Assessment of Tracer $^{99m}$Tc(V)-DMSA Uptake as a Measure of Tumor Cell Proliferation \textit{In Vitro}

Fatma J. Al-Saeedi$^{1,*}$, Princy M. Mathew$^1$, Yunus A. Luqmani$^2$

$^1$ Nuclear Medicine Department, Faculty of Medicine, Health Sciences Center, Kuwait University, Hawally, Jabriya, Kuwait, $^2$ Pharmaceutical Chemistry Department, Faculty of Pharmacy, Health Sciences Center, Kuwait University, Hawally, Jabriya, Kuwait

Abstract

\textbf{Purpose:} To examine whether $^{99m}$Tc(V)-DMSA could be used as a non-invasive measure of cancer cell proliferation.

\textbf{Methods:} Human breast cancer MCF-7, MDA-MB-231 and pl, and prostate cancer PC-3 cell lines were grown to 30, 50 and 100\% confluence and pulsed with $^{99m}$Tc(V)-DMSA in media for 60 min at 37°C. DNA synthesis was analysed by quantification of the S phase using flow cytometry, [methyl-$^3$H]thymidine incorporation and expression of proliferation markers PCNA and Ki-67 using realtime PCR. One way ANOVA was used to compare groups.

\textbf{Results:} In all cell lines rates of $^{99m}$Tc(V)-DMSA uptake were inversely related to cell density. This was paralleled by similar trends in S phase proportions, [methyl-$^3$H]thymidine incorporation and expression of PCNA and Ki-67.

\textbf{Conclusion:} Rates of $^{99m}$Tc(V)-DMSA uptake into different types of tumour cells correlate well with cell density that is useful as a non-invasive measure of tumour cellular proliferation \textit{in vivo}.

Introduction

The ability to non-invasively detect and image cell growth and proliferation throughout the body has long been recognised to be of significant value in the diagnosis, staging and treatment of cancer. The expansion of a tumour mass is directly related to its growth fraction, which can be assessed by measuring DNA synthesis by following incorporation of [$^3$H] or [$^{14}$C] labelled thymidine. This is acceptable for \textit{in vitro} model systems and in animals but as long lived beta emitters, neither radionuclide is suitable for imaging in humans. Thymidine also has the disadvantage that it is rapidly catabolised and produces large amounts of re-circulating labeled catabolites following administration \cite{1}, reducing tumour-to-normal-tissue signaling during detection and imaging of tracer incorporation into DNA by kinetic modeling \cite{2}.

Positron emission tomography (PET) has emerged as a very sensitive physiological, metabolic and molecular gamma ray detection technology that is used for imaging in many research and clinical applications but principally in oncology. For several decades the most commonly used PET tracer has been the cyclotron produced 2-[fluorine-18]Fluoro-2-deoxy-D-glucose ($^{18}$F-FDG). However, this is neither cell specific nor very appropriate for measurement of cell proliferation, besides which it has a short half-life of less than 2 h. Several non-catabolised thymidine analogues such as [$^{18}$F] 3'-deoxy-3'-fluorothymidine ($^{18}$F-FLT), have been enlisted into clinical use for lymphoma, non-small-cell lung cancer and brain tumors \cite{3-6}. However, incorporation of $^{18}$F-FLT proceeds only to the triphosphate nucleoside step in the DNA synthetic pathway and not into DNA itself \cite{7}. There are also some practical drawbacks of $^{18}$F-FLT for identifying sites of proliferative activity or malignancy in the liver and bone marrow due to the presence of high background radioactivity, and interference with pelvic lesions due to its significant excretion into the urinary bladder \cite{8-11}.

The short half-life of PET tracers has also limited their use to centers equipped with an on-site cyclotron. The practical need for longer-lived tracers has been resolved by the use of technetium-99m ($^{99m}$Tc). This radionuclide, is produced by a generator that is readily available in most hospital nuclear medicine departments and does not need an on-site cyclotron to produce it. It’s half-life of 6 h is long enough to perform nuclear medicine tests and short enough to minimize radiation exposure to the patient. Thus $^{99m}$Tc is a very useful isotope that has been incorporated into many tumor imaging agents such as $^{99m}$Tc-hexakis-2-methoxyisobutylisonitrile (sestamibi; $^{99m}$Tc-MIBI), used to detect metastatic breast cancer \cite{12-13}.

