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NETs: FROM DIAGNOSIS TO THERAPY – A THERAGNOSTIC APPROACH: PART I

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Abstract

Diagnostics and therapy in neuroendocrine tumors (NETs) has been heavily influenced by nuclear medicine progresses, both from a diagnostic and therapeutic perspective. The introduction of somatostatin-based peptides, labeled to radionuclides (constituting radiopharmaceuticals), such as the current golden standard 68Ga-DOTA-SSAs (SomatoStatin Analogues), has revolutionized the approach to these neoplasms. Peptides-based drugs in NENs' management have been introduced with OctreoScan® (111In-DTPA-octreotide) in combination with scintigraphy, but today techniques have deeply evolved, allowing better performance together with higher accuracy and sensitivity, in addition to image quality improvements (also in terms of details and anatomical data), especially thanks to the implementation of hybrid techniques, such as SPECT/CT and PET/CT. 68Ga-based radiotracers are not the only diagnostic option in NENs, because of the existence of several tumors (such as insulinomas or malignant neoplasms) that do not express SSAs' target molecules, thus the utilization of other radiocompunds is needed, such as 18F-FDG.

Keywords: *Neuroendocrine tumors, somatostatin receptors, peptides, theragnostics, octreotide*

Introduction

A key role in diagnosis and therapy of neuroendocrine tumors is molecular targeting specific membrane receptors: in this way, it is possible to identify and make use of overexpressed receptors on the surface of cell membrane, representing efficient targets for radiopharmaceuticals involved in different clinical steps, from diagnosis to therapy. (1-7) This is the pivotal concept which the *theragnostic* is based on. (8)

Let's introduce this term, often not so familiar even among healthcare professionals, although we are talking about a concept dated back to the Forties, but only nowadays, more precisely in the last decades, it is experiencing a renovated interest from several clinical fields. The word "*Theragnostic* "derives from the fusion of two other terms: "diagnosis" and "therapy". (9)

The main goal of theragnostic is finding a procedure that can work, firstly, as a diagnostic procedure, literally revealing where the specific targeted cells are located, then as a therapeutic strategy, binding the same previous target but this time carrying another radionuclide which can damage cancer cells and cause their death using different radiopharmaceuticals labelled to different radionuclides, each one with a specific characteristic/function. This way, diverse drugs can act for different purposes, even maintaining the same molecular target.

In other words, we can synthetize theragnostics in a simple *mantra*: "See what you treat and treat what you see". (10)

The first step is preparing the patient to receive the drug. After the radiotracer has been administrated by an intravenous injection and it distributes among the organism, the main event is the subsequent bond to targeted receptors (for which the radiotracer itself is designed for), then the internalization of the radiopharmaceutical-receptor complex takes place, realizing an endocytosis process, bringing the aforementioned complex into the cytoplasm of the targeted cell.

The retained drug will execute its effect inside the neoplastic cell. (11)

Depending on the radionuclide labelled to the tracer, the radiopharmaceutical can act as a diagnostic tool, making possible staging the patient. The technique, basing on its sensitivity and specificity, can localize both primary and metastatic lesions. (12)

On the other hand, there are available radiopharmaceuticals that have a therapeutic role in the management of advanced/inoperable NETs, because of the properties of certain radionuclides and their toxic effects on targeted cells. This is possible thanks to the intrinsic damaging action of the radionuclide labelled to the tracer. This is why PRRT (peptide receptor radionuclide therapy) is so powerful, effective and promising in this oncologic field. (13)

In addition, this is nothing but one of the possible future application of this theragnostic approach: several studies show how radiopharmaceuticals, PRRT and functional imaging could become far more applicable techniques, especially in a broader oncologic field and also in benign diseases. (14) As it will be better discussed later, studies show how not only neoplastic cells, but also inflammatory cells present on their surface some useful membrane receptors, some of these ones already studied in tumors like NETs, such as SSTR (Somatostatin Receptors), which are a good example of this concept. (15)

