reliable molecular imaging assesses cellular targets and therapeutic response at low cost, aids in the differential diagnosis of tumor versus benign tissue, aids in the prediction or selection of patients who would benefit from a particular regimen, and provides valuable dosimetry for internal radiation therapy. Stimulated by the success of the multi-sliced PET/CT systems, new hybrid SPECT/CT systems with advanced reconstruction software are being introduced. The new hybrid SPECT/CT systems come complete with an integrated diagnostic-quality CT scanner; these new hybrid SPECT/CT systems hold the potential to further improve attenuation correction and precisely correlate radionuclide signals with anatomic positions [48-53]. Molecular biological discoveries have great implications for prevention, detection, and targeted therapy. *In vivo* molecular imaging agents are able to provide cellular and molecular information. The combination of CT morphological/ anatomical information with these new mechanism-based agents should provide for accurate assessment of target function and image-guided therapy. Thus, it would be ideal to develop a molecular imaging agent suitable for PET/CT, SPECT/CT, PET/MRI and SPECT/MRI. The use of hybrid imaging agents should be more beneficial to patients by providing physicians with critical information that in turn could lead to more accurate diagnoses and more effective treatment regimens.

CHALLENGES IN MOLECULAR IMAGING

There are risks for industry to step in for molecular licensing based on patent information alone. Patents regarding molecular imaging technologies tend to review the structure composition, method of production, and potential applications of such technologies, while providing limited clinical information [54, 55]. The regulatory compliance issues for clinic phase I studies are related to chemistry, manufacturing and control (CMC), pharmacology and toxicology. In regards to CMC, some isotopes (^{68}Ga, ^{188}Re, ^{61}Cu, ^{64}Cu) are attractive for chelation, but the validation from laboratory development to clinic grade has not been well addressed. This includes physical (photon energy, mass spectrum) and chemical (metallic form, pH, radiochemical purity) characteristics. The reference cold standard needs be synthesized for radiochemical identity confirmation. In addition to assessment of radiochemical yield, assessment of *in vivo* stability is equally important. To manufacture an end product for clinical use, the site for current Good Manufacturing Practice (cGMP) (academic vs. industry) needs to be arranged. For pharmacology and toxicology data, drug uptake vs. target validation needs be addressed. In animal models, biodistribution and dosimetry studies need to be performed. The toxicology studies need to be conducted under Good Laboratory Practice (GLP).

