Is Technetium-99m dead or still alive?
An outlook to recent developments with special focus on myocardial perfusion imaging

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Abstract
Despite the advances associated with the introduction of PET technology, reasons to consider that SPECT, particularly with 99mTc radiopharmaceuticals will continue playing an important role in Nuclear Medicine, are presented. The details examined are the following ones. An improvement of the technology SPECT is appreciated with the development of new detection systems and the advantages of the appearance of the hybrid systems SPECT / TAC. The biggest half-life of the main SPECT radionuclides like 123I and 99mTc in comparison with PET radionuclides, facilitates its transportation at larger distances, allow the realization of studies with radiopharmaceuticals with different radionuclides in the same patient, and the possibility of the detection of lesions of low tracer accumulation. Finally the main base core for 99mTc complex formation with different ligands are reviewed, as a background that assure the appearance of new radiopharmaceuticals of practical interest. The oncological and cardiological potential applications are examined with emphasis in these last ones. It is clear that 99mTc radiopharmacy will continue playing an important role in nuclear medicine.

Key words: myocardium, Technetium 99, perfused organs, radiopharmaceuticals, nuclear medicine, single photon emission computed tomography

¿Murió o permanece con vida el Tecnecio 99m?
Una panorámica de desarrollos recientes con énfasis en la imagen de perfusión del miocardio

Resumen
En el trabajo se exponen razones para considerar que el SPECT, particularmente con radiofármacos de 99mTc continuará teniendo un importante papel en medicina nuclear, no obstante los avances asociados a la incorporación de la tecnología PET. Las razones que se examinan son las siguientes. Se aprecia un mejoramiento de la tecnología SPECT con el desarrollo de nuevos sistemas de detección y las ventajas de la aparición de los sistemas híbridos SPECT/TAC, la mayor vida media de los principales radionuclídos SPECT como 123I y 99mTc en comparación con los radionúclidos PET, lo que posibilita su traslado a mayores distancias, la realización de estudios con radiofármacos con más de un radionúclido en el mismo paciente y la posibilidad de detectar lesiones de baja captación, favorecido por lo indicado de las vidas medias. Finalmente se examinan los principales núcleos base de formación de complejos de 99mTc con distintos ligandos que aseguran la aparición de nuevos radiofármacos de interés. Se examinan las potenciales aplicaciones oncológicas y cardiológicas con énfasis en estas últimas. Se considera que la radiofarmacia del 99mTc continuará jugando un importante papel en medicina nuclear.

Palabras clave: miocardio, Tecnecio 99, órganos perfundidos, radiofármacos, medicina nuclear, tomografía de emisión computarizada de fotón único

Introduction
The radionuclide Technetium-99m has been the workhorse of nuclear medicine for decades. Nuclear imaging would not have been existed without this wonderful radionuclide having almost ideal nuclear properties for yielding functional images of the internal organs of the body. After so many successful years, as in any fairy tales, now it seems that time has come for it to disappear from the scene almost completely replaced by positron-emitting radionuclides. Is this really the end of the story? We will try here to bring some arguments that refute this conclusion and support the viewpoint that 99mTc radiopharmaceuticals may still have an important role in diagnostic nuclear medicine and molecular imaging. This short review aims to provide a few examples demonstrating how
the field is still active and producing new potentially useful radiopharmaceuticals selectively visualizing exactly the same biological end points that are currently the most widely pursued using positron-emitting tracers.

Some preliminary considerations

The nuclear imaging technologies, PET and SPECT are highly analogous. They both offer the sensitivity required to monitor drug distribution, pharmacokinetics and pharmacodynamics, and for imaging specific biomarkers and molecular end points. Depending on the ligands and radionuclides used, a myriad of molecular targets can be hit.