NENs AND IMAGING: A NUCLEAR MEDICINE RELATED STORY

Since the beginning, neuroendocrine neoplasms (NENs) have represented both a

diagnostic and therapeutic challenge. They are extremely various, spreading virtually everywhere in the organism. They can have very different behaviors, with subsequent very different prognoses, from a case to another, depending on their grade of differentiation. At a certain point of their natural history, NENs can show their presence: in some cases, they can clinically rise as an endocrine syndrome (functional NENs), depending on the hormone they are overproducing; let's think to all functional P-NETs, such as gastrinoma, insulinoma, glucagonoma or VIPoma, otherwise pheochromocytoma in adrenal glands, that can secrete catecholamines into the blood stream. In other cases, they show their presence only in advanced states, when metastases are already diffused in the organism at this point, since their silent and slow growth has not given any sign of their presence till that moment: these are non-functional NENs. (16)

When molecular receptors studies began, the approach to this type of disease changed forever. Thanks to this discovery, we are able to design specific radiopharmaceuticals that take advantage of this characteristic. But the reality is not as simple as that and the main reason lies in the vast heterogeneity of neuroendocrine tumors. (17)

The main discovery from a biological point of view was the identification and subclassification of SSTRs (Somatostatin Receptors), in five different subtypes (SSTR-1 to SSTR-5). The great news was that the majority of NENs overexpress SSTRs, especially SSTR-2. (18-21)

This knowledge could not be employed better: thanks to important efforts in research, it was possible to develop specific peptides that act as the physiological ligand of somatostatin receptor, making possible the bond between this molecule and the receptor itself. They were called SSAs (SomatoStatin Analogues) since their action could mimic somatostatin's one: they started to be used in clinical practice to control symptoms and also the growth of the tumor.

Then, the brilliant intuition: we had peptides that could bind a specific type of receptors, after being administered to the patient, travelling freely into the blood stream, reaching neoplastic cells that overexpressed exactly that receptor.

Why not "marking" these peptides? In this way we would have the chance to "follow" them. (22)

Did just exist a way to make them "shine" in order to communicate the precise position of certain cells with a specific and known characteristic? Additionally, was it possible to condensate all these data in just one clear and objective image?

The idea of creating a peptide (able to bind a specific receptor) labelled to a radionuclide (simply, a radioactive element, which spreads energy detectable by an appropriate machinery) started a totally new approach firstly in diagnosis, then in therapy, since from this foundation would was born the conception of peptide receptor radionuclide therapy (PRRT). (23)

So, there were in this way solved the diagnostic and therapeutic problems in NENs in generally? Unfortunately, the answer was not positive. In fact, the discovery that NENs don't always overexpress somatostatin receptors, mainly depending on their grade of differentiation, was an addition of a new level of complexity, because the higher the grading (tending to G3, so we are talking about poorly differentiated neoplasms), the lower is the density of SSTRs present on the membrane

surface of neoplastic cells: this condition is known as the "*escape phenomenon*", that will be discussed later, and is typical also in insulinoma (a benign functional NEN). In synthesis, using radiolabeled SSAs to localize tumor not expressing the right receptor (SSTRs) is not efficient, so new strategies were needed. (24)

Even if today nuclear medicine techniques represent an essential instrument in studying NENs, CT and, secondarily, MRI still cover an important role in NENs imaging. They remain first level procedures, in occasion of the first approach to the patient, allowing diagnosis of primary tumor (if detectable) and staging the patient. (25)

MSCT (spiral Multi-Slice Computed Tomography) is an established procedure in this context, thanks also to the use of a iodinated contrast media, realizing a three-phase technique able to assess the vascularization of the primary lesion and, eventually, metastases, localizing them among the organism. The sensitivity of this techniques reaches more than 80% in detecting primary lesion, while it stays around 50% in metastases research. (26)

Magnetic resonance Imaging (MRI), instead, is usually a second choice. It is employed in cases of counterindications of CT or its iodinated contrast media, or in specific condition where the absence of ionizing radiations is preferrable, like in younger subjects or in long-term surveillance. An added value is the higher capability of MRI (in respect to MSCT) in detecting and differentiating metastases in bones and liver. (27)

Overall, CT and MRI, even if they are capable to identify a generic "lesion", they are put in trouble especially in front of a non-functional and hypovascularized tumor: in this scenario, a drop in specificity can be easily observed. (28)

These techniques are just not able to provide more specific information about it, in particular they can only show morphostructural data, while functional ones are not supplied: this is, instead, the strength of nuclear medicine, because employing different radiopharmaceuticals, physicians are allowed to study in depth the lesions. This, today, is not considerable as marginal, because this type of information is firmly related to therapy and prognosis. (29)