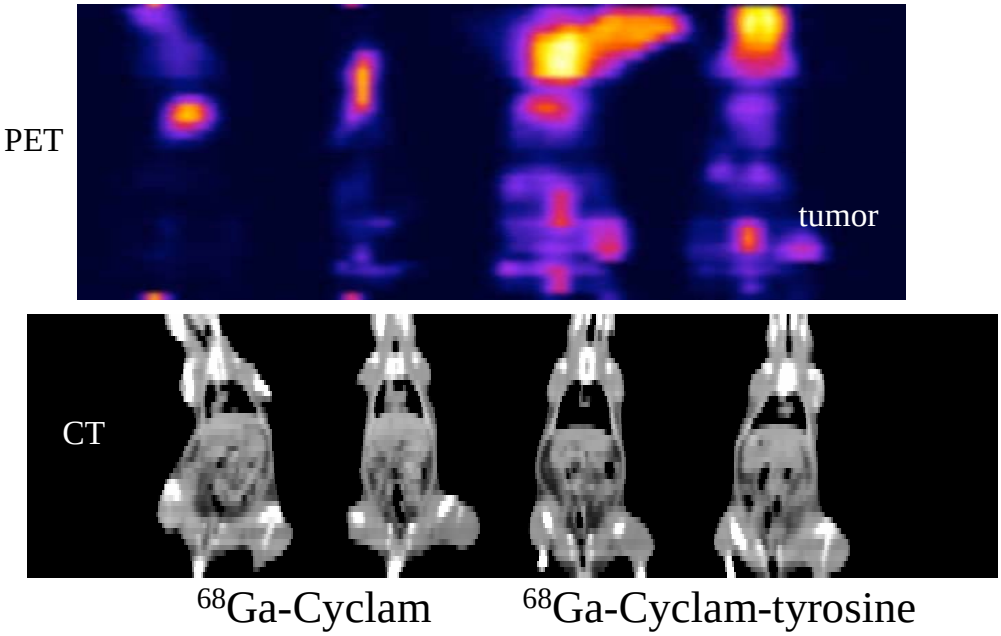


Fig. (6). Mammary tumor-bearing rats injected with 500 μCi ^{68}Ga-cyclam-tyrosine (500 μCi, iv) and the selected images at 45 min post-injection showed tumor could be visualized.

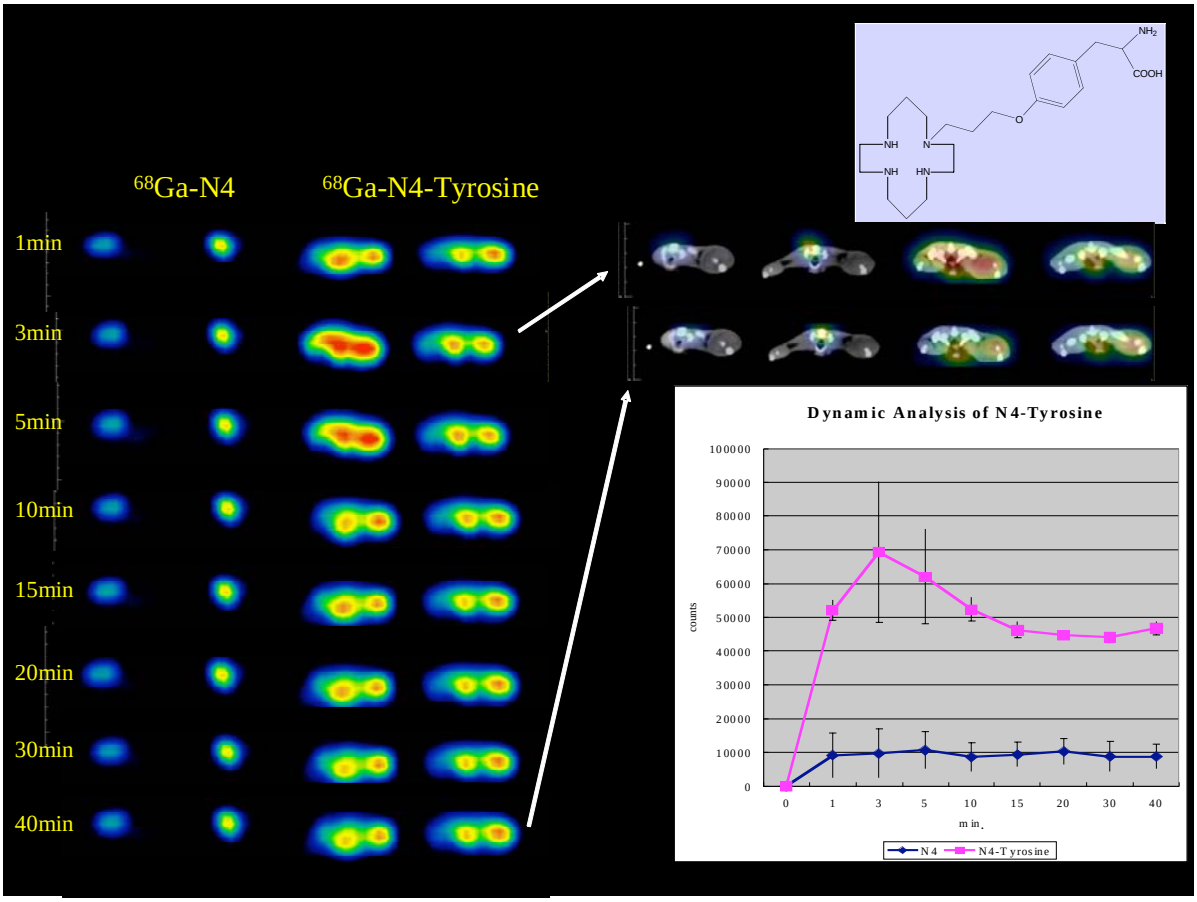


Fig. (7). Dynamic study of ^{68}Ga-cyclam-tyrosine in breast tumor-bearing rats.