Traditionally, PET has outperformed SPECT in terms of detection sensitivity and image resolution. Positron emitters have higher tissue penetration than the single photon emitters and the ability to localize positrons without the use of collimation techniques, results in better detection sensitivities. However, the recent emergence of hybrid systems, PET/CT and SPECT/CT, has narrowed some of the differences between the two modalities. Indeed, CT image data can be used to correct for tissue attenuation, and this anatomical information further contributes to improve the localization of single-photon emissions. Furthermore, the recent introduction of solid-state detectors and photomultipliers has dramatically improved the performance of SPECT.

Currently, the impact of these technological advancements has become apparent particularly in the field of imaging of smaller subjects such as mice and other animals. In fact, the recent invention of multiple pinhole collimation systems has resulted in very high imaging sensitivities with spatial image resolution in the nanometer range, far superior to PET.

Traditional single photon emitting radioisotopes such as 99mTc and 123I have radioactive half-lives of hours and are long enough to allow their production at a central site for subsequent distribution over a relatively large geographic region. In contrast most PET isotopes decay much faster and the distribution of their radiopharmaceuticals is impossible or usually requires building up a complex and expensive infrastructure. Conversely, SPECT is faster and cheaper when there is already a registered radiopharmaceutical for a specific diagnostic indication.

The ultra-high resolution nuclear imaging capabilities of novel SPECT/CT scanners offer unique advantages when the concentration of the target is relatively low. In these situations, detecting a signal sometimes requires a very low level of background noise that can be reached after hours necessary to eliminate background activity. For these applications, single photon emitters with longer half-lives can be more effective than short-lived positron-emitting isotopes.

Technetium 99m cores

The most interesting Tc-99m radiopharmaceuticals that have been developed in the last decade belong almost exclusively to three main categories of complexes each characterized by a specific metallic core. These cores constitute characteristic chemical motifs that control and shape the molecular structure of the resulting radiopharmaceutical and, ultimately, its biological properties. The main features of these cores and of the corresponding complexes are briefly outlined in the following.

A terminal $[^{99m}\text{Tc}]=\text{N}$ triple bond can be formally viewed as generated by the bonding interaction between a $[^{99m}\text{Tc}]=\text{N}$ ion and a $\text{N}^-\text{ nitrido nitrogen atom. This yields}$ the $[^{99m}\text{Tc}]=\text{N}$ functional moiety that, owing to its small molecular size, behave like a single atomic entity. Nitrido Tc-99m complexes usually possess a five-coordinated geometry in which the terminal Tc=O group spans an apical position. These compounds are commonly classified in two distinct subtypes: (a) symmetrical and (b) asymmetrical complexes [1-3].

Symmetrical complexes form when two identical bidentate chelating ligands bind the same Tc=O group (figure 1). This class can be further separated in two subsets depending on which molecular arrangement, among the two allowed for a five-coordinated species, is preferred by the symmetrical complex. Five-coordinated complexes can take on either a square pyramidal (sp) or a trigonal bipyramidal (tbp) arrangement Experimental evidence showed that square pyramidal complexes (figure 1a) are exclusively formed when two bidentate ligands having $\pi$-donor atoms are utilized as coordinating sites. In these complexes, the four $\pi$-donors are positioned on the basal plane of the square pyramid, the Tc=O moiety lying above this plane. Conversely, formation of tbp complexes (figure 1b) usually occurs when a combination of two $\pi$-donor and two $\pi$-acceptor atoms is placed around the Tc=O group. Obviously, symmetrical complexes can be obtained only when each bidentate ligand separately carries a set of one $\pi$-donor and one $\pi$-acceptor coordinating atoms. As a general rule, the two $\pi$-acceptor atoms on the two bidentate ligands have the tendency to occupy the two axial positions of the tbp geometry, whereas the two $\pi$-donors span the positions on the equatorial plane along with the nitrido nitrogen atom of the Tc=O group.