Nuclear medicine procedures have the ability to deeply characterize the lesions, both primary and (eventual) metastatic lesions, providing functional aspects and/or metabolic processes on a molecular level: a great strength of this capability is to recognize a neuroendocrine neoplastic lesion even before anatomical changes happen. (30-35)

Nowadays, NENs require a multidisciplinary diagnostic and therapeutic approach. Nuclear medicine plays a key role in this context thanks to its non-invasive procedures, aimed to study functional/molecular information: this allows an earlier diagnosis, even when traditional tomographic techniques cannot detect smaller lesions, in addition to the best therapeutic strategies, improving significantly the prognosis (also thanks to a more precise stratification of the patient). (36). The implementation of hybrid techniques has allowed the encounter between traditional radiology and nuclear medicine (such as PET/CT or SPECT/CT), defining revolutionary changes, with an important improvement in diagnostic accuracy: in fact, the fusion of both morphostructural and functional/metabolic data results in complete and very useful images. (37-39)

In this paper, we are going to describe older and newer techniques adopted to approach

these neoplasms. We will focus on peptides and radionuclides that allowed deep changes in NENs' medical approach. From octreotide to the latest DOTA-peptides and even newer molecules, we will dive into the nuclear medicine techniques panorama, but first let's define an important concept, that is at the base of this entire treatise: the structure of a radiopharmaceutical.

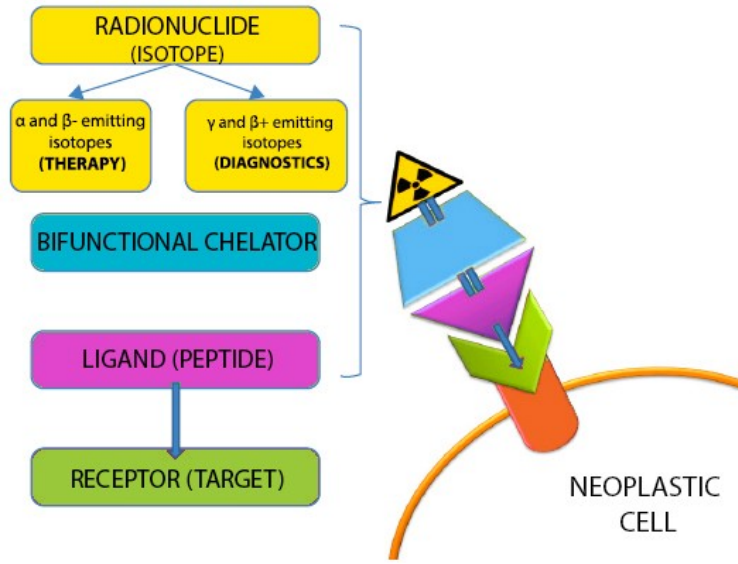
MEDICAL NUCLEAR IMAGING

Nuclear medicine (NM, as a comfortable abbreviation) is so called because it utilizes a different sort of "contrast medium" (radionuclides and radiopharmaceuticals) in respect to traditional radiology, where the latter is founded on a more "morphologic" point of view of the organism, while NM is more affiliated to physiology, making use of biological and physiopathological aspects of disease and ordinary processes, so applied to diagnosis and even therapy, realizing something of deep interest like PRRT, radiometabolic therapy or radiotherapy/brachytherapy. (40)

From an image quality perspective, traditional radiology has available machineries with a far higher power of resolution, resulting in more detailed images, but here is the advantage of NM: even if a lesion dimension is below the power of resolution of traditional radiology devices, it is visible anyway, because the accumulation of radiopharmaceutical allows the emission of a signal that is captured and interpretated, showing even the smallest lesion that would have passed unnoticed, instead, if it was only utilized tradition radiology. (41-42)

Speaking of NENs, the most important diagnostic instruments utilized in nuclear medicine are scintigraphy (often it is indicated as "SRS", meaning "Somatostatin Receptor Scintigraphy"), SPECT and PET, taking advantage of different radionuclides depending on the purpose. Moreover, as aforementioned radionuclides are involved also in therapies. Therefore, in synthesis: β^+ or γ-emitters radionuclides find a diagnostic employ, while β^- or α-emitters are utilized as therapeutic strategies, such as PRRT (Peptide Receptor Radionuclides Therapy), radiotherapy/brachytherapy and radiometabolic therapy.