Pentavalent $^{99m}$Tc-dimercaptosuccinonic acid ($^{99m}$Tc(V)-DMSA) formed from labelling of meso-2,3-dimercaptosuccinic acid (DMSA) with $^{99m}$Tc under alkaline conditions, has been found to be a tumor-seeking agent. It was introduced to evaluate, image and manage medullary carcinoma of the thyroid \cite{14-15}. Also it has been reported to be useful in detecting many other types of cancers such as head and neck, soft tissue tumors \cite{16-17}, breast...
and compared this with assessment of other known proliferation-related activity under these different conditions (this has also been shown to express type III NaPi co-transporters) several different cancer cell lines under these different conditions and activities could be very useful in predicting the progression of metastatic carcinoma of the prostate [24–25] and melanoma [26].

In this study we have sought to determine whether \( ^{99m} \text{Tc(V)} \)-DMSA uptake could be correlated with the rate of cell proliferation using an \( in vitro \) model system. Typically, attaching monolayer cultures undergo a lag phase followed by a period of rapid growth that then slows as the cells in the culturing vessel reach confluence. Confluency is the measure of % coverage of the culturing vessel. We measured \( ^{99m} \text{Tc(V)} \)-DMSA uptake into several different cancer cell lines under these different conditions and compared this with assessment of other known proliferation-associated markers.

**Materials and Methods**

**Materials**

All general chemical reagents were purchased from Sigma (UK). Tissue culture reagents were purchased from Life Technologies (USA). Propidium iodide (PI)-ribonuclease (RNase) staining buffer (BD staining kit) was obtained from BD Biosciences (UK). Radionuclide, \( ^{99m} \text{Tc} \), was obtained from a molybdenum-99-technetium-99m (\( {^{99m} \text{Mo}}-{^{99m} \text{Tc}} \)) generator located in the Clinical Nuclear Medicine Department of Mubarak Al Kabeer Hospital, Kuwait. [Methyl-\( ^3 \text{H} \)]thymidine (specific activity 0.93 TBq/mmol) was obtained from Amersham. 2-[fluorine-18]Fluoro-2-deoxy-D-glucose (18F-FDG) was obtained from Kuwait Cancer Center (Kuwait). Dimercaptosuccinic acid (DMSA, Succimer) kit was purchased from Mallinkrodt Medical BV (Holland). Sodium bicarbonate was purchased from Pharmaceutical Solutions Industries (Saudi Arabia). Imatinib was purchased from LC Laboratories (USA).

**Cell culture and culture media**

Human breast cancer MCF-7, MDA-MB-231 and prostate cancer PC-3 cell lines were purchased from Cell Lines Service (CLS, Germany). pII is an estrogen receptor down-regulated transfected cell line derived from MCF-7 [30]. All cell lines were maintained in Advanced Dulbecco’s Modified Eagle’s Medium (Advanced DMEM) supplemented with 5% fetal bovine serum (FBS), 10,000 units/ml penicillin, 10,000 \( \mu \)g/ml streptomycin, 200 mM L-glutamine and non-essential amino acids. All cells were routinely incubated in a 37°C humidified incubator in an atmosphere of 5% CO2-95% air. For sub-culturing, cell monolayers were harvested with trypsin-EDTA. Cell counting was carried out using a haemocytometer (Asistent, Germany) and Cell Viability Analyzer (Beckmann Coulter Vi-cell \( ^{TM} \) XR, USA). For most experiments cells were seeded into 25 cm flasks and grown to 70% density in a 24 well plate. These were pretreated for either Pu-dependent phosphate cotransporter (NaPi) inhibitors.

**Preparation of \( ^{99m} \text{Tc(V)} \)-DMSA**

The commercial meso-2,3-diaminocapto-succinic acid (DMSA) kit (Succimer), for the preparation of trivalent technetium-99m DMSA (\( ^{99m} \text{Tc(III)} \)-DMSA), was used here for the preparation of technetium-99m (\( ^{99m} \text{Tc} \)) complex with DMSA. \( ^{99m} \text{Tc(III)} \)-DMSA complex was prepared by addition of sodium bicarbonate solution to the kit vial followed by the addition of \( ^{99m} \text{Tc} \)-chloride (\( ^{99m} \text{TcCl}_4 \), approximately 500 MBq). The reaction mixture reached a \( \rho \)H value of approximately 8 (as determined with \( \rho \)H indicator strips) and was then left for 15 min at room temperature. This value of \( \rho \)H indicates the optimum conversion to \( ^{99m} \text{Tc(V)} \)-DMSA [37–40].