Asymmetrical nitrido complexes [4-8] arise when the two $\pi$-acceptor and the two $\pi$-donor atoms are separated in two distinct sets belonging to two different bi-
dentate ligands. In this situation, upon coordination, the TcN core becomes surrounded by two bidentate ligands that are not identical since one carries only π-acceptor atoms and the other only π-donor atoms. Accordingly, the final nitride complex (heterocomplex) has an asymmetrical structure as illustrated figure 2 below.

Figure 2. Common bidentate π-acceptor ligands utilized in the preparation of asymmetrical complexes are heterodiphosphane ligands of the type shown in figure 3 (PNP ligands). Examples of bidentate ligands having S-, S, NH2, and O- as π-donor atoms (XY ligands) are illustrated in figure 4. Asymmetrical nitrido heterocomplexes, therefore, can be represented by the general formula \([99mTc(N)(PNP)(XY)]^{0/+}\) where the neutral or monopositive charge depends on whether monoanionic or dianionic bidentate XY ligands are employed in the preparation.

Figure 3. Figure 4.

Another important Tc-99m core is the monocatio
nic organometallic fragment \([99mTc(CO)3]^+\). It represents a building block particularly suitable for the labelling of biomolecules since it contains three fixed CO ligands bound to a formally Tc+ ion, that constitutes the invariant and stable region of the resulting radiopharmaceuticals. The metallic fragment is completed by the coordination of three weakly bound water molecules, which can be easily exchanged by other chelators for labelling of targeting biomolecules or for the preparation of technetium essential radiopharmaceuticals. The preparation of the intermediated precursor \([99mTc(OH2)3(CO)3]^+\) is not straightforward, but it can be prepared through a kit formulation that is currently available commercially [9-11]. The approach is of broad applicability and many biomolecules have been labelled with the \([99mTc(CO)3]^+\) core. Among these are peptides [12-19], antibodies [18], glucose, CNS receptor ligands and many small molecules [20-24]. The principle of the precursor preparation is given in figure 5 below.

Figure 5.

The advantages and disadvantages of the \([Tc(CO)3]^+\) labelling approach have been discussed in more detail in a controversial article [25,26]. A major advantage is certainly the very wide variety of ligands, which bind very efficiently to the Tc(I) metallic center. Complex stabilities are governed by kinetic stability or inertness. The complexes are all highly robust and, commonly, do not decompose in serum or in vivo. Many different ligands have been exploited and among them are hydrides [27], and cyclopentadienyl [28]. Many combinations of mono- bi- and tridentate ligands are possible, but tridentate chelators turned out to be most versatile [29]. Labelling can be performed at low biomolecule concentration, but heating is always required in order to achieve quantitative labelling. The resulting complexes are usually lipophilic, which could be a disadvantage when attempting to label hydrophilic substrates such as peptides. Despite of this, a few works described the preparation of very hydrophilic Tc-99m tris-carbonyl complexes [19].

Heart Imaging

Myocardial perfusion imaging (MPI) still constitutes a major area of diagnostic nuclear medicine where Tc-99m
cardiac tracers play a fundamental role. Actually, MPI using Tc-99m radiopharmaceuticals remains the most common, non-invasive imaging tool for risk stratification of patients with coronary artery disease (CAD). Recent progress in SPECT technology with the introduction of ultrafast dedicated cardiac scanner paralleled the continuous development of new Tc-99m heart imaging agents having biodistribution properties approaching the requirements for an ideal perfusion tracer. Ultra-fast cardiac SPECT cameras have been created to meet current evolutionary challenges in nuclear cardiology [7,8]. These new devices feature high sensitivity as well as improved spatial, temporal and energy resolution. They enable reduction of acquisition time and fast protocols. Most importantly, they are inherently tomographic imaging characterized by high count rate linearity and, therefore, are potentially capable of dynamic 3-D acquisition. In the near future, increased resolution will be a key factor to allow nuclear cardiology entering the field of molecular imaging. Unlike PET, SPECT is capable of simultaneous multiple isotope imaging and, in principle, a better spatial resolution may ultimately be achieved with SPECT. Critical research targets include imaging of vulnerable plaques at high risk for rupture, left ventricular remodelling, angiogenesis, apoptosis and hypoxia, gene expression, and stem cell therapy. Thus a steady evolution of nuclear cardiology beyond the assessment of myocardial perfusion, towards the characterization of molecular events can be envisaged, thus linking molecular biology science and clinical cardiology.