"ANATOMY" OF A RADIOPHARMACEUTICAL



What is exactly a radiopharmaceutical?

Essentially, it is a drug that contains a radiation emitting component, with a very specific bond capability toward a certain molecular target. (43)

From a structural point of view, it is constituted by:

1. a **binding molecule**: this is the component, usually a peptide, that functions as a ligand for a specific

membrane receptor; in this way, the drug is "addressable" to very specific targeted molecules, guaranteeing that and only that receptor binding event;

2. a **radionuclide**: simply, the radiation emitting component. It can deeply vary according the precise purpose of the drug, because different radionuclides/isotopes allow different diagnostic or therapeutic results: in particular, α- or β^--emitters (like ^{68}Ga) are used in diagnostic imaging, while γ- or β^+-emitters (such as ^{177}Lu) report great outcomes in therapeutic applications.
3. A **linking molecule**: also definable as "Bi-Functional Chelator" (BFC, "chelator" derives from the Greek word "*χηλή*, chēlē", meaning "*claw*", because it "captures" the molecule as a crab would do with its prey), which has the main purpose of linking together both the radionuclide and the peptide (using two different functional groups, one for each component, giving reason to the term "bifunctional"), without substantially altering the pharmacokinetics and safety profile of the drug in its complex; mainly, its functions are essentially two: firstly, it necessarily has not to modify biological properties and receptor affinity of the peptide itself (since it would not be as efficient in binding its molecular target); secondly, it should be resistant to radiolysis, since this phenomenon would irreversibly damage the radiopharmaceutical, making it unusable.
   Important note: there is no "universal" BFC that can function or excel for every purpose and in every situation, so each one has to be tailored to radionuclide characteristics and to the role it has to cover. Examples of BFCs are DTPA (pentetic acid or DiethyleneTriaminePentaacetic Acid) and DOTA (tetraxetan or DOdecane Tetraacetic Acid).
4. A **spacer**: nevertheless, there is always a consequence from a chemical point of view by using a BFC, so it is possible to use a "spacer" to further influence the kinetics of the newly synthetized compound to make it as whished, essentially another molecule that allows fine-tuning chemical aspects of the radiopharmaceutical. Although, its involvement is not mandatory, so it can be absent in the composition of a radiopharmaceutical.

In conclusion, here we report a practical example of what mentioned above. Let's consider ^{111}*In-DTPA-octreotide*, a well-known radiopharmaceutical largely used in a technique called OctreoScan®, and let's analyze it:

- ^{111}In: this is the radionuclide, in particular it has a diagnostic purpose;
- DOTA: this is the bifunctional chelator (BFC), that is able to bind at the same time both the radionuclide and the peptide;
- octreotide: finally, this is peptide itself, capable of the bond with the membrane receptor (SSTR in this specific case).

NENs IMAGING: CURRENT APPROACHES

SOMATOSTATIN AND SSTRs OVERVIEW

When it was discovered the characteristic overexpression of somatostatin receptors (SSTRs) in NENs, the related nuclear imaging underwent to a complete revolution. Thanks to this knowledge, new procedures were

elaborated, conducing to SRS (Somatostatin Receptor Scintigraphy), SPECT, PET and, later, hybrid techniques (SPECT/CT and PET/CT), with every time better results and higher accuracy in depicting lesions. (45)

SSTRs belong to a particular class of membrane receptors, called GPCRs (G Protein Coupled Receptors), showing 7 transmembrane domains, with an extracellular extremity and also an intracellular one; the first component makes the bond with the ligand possible (in this case the somatostatin or a SSA), whereas the other extremity allows the interaction with G protein: this little enzyme has an intrinsic GTPase activity, showing the peculiarity of being activated only when the coupled receptor changes its conformation (an event that occurs when the ligand interacts with it), then it binds a GTP molecules and starts to function; only when the automatic GTPase activity occurs, the protein degrades GTP into GDP and a phosphate group, interrupting its functioning. (46)

Somatostatin receptors have been distinguished in five different subtypes, each one with some biological differences. Not all of them are necessarily overexpressed on a NET cell, complicating the employment of somatostatin analogues, especially because not all the latter have the same affinity for each subtype. SSTRs' subtypes are identified as SSTR-1, SSTR-2, SSTR-3, SSTR-4 and SSTR-5, but biological studies on NENs have shown the prevalent overexpression of SSTR-2 and SSTR-5 subtypes in the majority of cases. With the effort of researchers, several SSAs and techniques have been elaborated to obtain the best result nowadays possible, and this behavior continues to go on, improving time after time.