In summary, noninvasive imaging assessment of tumor cell proliferation could be helpful in the evaluation of tumor growth potential/aggressiveness and be used to provide an early assessment of treatment response prior to changes in tumor size as determined by CT, MRI, or ultrasonography. Understanding of tumor proliferative activity, in turn, could aid in the selection of optimal

therapy by estimating patient prognosis and assisting in the selection of a proper management strategy. Aiming to develop new radiolabeled ligands for metabolic imaging of tumors, a series of new ligands have been developed for PET and SPECT imaging of neoplasms. For apoptosis assessment, radiolabeled EC-annexin V has been shown to have potential in the prediction of treatment response. For DNA/RNA markers, EC-Guan has shown promising results in differentiating inflammation versus tumor. For angiogenesis imaging, EC-endostatin, EC-CBX and EC-C225 are adequate to assess specific targets. For tumor hypoxia imaging, a classic nitroimidazole agent such as metronidazole is useful to monitor treatment response and may have potential in drug resistance or radioresistance imaging. Our metabolic imaging agent, radiolabeled EC-DG could assess tumor growth. Radiolabeled tyrosine could be used to monitor metabolic kinase activity. All of these functional ligands should provide information for prediction or monitoring treatment response of tumors. Thus, they may improve the diagnosis, planning and monitoring of cancer treatment. Challenges in radio-metallic chemistry promote opportunities in drug development.

CURRENT AND FUTURE DEVELOPMENTS

Currently multiple isotopes and chelators and synthetic techniques are being used by investigators. Without uniformity, it is difficult to draw conclusions regarding the best technology to use. Even if labeling a single molecule, each change in chelator or isotope could lead to changes in the metabolic profile. Ideally, more of the variables would be controlled and deeper testing performed. Due to the wide variety of targets, isotopes, and chelator agents in use, we would recommend that future focus be on the development and universal adoption of accessible, affordable, reproducible, and accountable techniques to assess the relative value of these technologies.

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REFERENCES

[1] Zareneyrizi F, Yang DJ, Oh CS, *et al.* Synthesis of ^{99m}Tc-ethylenedicysteine-colchicine for evaluation of antiangiogenic effect. Anticancer Drugs 1999; 10: 685-92.

[2] Yang DJ, Kim KD, Schechter NR, *et al.* Assessment of antiangiogenic effect using ^{99m}Tc-EC-endostatin. Cancer Biother Radiopharm 2002; 17: 233-45.

[3] Yang DJ, Bryant J, Chang JY, *et al.* Assessment of COX-2 expression with ^{99m}Tc-labeled celebrex. Anti-Cancer Drugs 2004; 15: 255-63.

[4] Ito M, Yang DJ, Mawlawi O, *et al.* PET and planar imaging of tumor hypoxia with labeled metronidazole. Academic Radiology 2006; 13(5): 598-609.

[5] Ohtsuki K, Akashi K, Aoka Y, *et al.* Technetium-99m HYNIC-annexin V: a potential radiopharmaceutical for the *in-vivo* detection of apoptosis. Eur J Nucl Med 1999; 26(10): 1251-8.

[6] Vriens PW, Blankenberg FG, Stoot JH, *et al.* The use of technetium ^{99m}Tc annexin V for *in vivo* imaging of apoptosis during cardiac allograft rejection. J Thorac Cardiovasc Surg, 1998; 116: 844-53.

[7] Kubota K, Ishiwata K, Kubota R, *et al.* Tracer feasibility for monitoring tumor radiotherapy: A quadruple tracer study with fluorine-18-fluorodeoxy-glucose or fluorine-18-fluorodeoxyuridine, L-[Methy1-^{14}C]methionine,[6-^{3}H]thymidine, and gallium-67. J Nucl Med 1991; 32: 2118-23.