In the following, a brief overview of recent progress in the discovery of new Tc-99m cardiac agents using the nitrido and tris-carbonyl Tc-99m cores is reported. Symmetrical nitrido Tc-99m complexes have proven to be particularly useful for heart imaging the most important class of cardiac tracers being that of bis(dithiocarbamato) nitrido Tc-99m complexes formed by complexes containing two dithiocarbamate ligands bound to a Tc\(\text{N}\) core [30]. These complexes are neutral and accumulate in myocardium of various animal species and of humans to a different extent depending on the nature of the lateral groups appended to the nitrogen atom of the dithiocarbamate ligand. Extensive clinical studies have been carried out with the complex bis[(N-ethoxy, N-ethyl)dithiocarbamato] nitrido Tc-99m (99mTcN-NOET) (figure 6) [30,31]. 99mTcN-NOET was found to accumulate human myocardium with uptake as high as 4% of the injected activity at 5 min post injection.

This compound showed high first-pass extraction (> 86%) in a canine and isolated rabbit heart models, and a myocardial uptake that correlates with myocardial blood flow over a wide range of flow. According to the observed kinetic behavior, 99mTcN-NOET appears much closer to 201Tl than other technetium complexes. These similarities include high first-pass extraction, good correlation with coronary blood flow, and redistribution. In fact, as observed for 201Tl, experimental studies showed that 99mTcN-NOET has differential clearance from ischemic and normal myocardium resulting in normalization of initial ischemic defect over time. Models of occlusion and reperfusion demonstrate significant redistribution of 99mTc N-NOET within 15 minutes after injection similar to that observed for the neutral lipophilic compound 99mTc Teboroxime.

A relevant feature of symmetrical bis(dithiocarbamato) 99mTc nitrido tracers lies in the possibility to achieve a fine tuning of their pharmacokinetics properties by simply modifying the chemical nature of the lateral groups bound to the dithiocarbamate \(\text{[N−C(=S)S]}\) moiety. This allowed obtaining a series of perfusion tracers showing a variable residence time in myocardial tissue ranging from a few minutes to hours. For instance, the three derivatives represented in figure 7a only differ in their lateral groups, but their heart washouts are dramatically different. In figure 7b, the time-variation of heart uptake in monkeys for these compounds is reported in comparison with 201Tl+, 99mTc-MIBI and 99mTcN-NOET.
The first-pass extractions of these tracers were comparable to that of $^{201}$Tl$^+$ and $^{99m}$TcNOET, as well as the high initial cardiac accumulation. However, after 20 min post injection, approximately 50% of the initial activity was washed out. A representative example of planar images recorded in baboons at different times [5 min (a) and 25 min (b)] for the complex $^{99m}$Tc-TMIP is reported in figure 8.

![Figure 8](image1.png)

The high first-pass extraction combined with a fairly linear relationship between uptake and flow suggest that this class of myocardial tracers could be particularly useful when used in combination with the new dedicated ultrafast cardiac scanners able to collect high-quality SPECT images in less than 2 minutes.

Replacement of one dithiocarbamato ligand with an heterodiphosphane ligand PN(R)P led to the formation of asymmetrical species containing two different bidentate ligands bound to the same Tc-N group. This change yielded dramatic consequences on the observed cardiac uptake. The resulting monocationic nitrido Tc(V) heterocomplexes, $\text{[Tc(N)(PN(R)P)[R(R')N-CS$_2$]]}^+$, constitute a novel class of myocardial tracers exhibiting superior imaging qualities was obtained [11, 41]. In particular, using the heterodiphosphane ligand bis[(dimethoxypropylphosphanyl)ethyl]ethoxyethyamine (PNP5) a novel class of myocardial tracers exhibiting superior imaging qualities was obtained [47, 48]. Within this class, the derivative $^{99m}$Tc(N)(PNP5)(DBODC) $^{99m}$TcN-DBODC (DBODC = diethoxyethylthiocarbamato, PNP5 = bis[(dimethoxypropylphosphanyl)ethyl]ethoxyethyamine) has recently raised much interest due to its unprecedented imaging properties. The structure of this complex is reported in figure 9.