So, the introduction of radiolabeled SSAs now rules in NET's imaging scenario. But there is a question that could arise spontaneously: why not directly using a radiolabeled version of somatostatin? It was really necessary designing somatostatin analogues?

The answer lies into the half-life of somatostatin itself: estimated at about 1-3 minutes, it is absolutely not suitable for diagnostic (or even therapeutic) purposes. This phenomenon is related to the presence of plasmatic enzymes dedicated to somatostatin degradation (hydrolytic enzymes).

We could simply think that SSAs have a far longer half-life, in addition to our capability of completely redesign them with specific pharmacokinetics properties and also control their receptor affinity, making them a far more versatile choice. (47)

SSTRs AND NUCLEAR MEDICINE: A WINNING COMBINATION

Somatostatin Receptor Scintigraphy (SRS) has been the first nuclear imaging technique applying SSAs, octreotide in particular, utilizing OctreoScan® (^{111}In-Pentetreotide) as radiotracer. Later, the same drug would have been employed also in SPECT imaging, with higher quality and accuracy images than SRS.

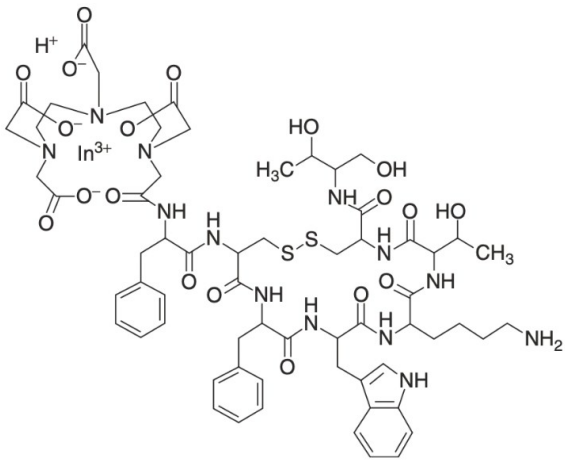


Figura 1 OctreoScan® (OCT or ^{111}In-Pentetreotide or ^{111}In-DTPA-octreotide) chemical structure

After intravenous administration of the radiopharmaceutical, both planar (scintigraphic) and tomographic (SPECT) scans are acquired with a gamma camera, usually 24 and 48 hours after the injection of the radiotracer.

^{111}In-Pentetreotide displays selective affinity in respect of SSTR-2 and SSTR-5, the two most commonly overexpressed receptors in NENs, but it is important to consider that there are also other non-neoplastic sites of collection of the tracer itself, that can potentially represent pitfalls. In particular, physiological retention of the radiopharmaceutical happens in thyroid, spleen, live, kidneys and in the pituitary gland, in addition to other organs that are depicted after a certain period of time: these are the renal collecting system, urinary bladder, gallbladder and bowel. This regard, the particular composition of the tracer can deeply change its pharmacokinetic: as an example, when octreotide (the first SSA largely employed in NETs management) is employed as ^{123}I-Tyr-octreotide, its excretion is mainly related to liver, causing a collection of radiotracer into the bowel, making difficult the study of abdominal masses; this drawback can be solved acquiring an early scan (2-4 hours after drug administration) or with the employment of SPECT, making possible to evaluate without excessive problems also the abdomen and the pelvis districts: in this way it is possible to detect also small pancreatic NETs, even if, sometimes, a false negative results could occur, especially when facing a NEN smaller than 1 cm. After all, labelling octreotide to ^{111}In and DTPA (forming ^{111}In-DTPA-octreotide or ^{111}In-Penteotride), the tracer shows a prevalent renal excretion, solving the previous problem. (48)