[8] Schechter NR, Yang DJ, Azhdarinia A, *et al.* Assessment of epidermal growth factor receptor with ^{99m}Tc-ethylenedicysteine-C225 monoclonal antibody. Anticancer Drugs 2003; 14: 49-56.

[9] Yoo IeR, Park HJ, Hyun J, *et al.* Two cases of pulmonary paragonimiasis on FDG-PET CT imaging. Ann Nucl Med 2006; 20(4): 311-5.

[10] Wyss MT, Spaeth N, Biollaz G, *et al.* Uptake of 18F-Fluorocholine, 18F-FET, and 18F-FDG in C6 gliomas and correlation with 131I-SIP(L19), a marker of angiogenesis. J Nucl Med 2007; 48(4): 608-14.

[11] Personal Communication, Osama Malawi, Ph.D., Assoc. Professor, Imaging Physics, M. D. Anderson Cancer Center, 2007.

[12] Hofmann M, Maecke H, Borner R, *et al.* Biokinetics and imaging with the somatostatin receptor PET radioligand (68)Ga-DOTATOC: preliminary data. Eur J Nucl Med 2001; 28(12): 1751-7.

[13] Canet EP, Casali C, Desenfant A, *et al.* Kinetic characterization of CMD-A2-Gd-DOTA as an intravascular contrast agent for myocardial perfusion measurement with MRI. Magn Reson Med 2000 43(3): 403-9.

[14] Van Nerom CG, Bormans GM, De Roo MJ, Verbruggen AM. First experience in healthy volunteers with technetium-99m L,L-ethylenedicysteine, a new renal imaging agent. Eur J Nucl Med 1993; 20: 738-46.

[15] Laissy JP, Faraggi M, Lebtahi R, *et al.* Functional evaluation of normal and ischemic kidney by means of gadolinium-DOTA enhanced TurboFLASH MR imaging: a preliminary comparison with ^{99m}Tc-MAG3 dynamic scintigraphy. Magn Reson Imaging 1994; 12(3): 413-19.

[16] Wu HC, Chang CH, Lai MM, Lin CC, Lee CC, Kao A. Using Tc-99m DMSA renal cortex scan to detect renal damage in women with type 2 diabetes. J Diabetes Complications 2003; 17:297-300.

[17] Anderson CJ, John CS, Li YJ, *et al.* N,N'-ethylene-di-L-cysteine (EC) complexes of Ga(III) and In(III): molecular modeling, thermodynamic stability and *in vivo* studies. Nucl Med Biol 1995; 22:165-73.

[18] Sun Y, Anderson CJ, Pajeau TS, *et al.* Indium (III) and gallium (III) complexes of bis(aminoethanethiol) ligands with different denticities: stabilities, molecular modeling, and *in vivo* behavior. J Med Chem 1996; 39: 458-70.

[19] Zheng YY, Saluja S, Yap GP, Blumenstein M, Rheingold AL, Francesconi LC. Gallium and Indium Complexes of Bis(amino thiol) (N(2)S(2)). Ligands. Inorg Chem 1996; 35: 6656-66.

[20] Yang DJ, Azhdarinia A, Kim EE. Tumor specific imaging using Tc-99m and Ga-68 labeled radiopharmaceuticals. Current Medical Imaging Reviews 2005; 1: 25-34.

[21] Li S, Liu J, Zhang H, Tian M, Wang J, Zheng X. Rhenium-188 HEDP to treat painful bone metastases. Clin Nucl Med 2001; 26(11): 919-22.

[22] Knapp FF Jr, Beets AL, Guhlke S, *et al.* Availability of rhenium-188 from the alumina-based tungsten-188/rhenium-188 generator for preparation of rhenium-188-labeled radiopharmaceuticals for cancer treatment. Anticancer Res 1997; 17(3B): 1783-95.