![Figure 9](image2.png)

In figure 10a, whole-body image of a rat obtained at 30 min after administration of the tracer is reported. It clearly shows a high accumulation of activity in the myocardium, but with negligible uptake in background tissues, particularly in lungs and liver. As a result, heart region appears very well delineated allowing the acquisition of SPECT images of superior quality. The difference in the liver washout kinetic between the new tracer and $^{99m}$Tc-Sestamibi is clearly evidenced in figure 10b, which reports rat planar whole-body image for $^{99m}$Tc-Sestamibi collected at the same time.

![Figure 10](image3.png)

Biodistribution data of $^{99m}$TcN-DBODC in rats [49–51] showed that the heart is the main target organ and liver activity is rapidly washed out into the intestine leaving the cardiac region completely free of background activity. This result appeared particularly evident when time variation of heart/liver ratios is considered in comparison with $^{99m}$Tc-sestamibi and $^{99m}$Tc-tetrofosmin (figure 11). Heart/liver ratio of $^{99m}$TcN-DBODC rises dramatically over time whereas those of the other two agents remain almost constant.

![Figure 11](image4.png)

First-pass extraction fraction of $^{99m}$TcN-DBODC, as determined in dogs [52], was found to be comparable to $^{99m}$Tc-sestamibi and $^{99m}$Tc-tetrofosmin. The new tracer has been tested in healthy human volunteers and results confirmed the favorable kinetic behavior and fast
and quantitative liver washout as early as 30 min post injection [53].

Preliminary biological evaluation of $^{99m}\text{Tc-N-MPO}$ in rats [54, 55] showed a high initial heart uptake with long myocardial retention. The heart uptake of $^{99m}\text{Tc-N-MPO}$ was between that of $^{99m}\text{Tc-sestamibi}$ and that of $^{99m}\text{Tc-DBODC}$. Clearance from the liver and lungs was rapid, resulting in high heart–liver and heart–lung ratios.

In the family of Tc-99m tris-carbonyl complexes, recently a new promising heart imaging agent has been described [56, 57]. The complex $\text{fac-}[^{99m}\text{Tc}(\text{CO})_3\{\text{HC}[3,4,5-(\text{CH}_3\text{OCH}_2)\text{pz}]_3\}]^+$ containing a functionalized tris(pyrazolyl) methane chelators with methoxymethyl groups at the azole rings (figure 14) exhibits a significantly high and stable heart uptake associated with very fast liver clearance.

Cancer imaging

Somatostatin receptors (SSTs) are integral membrane glycoproteins that are distributed in a variety of tissues throughout the body [22]. They have multiple physiological functions, including an inhibition of secretion of a growth hormone, glucagon, insulin, gastrin and other human peptide hormones. In addition, somatostatin plays an inhibitory role in the immune system and acts as a neuromodulatory peptide in the central nervous system. Alterations of somatostatin receptor expression during disease, such as overexpression in many tumours can be exploited by imaging techniques. Receptor-positive tumours may be originated in the neuroendocrine system, such as pituitary adenomas, gastroenteropancreatic tumours, and pancreatic islet-cell tumours, but other tumours, such as lymphomas and breast cancer, may possess these receptors as well. Of the six SST subtypes, SST1, SST2A, SST2B, SST3, SST4, and SST5, SST2A is the most important one because of its high overexpression in malignant tumours and its high affinity for the clinically available somatostatin analogues.