The efficiency of radiolabeling of DMSA was assessed by ascending thin-layer chromatography using Merck silica gel 60F TLC-SG 60; Merck, Germany) and n-butanol:acetic acid:H2O (3:2:3) developing mixture. The retention factor (Rf) value for \( ^{99m} \text{Tc(V)} \)-DMSA was 0.7 while for \( ^{99m} \text{Tc(III)} \)-DMSA it was 0.1 giving a very clear separation [41]. The radiochemical purity was calculated as the percentage of \( ^{99m} \text{Tc(V)} \)-DMSA relative to total activity on the plate. Typically we obtained efficiencies in excess of 95% conversion of \( ^{99m} \text{Tc(III)} \)-DMSA to \( ^{99m} \text{Tc(V)} \)-DMSA. The \( ^{99m} \text{Tc(V)} \)-DMSA mixture was used within 3 h of preparation.

It has previously been reported [42] that almost all of the trivalent technetium-99m DMSA (\( ^{99m} \text{Tc(III)} \)-DMSA) remaining in the labeled preparation can be changed into \( ^{99m} \text{Tc(V)} \)-DMSA by bubbling with pure oxygen. We therefore performed comparative uptake experiments using \( ^{99m} \text{Tc(V)} \)-DMSA prepared with oxygen bubbling (100% oxygen) for 10 min or with no bubbling (0% oxygen).

The \( ^{99m} \text{Tc(V)} \)-DMSA was also used for \( in vivo \) imaging in patients showing significant uptake into breast tumors (data not presented).

**Determination of \( ^{99m} \text{Tc(V)} \)-DMSA uptake into cell cultures**

All the cell lines were pulse labeled with \( ^{99m} \text{Tc(V)} \)-DMSA (74 MBq/ml medium) for 60 min at 37°C. The culture medium was removed and counted to determine effluxed radioactivity using a dose calibrator (ATOMLAB 100, USA). The cell monolayers were rapidly washed three times with ice-cold phosphate buffered saline (PBS) and detached with 0.5 ml trypsin-EDTA followed by re-suspension in 5 ml of medium. The cells were centrifuged at 10,000 g for 5 min at 4°C and solubilized with 1% sodium dodecyl sulphate (SDS) in 10 mM sodium borate. The \( ^{99m} \text{Tc(V)} \)-DMSA incorporated into the cellular lysate was counted as above.

**Results**

were expressed as the total \( ^{99m} \text{Tc(V)} \)-DMSA radioactivity uptake in megabecquerel (MBq) per mg of protein.

**Effect of Na+ dependent phosphate cotransporter (NaPi) inhibitors**

In other experiments, MCF-7 cells were grown to about 60–70% density in a 24 well plate. These were pretreated for either
24 h or 48 h with 50 μM imatinib (prepared as a 10 mM stock solution in DMSO), or for 15 min with 5 mM and 10 mM phosphonomiformic acid (PFA) prior to uptake determination of 99mTc(V)-DMSA as described above.

Extraction of radio-labeled metabolites and lipids
The intracellular fate of the 99mTc(V)-DMSA (phospholipid synthesis) was determined by extraction of cell pellets with chloroform/methanol tris (hydroxymethyl) aminomethane buffer solvent system as described by Bligh and Dyer [43] and Al-Saeedi et al [34]. Cell pellets were re-suspended in 0.2 ml PBS in eppendorf tubes to which 0.5 ml of methanol and 0.25 ml of solvent system as described by Bligh and Dyer [43] and Al-Saeedi et al [34]. Cell pellets were re-suspended in 0.2 ml PBS in eppendorf tubes to which 0.5 ml of methanol and 0.25 ml of solvent system as described by Bligh and Dyer [43] and Al-Saeedi et al [34]. Cell pellets were re-suspended in 0.2 ml PBS in eppendorf tubes to which 0.5 ml of methanol and 0.25 ml of.