[23] Azhdarinia A, Yang DJ, Yu D-F, *et al.* Regional Radiochemotherapy Using In Situ Hydrogel. Pharmaceutical Research 2005; 22(5): 776-83.

[24] Yang DJ, Azhdarinia A, Wu P, *et al. In vivo* and *in vitro* measurement of apoptosis in breast cancer cells using ^{99m}Tc-EC-annexin V. Cancer Biother Radiopharm 2001; 16: 73-83.

[25] Proks P, Reimann F, Green N, Gribble F, Ashcroft F. Sulfonylurea stimulation of insulin secretion. Diabetes 2002; 51: S368-76.

[26] Yang DJ, Wallace S, Cherif A, *et al.* Development of F-18-labeled fluoro-erythronitroimidazole as a PET agent for imaging tumor hypoxia. Radiology 1995; 194: 795-800.

[27] Cherif A, Wallace S, Yang DJ, *et al.* Development of new markers for hypoxic cells: [^{131}I]iodomisonidazole and [^{131}I]iodoerythronitroimidazole. J Drug Targeting 1996; 4: 31-9.

[28] Yang DJ, Ilgan S, Higuchi T, *et al.* Noninvasive assessment of tumor hypoxia with ^{99m}Tc-labeled metronidazole. Pharm Res 1999; 16(5): 743-50.

[29] Song HC, Bom HS, Cho KH, *et al.* Prognostication of recovery in patients with acute ischemic stroke using brain spect with ^{99m}Tc-metronidazole. Stroke 2003; 34: 982-86.

[30] Marshall S, Bacote V, Traxinger RR. Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. J Biol Chem 1991; 266: 4706-12.

[31] Wells L, Gao Y, Mahoney JA, *et al.* Dynamic O-glycosylation of nuclear and cytosolic proteins: further characterization of the nucleocytoplasmic beta-N-acetylglucosaminidase, O-GlcNAcase. J Biol Chem, 2002; 277: 1755-61.

[32] Pal S, Claffey KP, Cohen HT, Mukhopadhyay D. Activation of Sp1-mediated vascular permeability factor/vascular endothelial growth factor transcription requires specific interaction with protein kinase C zeta. J Biol Chem 1998; 273: 26277-80.

[33] Black AR, Black JD, Azizkhan-Clifford J. Sp1 and kruppel-like factor family of transcription factors in cell growth regulation and cancer. J Cell Physiol 2001; 188: 143-60.

[34] Yang DJ, Kim CG, Schechter NR, *et al.* Imaging with ^{99m}Tc ECDG targeted at the multifunctional glucose transport system: feasibility study with rodents. Radiology 2003; 226: 465-73.

[35] Yang DJ, Yukihiro M, Oh C-S, *et al.* Assessment of Therapeutic Tumor Response Using ^{99m}Tc-Ethylenedicysteine-Glucosamine. Cancer Biotherapy and Radiopharm 2004; 19(4): 444-58.

[36] Goethals P, Eijkeren MV, Lodewyck W, Dams R. Measurement of [methyl-carbon-11]thymidine and its metabolites in head and neck tumors. J Nucl Med 1995; 36: 880-2.

[37] Tjuvajev J, Macapinlac HA, Daghighian F, *et al.* Imaging of brain tumor proliferative activity with iodine-131-iododeoxyuridine. J Nucl Med 1994; 35: 1407-17.

[38] Abe Y, Fukuda H, Ishiwata K, *et al.* Studies on ^{18}F-Labeled Pyrimidines Tumor Uptakes of ^{18}F-5-Flourouracil, ^{18}F-5-Flourouridine, and ^{18}F-5-Flourodeoxyuridine in Animals. Eur J Nucl Med 1983; 8:258-61.

[39] Kim CG, Yang DJ, Kim EE, *et al.* Assessment of tumor cell proliferation using [^{18}F]fluorodeoxyadenosine and [^{18}F]fluoroethyluracil. J Pharm Sci 1996; 85: 339-44.