The first successful Tc-99m radiopharmaceutical targeting SST receptors that has been introduced into the clinical use was P829 (Depreotide, NeoSPECT) [58]. It is based on the oxo-Tc-99m core coordinated to a $\text{N}_2\text{S}$ monothiol-bisamide-monoamine ligand bearing an additional lysine residue. In patients, it has proven suc-
cessful in imaging solitary pulmonary nodes but failed in gastrointestinal tumours. Further improvement of the peptide (99mTc-P2045) resulted in a dramatic increase in tumour uptake with improved biodistribution pattern suitable for 188/186Re labelling for therapeutic applications [59].

HYNIC (6-hydrazinonicotinamide) is of particular interest due to its high 99mTc-labeling efficiency (rapid and high yield radiolabeling), the high solution stability of its 99mTc complexes, and the easy use of coligands for modification of biodistribution characteristic of the 99mTc-labeled small biomolecules [60]. HYNIC-conjugated octreotide analogues have been proven to be particularly suitable for 99mTc labelling [61]. Among them, [HYNIC-DPhe¹,Tyr³]-octreotide (figure 15) was proved to be superior in patients compared to ¹¹¹In-DTPA octreotide. Especially when EDDA (ethylenediaminodiacetic acid) was used as the coligand, the resulting 99mTc-labeled peptide becomes highly stable and hydrophilic. So far 99mTc-EDDA/HYNIC-TOC can be considered the most widely used 99mTc labelled octreotide derivative [62].

Tumors produce many angiogenic factors, which are able to activate endothelial cells in the established blood vessels and induce endothelial proliferation, migration, and new vessel formation (angiogenesis). The angiogenic process is regulated by cell adhesion receptors. Integrins are a family of proteins that facilitate cellular adhesion and migration on the extracellular matrix proteins found in intercellular spaces and basement membranes, and regulate cellular entry and withdraw from the cell cycle [63]. Integrin γβ3 serves as a receptor for the extracellular matrix proteins, including vitronectin, fibronectin, fibrinogen, lamin, collagen, Von Willibrand’s factor, osteopontin and adenovirus particles, with the exposed arginine-glycine-aspartic (RGD) tripeptide sequence [64,65]. Integrin γβ3 is expressed at low levels on epithelial cells and mature endothelial cells, but it is highly expressed on the activated endothelial cells in the neovascularisation of tumors. The highly restricted expression of integrin γβ3 during tumor growth, invasion and metastasis present an interesting molecular target for early diagnosis of rapidly growing and metastatic tumors. During the last several years, a number of radiolabeled RGD peptides have been evaluated as integrin γβ3-targeted radiotracers by single photon emission computed tomography (SPECT) or positron emission tomography (PET) in pre-clinical animal models and human clinical trials.

Cyclic RGD peptides, such as c(RGDfK) and E[c(RGDfK)], have been used to develop Tc-99m radiotracers for imaging integrin γβ3 expression in tumors [66,67]. The results from biodistribution studies showed that [99mTc(HYNIC-E[c(RGDfK)])(tricine)(TPPTS)] (RP593) (figure 16) has the best biodistribution characteristics in terms of tumor uptake, tumor/liver and tumor/lung ratios. RP593 showed much higher tumor uptake and longer retention than [99mTc(HYNIC-c(RGDfK))(tricine)(TPPTS)] (RP582). The tumor uptake of RP593 can be blocked by co-injection of excess c(RGDfV), suggesting that its tumor localization is indeed due to the integrin γβ3 binding. RP593 and RP582 were also evaluated in the female Balb/c mice with subcutaneously growing OVCAR-3 ovarian carcinoma xenografts [68,69]. The tumor uptake of RP593 was significantly higher than that of RP582 at different time points. These results strongly suggest that cyclic RGD dimer E[c(RGDfK)] [70] has a significant advantage over its monomer counterpart with respect to the tumor uptake and retention of their 99mTc radiotracers.

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