Fluorine-18-FluoroDeoxy Glucose (18F-FDG) uptake
MCF-7 cells were pulse labeled with 18F-FDG (13.283 MBq) for 15 min at 37°C and processed as described above for 99mTc(V)-DMSA uptake.

Effect of Na+ on 99mTc(V)-DMSA uptake
The effect of Na+ on the uptake of 99mTc(V)-DMSA was assessed by incubating cells for 1 h, prior to performing the assay, in two types of media in place of the standard DMEM. The high Na+ medium was composed of 137 mM NaCl, 5.4 mM KCl, 2.3 mM CaCl2, 1.2 mM MgSO4, 14 mM Tris (pH 7.4) and 0.1 mM KH2PO4. The Na+ -free medium was similarly composed except that the NaCl was substituted with 137 mM N-methyl-D-glucamine.

[Radiolabelled]thymidine incorporation into DNA
MCF-7, MDA-MB-231, pII and PC-3 cell lines grown to different cell densities (30, 50, 100%, were pulse labeled with [methyl-3H]thymidine (51.8 MBq/ml of medium per flask) for 1 h at 37°C in order to assess DNA synthesis. Cells were washed thrice with ice cold PBS, trypsinized and centrifuged at 10,000 g for 5 min at 4°C. Cell pellets were suspended with ice cold PBS and left on ice for 15 min before centrifugation. Pellets were resuspended in 1 ml of 4% trichloroacetic acid (TCA) (Fluka, UK) at 4°C and again centrifuged. Washes were repeated in 4% TCA at 4°C until the radioactivity in the supernatant was reduced to background level. To solubilise the DNA (acid-insoluble fraction), the pellet was resuspended in 0.5 ml of 4% TCA and heated to 90°C for 1 h. Then the cell debris was removed by centrifugation and washed with 0.5 ml of 4% TCA. The resulting supernatants and pellets were pooled, then suspended with Ultima Gold™ scintillation fluid (PerkinElmer, USA) and counted in a Beckman LS 6000 TA liquid scintillation counter (Beckman, USA).

RNA extraction and measurement of gene expression
Total cellular RNA was extracted from frozen cell pellets of MCF-7, MDA-MB-231, pII and PC-3 cells using the RNaseasy Plus Mini kit (Qiagen, USA) according to the manufacturer’s protocol, quantitated by spectrometry and checked for integrity by standard agarose gel electrophoresis. cDNA synthesis was performed with 2 μg RNA in 20 μl using the High Capacity Reverse Transcrip-
a technically difficult procedure to maintain sterility, its use was discontinued.

Fig. 2 shows 99mTc(V)-DMSA uptake for each of the four cell lines used in this study. For comparison, the uptake at 30% confluency was assigned as 1 and uptake at other densities was expressed relative to this. In all cases the relative rate of uptake was inversely related to cell density. At 50 and 100% confluency, the values were 0.94±0.002 and 0.93±0.001 for MCF-7 (p = 0.01), 0.67±0.002 and 0.49±0.004 for MDA-MB-231 (p<0.0001), 0.67±0.02 and 0.41±0.07 for pII (p<0.0001) and 0.79±0.009 and 0.74±0.005 for PC-3 (p = 0.001).

The intracellular fate of the 99mTc(V)-DMSA was determined in MCF-7 cells by partitioning the pelleted cellular material, following the uptake experiment, into aqueous and lipid soluble phases (phospholipid synthesis). Both phases were counted for 99mTc(V)-DMSA content. Less than 5% of the label appeared in the lipid fraction indicating that almost all of the 99mTc(V)-DMSA that enters the cell remains unbound to lipid during the time of the experiment.

Uptake of [methyl-3H]thymidine
This was determined under the same conditions as for 99mTc(V)-DMSA uptake and the results are shown in Fig. 3. With all four lines, relative uptake significantly decreased with increasing cell density.

S-phase distribution
Flow cytometry was used to determine the proportion of cells in S-phase at the different densities. Significant decrease was noted as cells reached 100% confluency, in all the four cell lines (Fig. 4).