[40] Buck AK, Halter G, Schirrmeister H, *et al.* Imaging Proliferation in Lung Tumors with PET: ^{18}F-FLT Versus ^{18}F-FDG.J Nucl Med 2003; 44:1426-31.im CG, Yang DJ, Kim EE, *et al.* Assessment of tumor cell proliferation using [^{18}F]fluorodeoxyadenosine and [^{18}F]fluoroethyluracil. J Pharm Sci 1996; 85: 339-44.

[41] Francis DL, Visvikis D, Costa DC, *et al.* Potential impact of [^{18}F]3'-deoxy-3'-fluorothymidine versus [^{18}F]fluoro-2-deoxy-D-glucose in positron emission tomography for colorectal cancer. Eur J Nucl Med Mol Imaging 2003; 30: 988-94.

[42] Yang DJ, Ozaki K, Oh C-S, *et al.* ^{99m}Tc-EC-Guanine: Synthesis, Biodistribution and Tumor Imaging in Animals. Pharmaceutical Research 2005; 22(9): 1471-9.

[43] Gong J, Yang DJ, Kohanim S, Humphreys R, Broemeling L, Kurzrock R. Novel *in vivo* imaging demonstrates upregulation of death receptors by paclitaxel and correlates with enhanced antitumor effects of receptor agonist antibodies. Molecular Cancer Therapeutics 2006; 5(12): 2991-3000.

[44] Jager PL, Plaat BE, de Vries EG, *et al.* Imaging of soft-tissue tumors using L-3-[iodine-123]iodo-alpha-methyl-tyrosine single photon emission computed tomography: comparison with proliferative and mitotic activity, cellularity, and vascularity. Clin Cancer Res 2000; 6: 2252-9.

[45] McConathy J, Martarello L, Malveaux EJ, *et al.* Synthesis and evaluation of 2-amino-4-[(18)F]fluoro-2-methylbutanoic acid (FAMB): relationship of amino acid transport to tumor imaging properties of branched fluorinated amino acids. Nucl Med Biol 2003; 30: 477-90.

[46] Karacalioglu AO, Yang DJ, Azhdarinia A, *et al.* Radiolabeled L-lysine for tumor imaging. Academic Radiology 2006; 13(11): 1327-37.

[47] Zaidi, H. and Koral, K. F. 2004. Scatter modelling and compensation in emission tomography. Eur J Nucl Med Mol Imaging 31: 761-782.

[48] Tagliabue L, Schillaci O. SPECT/CT in oncology: the fusion of two imaging modalities is a new standard of care. Q J Nucl Med Mol Imaging 2007; 51(4): 285-9.

[49] Nakahara T, Shigematsu N, Fujii M, *et al*. Value of CT thallium-201 SPECT fusion imaging over SPECT alone for detection and localization of naso-pharyngeal and maxillary cancers. AJR Am J Roentgenol 2006; 187(3): 825-9.

[50] Kramer EL, Noz ME. CT-SPECT fusion for analysis of radiolabeled antibodies: applications in gastrointestinal and lung carcinoma. Int J Rad Appl Instrum B 1991; 18(1): 27-42.

[51] Bailey DL, Roach PJ, Bailey EA, Hewlett J, Keijzers R. Development of a cost-effective modular SPECT/CT scanner. Eur J Nucl Med Mol Imaging 2007; 34(9): 1415-26.

[52] Roach PJ, Schembri GP, Ho Shon IA, Bailey EA, Bailey DL. SPECT/CT imaging using a spiral CT scanner for anatomical localization: Impact on diagnostic accuracy and reporter confidence in clinical practice. Nucl Med Commun 2006; 27(12): 977-87.

[53] Hillel PG, van Beek EJ, Taylor C, *et al*. The clinical impact of a combined gamma camera/CT imaging system on somatostatin receptor imaging of neuroendocrine tumours. Clin Radiol 2006; 61(7): 579-87.

[54] Achilefu, S.I., Rajagopalan, R., Dorshow, R.B., Bugaj, J.E., Periasamy, M.P.: US20077198778 (**2007**).

[55] Artemov, D., Kato, Y.: WO2007028141 (**2007**).