Effect of imatinib and phosphonoformic acid on 99mTc(V)-DMSA uptake
MCF-7 cells were exposed to either imatinib for 24 h and 48 h or to PFA for 15 min prior to measuring uptake of 99mTc(V)-DMSA. Uptake was significantly decreased in the presence of imatinib by about 70% after the 24 h exposure (data not shown) and by about 60% after the 48 h exposure as compared with untreated controls, indicating that 99mTc(V)-DMSA enters the cell mainly through transporters rather than simple diffusion (Fig. 5).
This was further confirmed by the inhibition of uptake by PFA, a direct competitive inhibitor of NaPi cotransporters.

Effect of cell density on expression of proliferation markers

PCNA and Ki-67 expression determined by Taqman quantitative PCR showed significant decrease as cells reached 100% confluency in all the four cell lines (Fig. 6). Expression in 50% confluent MCF-7 however was higher than at 30% and then decreased at 100% for both genes. This was not seen with the other cell lines.

Fluorine-18-FluoroDeoxy Glucose (18F-FDG) Uptake

18F-FDG uptake was determined in MCF-7 cells grown to different confluencies and was found to be the same at all densities.

Effect of extracellular Na+ on 99mTc(V)-DMSA uptake

99mTc(V)-DMSA uptake was decreased by approximately 50%, by replacing sodium with N-methyl-D-glucamine in the culture medium prior to measuring uptake.

Discussion

In agreement with many studies reporting that 99mTc(V)-DMSA prepared under alkaline conditions showed increased uptake into a variety of tumors [14 15, 17, 19, 27] we observed active uptake into several cell lines in vitro.

This radiotracer is not only used as a diagnostic tool but even further as a therapeutic agent. Pentavalent rhenium-188 dimer-captosuccinic acid (188Re(V)-DMSA) is a beta-emitting analogue of 99mTc(V)-DMSA, a tracer that is taken up into a variety of tumors and bone metastases that is used on a therapeutic scale for cancer therapy [45–48]. For example, the treatment with 188Re(V)-DMSA in cases of advanced disease with osseous and soft tissue metastatic spread could be considered if these lesions are depicted by breast and whole-body 99mTc(V)-DMSA scintigraphy [31]. In some reports, 188Re(V)-DMSA was loaded with poly(lactic-co-glycolic)acid microspheres for targeted radiotherapy and for the delivery of a radiation dose to tumors [31,47].

The technetium is pentavalent and coordinated by an oxo-ligand and four thiolate sulfurs of two DMSA ligands. 99mTc(V)-DMSA forms a small complex as [MO(DMSA)2]-, in which the M (99mTc, 188Re) is coordinated square-pyramidically by the four thiolates and by an apical oxo-ligand and consists of mixtures of three stereo isomers of the square pyramidal mononuclear complex [49–50]. The negatively charged Tc(V)-oxo complex with DMSA consists of a TcO core with four sulfur atoms of the bidentate DMSA ligands arranged in a plane. In other words, the chemical structure or formula of the 99mTc(V)-DMSA complex possesses four negatively charged carboxylate groups, and a central anionic technetium oxobis (dithiolato) core, i.e., [TcO(DMSA)2]⁻[50–51].

Reports as recent as 2005 and 2007 describe the use of 99mTc(V)-DMSA for imaging [20,26] and the group of Papantoniou have performed several studies on breast lesions, extensively reviewed earlier this year [31]. In this publication it is also mentioned that a protocol for use of 99mTc(V)-DMSA as a guide for therapeutic administration of 188Re(V)-DMSA for advanced breast cancer is underway at the Vince Institute of Nuclear Sciences in Belgrade. This agent has advantages over currently employed PET radiopharmaceuticals. It is safe, cheap and readily available in many nuclear medicine centers, and can be prepared...
as an in house radiopharmaceutical. Unlike for example the more commonly used imaging agent ¹⁸F-FDG, there is no need for a cyclotron to produce it so it would be useful for medical centers that have no or limited access to such facilities for generating the current range of PET tracers. PET is not currently well-established in Kuwait; this study could provide some justification for considering a future trial to examine the utility of ⁹⁹mTc(V)-DMSA as a nuclear medicine marker that can be synthesized locally without the need for an expensive cyclotron.

The label was predominantly found in the aqueous fraction of cell extracts indicating actual uptake into the cell rather than immediate incorporation into membrane or other phospholipid. The comparative rates of uptake differed between the three breast and one prostate line, being higher in the more aggressive estrogen receptor negative MDA-MB-231 and pII lines. Interestingly, and one prostate line, being higher in the more aggressive estrogen type that is characteristically slower growing. In all cases however, we observed an inverse correlation between ⁹⁹mTc(V)-DMSA uptake and cell density. It is generally the case that the growth rate of tumors slows as they increase in size, mainly as a result of poorer vascularization and necrosis at the centre. As this is an important consideration in the design of chemotherapeutic strategies, information regarding growth rates can be clinically useful, as well indicating prognosis. Our simple in vitro model aims to simulate slow and faster growing groups of cells and the results suggest that the imaging tracer ⁹⁹mTc(V)-DMSA can provide additional information in this respect.

It is considered that ⁹⁹mTc(V)-DMSA utilizes the type III NaPi co-transporters [35–36] as phosphonoformic acid, a competitive inhibitor of NaPi co-transport, also affects ⁹⁹mTc(V)-DMSA uptake. Our data is consistent with studies showing that this transport is largely Na⁺ dependent.

Activated platelet-derived growth factor receptor (PDGF-R) pathway is involved in this transport mechanism. The addition of the PDGF-R inhibitor, imatinib, at least partially inhibited ⁹⁹mTc(V)-DMSA in our study after both 24 and 48 h of exposure to the drug, an agreement with previous studies. This drug has also been shown previously to reduce expression of these transporters [53–57]. Several studies reported that imatinib mesylate, a small molecule inhibiting the PDGF-R tyrosine kinase, suppressed NaPi co-transporter expression sufficiently during the time-frame of the experiment, the 24 h pre-incubation [32,58,59].

A recent study on U87-MG glioblastoma cells investigated an issue with obvious clinical implications, the ability of ⁹⁹mTc(V)-DMSA to trace the antiproliferative effects of imatinib mesylate [60]. Cells treated with imatinib for 48 h showed significant decreases in proliferation, invasion, migration and PDGF-R expression. ⁹⁹mTc(V)-DMSA cellular uptake studies showed that the specification of imatinib on PDGF-R signal pathway, in the human glioblastoma cell line U87-MG, could be followed by radioactive tracer. Furthermore, strong correlations between cellular ⁹⁹mTc(V)-DMSA uptake and the effect of imatinib therapy on U87-MG proliferation, invasion and migration were obtained, likewise for ⁹⁹mTc(V)-DMSA uptake and PDGF-R expression [60]. Besides, imatinib mesylate was initiated to treat the temozolomide-refractory tumor [53,61].

The uptake of [methyl-¹H]thymidine into cells and its subsequent incorporation into DNA is a common measure of cell proliferation. We have shown that its uptake into all four of our cell lines parallels that of ⁹⁹mTc(V)-DMSA, decreasing with increasing cell density. Similarly, flow cytometry showed that the proportion of cells in S-phase was also inversely correlated with cell density. One study has reported that ⁹⁹mTc(V)-DMSA brain scintitomography is a plausible non-invasive measure of glioblastoma proliferation and therapy response [50]. Recent papers have demonstrated that in vivo ⁹⁹mTc(V)-DMSA uptake has been correlated with the proliferation index measured by Ki-67 expression and phosphorylated focal adhesion kinase [19,29,59]. We found that expression of Ki-67 as well as of PCNA, another common proliferation marker, inversely correlated with cell density. As a comparison, we also measured uptake of ¹⁸F-FDG, a commonly used imaging tracer, and found that unlike ⁹⁹mTc(V)-DMSA, its transport into cells was unrelated to proliferation rate, as has previously been reported [62].

In summary, we have demonstrated carrier mediated uptake of ⁹⁹mTc(V)-DMSA into 4 independent cancer cell lines and shown that this correlates well with proliferation rate using cells under conditions of fast and slow growth. The model has been validated with measurement of several parameters commonly accepted as markers of proliferation rate.

**Conclusion**

Our data indicates that it would be worthwhile to conduct further studies to examine the extent of ⁹⁹mTc(V)-DMSA uptake into tumors with respect to their rates of growth so that it may have an additional use beyond detection/localization of tumours.

**Author Contributions**

Conceived and designed the experiments: FA YL. Performed the experiments: FA YL. Analyzed the data: FA PM. Contributed reagents/materials/analysis tools: FA YL. Wrote the paper: FA YL.

**References**

1. Conti PS, Hilton J, Wong DF, Alaudin MM, Dannals RF, et al. (1994) High performance liquid chromatography of [¹¹C-methyl]-thymidine and its major catabolites for clinical PET studies. Nucl Med Biol 21: 1045–1051.
2. Mankoff DA, Shields AF, Link JM, Graham MM, Muzi M, et al. (1999) Kinetic analysis of 2-[¹¹C]thymidine PET imaging studies: validation studies. J Nucl Med 40: 614–620.
3. Buck AK, Hetzel M, Schirrmeister H, Halter G, Möller P, et al. (2005) Clinical relevance of imaging proliferative activity in lung nodules. Eur J Nucl Med Mol Imaging 32: 325–333.
4. Buck AK, Bommer M, Stilgenbauer S, Jiweid M, Glätting G, et al. (2006) Molecular imaging of proliferation in malignant lymphomas. Cancer Res 66: 11053–11061.
5. Muzi M, Veselh H, Grierson JR, Mankoff DA, Schmidt RA, et al. (2005) Kinetic analysis of 3-deoxy-3-fluorothymidine PET studies: validation studies in patients with lung cancer. J Nucl Med 46: 274–282.
6. Muzi M, Spence AM, O’Sullivan F, Mankoff DA, Wells JM, et al. (2006) Kinetic analysis of 3-deoxy-3-¹⁸F-fluorothymidine in patients with gliomas. J Nucl Med 47: 1612–1621.
7. Shields AF, Grierson JR, Dohmen BM, Machulla HJ, Stayanof JC, et al. (1998) Imaging proliferation in vivo with [¹⁸F]FLT and positron emission tomography. Nat Med 4: 1334–1336.
8. Okada J, Yoshiwaka K, Itami M, Inaseki K, Uno K, et al. (1992) Positron emission tomography using fluorine-18-fluorodeoxyglucose in malignant lymphoma: a comparison with proliferative activity. J Nucl Med 33: 323–329.
9. Vesselle H, Grierson J, Peterson LM, Muzi M, Mankoff DA, et al. (2005) ¹⁸F-Fluorothymidine radiation dosimetry in human PET imaging studies. J Nucl Med 44: 1492-1498.
10. Shields AF (2006) Positron emission tomography measurement of tumor metabolism and growth: its expanding role in oncology. Mol Imaging Biol 8: 141–150.
11. Sahar A, Tammiuitti VS, Grierson J, Veselh H (2007) FLT: measuring tumour cell proliferation in vivo with positron emission tomography and 3'-deoxy-3-¹⁸F-fluorothymidine. Semin Nucl Med 37: 429–439.
12. Papantonious V, Christodoulidou J, Papadaki E, Valotassiou V, Svoratzzoglou M, et al. (2002) Uptake and washout of ⁹⁹mTc-(V)dimercaptosuccinic acid and ⁹⁹mTc-((V)DMSA Assessment and Cell Proliferation
38. Washburn LC, Binakiewicz DS, Masson HR, 3rd (1995) Reliable preparation of 
\(^{99m}\text{Tc}\)-DMSA by a simple radiochemical method using a commercial kit for 
\(^{99m}\text{Tc}\) (III) DMSA. Nucl Med Biol 22: 689-691.

40. Babbar A, Kashyap N, Upadhyay UK, et al. (1991) A convenient method for the preparation of 
\(^{99m}\text{Tc}\)- labelled pentavalent DMSA and its evaluation as a tumour imaging agent. J Nucl Med Biol 35: 100-104.

41. Saha GB (1992) Fundamentals of Nuclear Pharmacy. New York: Springer Verlag. 150 p.

42. Kobayashi H, Suzuki KH, Sakahara H, Yano SY, Yokoyama A, et al. (1995) Oxygen  
  breathing can improve the labelling of pentavalent technetium-99m  
  dimercaptosuccinic acid. Eur J Nucl Med 22: 559-562.

43. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and  
  purification. Can J Biochem Physiol 37: 911-917.

44. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time 
  RT-PCR. Nucl. Acids Res. 29: e5.

45. Garnasek P, Padák D, Mauzer Jčovčík D, Karčmarczuk V, et al. (2012) Comparison of 
  chromographic methods for quality control of DMSA complexes with 
  \(^{99m}\text{Tc}\) and \(^{99m}\text{Re}\) at (III) and (V) oxidation states. Nucl Med Biol 39: 1029-1036.

46. Shukla J, Bandopadhyay GP, Varma IK (2005) \(^{188}\text{Re}\)dimercaptosuccinic acid loaded poly( 
  lactic-co-glycolic)acid microspheres for targeted  
  radiotherapy: production and effectiveness. Pharmazie 60: 583-587.

47. Kothari K, Pillai MR, Umri PR, Shigmi HH, Noronha OP, et al. (1999) Preparation of 
  \(^{99m}\text{Tc}\)Re-DMSA and its biotransdistribution. Appl Radiat Isot 51: 43-49.

48. Davison A, Orvig C, Trop H, Sohn M, DePamphilis BV, et al. (1800)  
  Preparation of oxoid (dihalide) complex of technetium(V) and rhenium(V). 
  Inorg Chem 19: 1998-1992.

49. Blower PJ, Singh J, Clarke SEM (1991) The chemical identity of pentavalent 
  technetium-99m-dimercaptosuccinic acid. J Nucl Med 32: 845-849.

50. Saha GB (2004) Characteristics of specific radiopharmaceuticals. In: Saha GB 
  editor. Fundamentals of nuclear Pharmacy New York: Springer. 124-125.

51. Al Saleh S, Al Mulla F, Luqmani YA (2011) Estrous estrogen silencing induces 
  epithelial to mesenchymal transition in human breast cancer cells. PLoS One 6: 21-30.

52. K딩hoff M, Kreuzer KA, Appel C, Scholz R, Na IK, et al. (2005) Imatinib 
  mesylate radiosensitizes human glioblastoma cells through inhibition of 
  platelet-derived growth factor receptor. Blood Cells Mol Dis 34: 181-185.

53. Koning L, van der Meer A, van der Spek JG, et al. (1998) In vitro comparison with 
  DNA flow cytometry activity of human cancer cells? In vitro comparison with DNA flow cytometry activity of human cancer cells? Int J Radiat Biol 73: 17-24.

54. Heldin CH, Westmark B (1990) Platelet-derived growth factor, mechanism of action and possible in vivo function. Cell Regul 1: 355-366.

55. Tsuorit S, Pernettis I, Chatzipanagiotou T, Ptohis N, Papantoniou V (2007) Pentavalent 
  technetium-99m dimercaptosuccinic acid \(^{99m}\text{Tc}\)DMSA brain 
  scintigraphy in patients with head and neck squamous carcinoma: experience in imaging. J Nucl Med 30: 174-180.

56. Cortajada FM, Puzenat E, Fabregas R, et al. (1992) Evaluation of pentavalent 
  technetium-99m DMSA scintigraphy in small cell and non small cell lung cancers. Nuklearmedizin 36: 223-227.

57. Lam AN, Kettle AG, O’Doherty MJ, Coakley AJ, Barrington SF, et al. (1997) 
  Pentavalent 99Tc(V)-DMSA imaging in patients with bone metastases. Nucl Med Commun 18: 907-914.

58. Kiran H, Kirath PO, Ercan MT (1998) Scintigraphic evaluation of tumour metastatic to the choroid using technetium-99m(V)-dimercaptosuccinic acid. Acta 
  Radiol 39: 365-367.

59. Al-Saeedi F, Welch AE, Smith TAD (2005) \(^{[18F]}\text{F-FDG}\) incorporation is enhanced by attenuation of p53 function in breast cancer cells in 
  vivo. Clin Exp Metastasis 22: 191-198.

60. Saha GB (2004) Characteristics of specific radiopharmaceuticals. In: Saha GB 
  editor. Fundamentals of nuclear Pharmacy New York: Springer. 124-125.

61. Raymond E, Brandes AA, Dittrich C, Fumoleau P, Coudert B, et al. (2008) 
  Preparation of \(^{99m}\text{Tc}\)Re-DMSA and its bio-distribution studies. Appl Radiat Isot 66: 359-367.