The most important parameters in the efficacy of these technique are represented by the density and the subtype of receptor expressed by neoplastic cells: if they do not express enough receptor or the expected subtypes, they could cause troubles. The nuclear imaging, in this way, represents also a technique that allows a functional and biological study of the neoplasia, both considering the primary lesions and eventual metastases. It can happen, due to their heterogeneity, that the primary lesion differs in grading in respect to secondary ones: so, if the primary lesion expresses the receptors, but metastases do not, the latter will not be depicted in first place. This finding is known as "escape phenomenon" (mainly referrable to dedifferentiation some lesions can manifest), because lesions not expressing the appropriate amount or subtype of receptors simply cannot be recognized in imaging; typically, this condition negatively influences the prognosis, due to the underestimation of disease extension. (49)

The most important and relevant studies about SRS and SPECT with octreotide

radiopharmaceuticals have always shown good results in the accuracy of this technique, reporting a sensitivity up to 80% for primary lesions and 90% for secondary lesions in gastroenteropancreatic neuroendocrine tumors (GEP-NETs), but there is an important presupposition which these papers are based on: since from the beginning, all the patients examined already had a high probability of a NEN diagnosis, so it is possible to consider that, from a certain perspective, there was a sort of a bias in this evaluation. But what about the results of SRS and SPECT with octreotide in patients with an intermediate-high risk of disease? The answer is, unfortunately, a lower sensitivity detecting primary lesions.

Later, Hillel et al. decided to study a new group of NET patients with a different technique: SPECT/CT with OctreoScan®. The idea is to fuse morphostructural and functional imaging, resulting in a more complete result in terms of data, combining the best of two worlds in a single procedure. The outcomes were very promising, showing unknown tumors in 7 of 11 patients, in addition to a different tumor site (in respect the localization provided by planar imaging) in 4 of 11 patients. These findings further reinforced the improvement provided by hybrid techniques in comparison to SRS or SPECT only investigations. According to this, the next evolution would have been the employ of PET, especially combined with CT to provide far better and more accurate image data. (50)

As previously enlightened, the density and the subtype of receptors expressed on neoplastic cell surface is determining the outcome of nuclear imaging procedures. So, what happens in very specific cases, like the insulinoma?

This functional NEN has a pretty peculiar characteristic: although it usually is a benign tumor, it expresses far less SSTRs than other NENs (SSTR-2 density is particularly low). So, techniques based on SSAs are quite useless, because they will simply not work appropriately: results shown by several studies report a very low sensitivity of up to 50%, further worsened by their typical small dimension, usually under 1 cm.

Encouraging outcomes are provided by ^{18}F-FDG and, even more, ^{18}F-DOPA PET with carbidopa premedication, based on the mechanism of concentrations in areas of increased catecholamines metabolism, not influenced by SSTRs expression. (51)

PET

Although SRS and SPECT provided significant results in NETs' nuclear imaging, it is undeniable that some (sometimes important) limits are typical of these techniques. For this reason, several efforts have been done with PET, especially about the introduction of new peptides and radionuclides, that showed very promising results, with the improvement of imaging accuracy and the capability of a biological and functional assessment of the neoplasia (both about the primary and secondary lesions).

PET (Positron Emission Tomography) uses specific β^+ emitting radiopharmaceuticals developed to improve the quality and accuracy of imaging, especially because since the beginning of its employment it made possible to obtain more conspicuous information on the disease itself, functioning as a complementary investigation next to SRS and SPECT. (52)

Later, with the introduction of new SSAs (specifically designed with PET in mind), the improvements have been very remarkable, in fact the better sensitivity and resolution distinctive of this technique encouraged a renovated interest. (53)

^{18}F-FDG (Fluorine-18 Fluorodeoxyglucose) was the first radiotracer involved in this approach, but unfortunately it provided inadequate results: in fact, due to the usually well-differentiated anatomopathological aspect of NENs, these tumors did not depict adequately, being low detectable by the machinery. This condition is referrable to their only slightly increased metabolic activity, in contrast to those neoplasms that, instead, would goodly be displayed because of their aggressive behavior: the rationale of using ^{18}F-FDG is that a site with aggressive neoplastic cells has an increased metabolic activity, due to the high rate of mitotic activity and fast growing attitude of the lesion, so a higher amount of glucose is needed to these cells to support such a high rhythm of replication (expression of an increased glycolytic activity); the idea is to "send" a certain quantity of radiomarked glucose to these neoplastic cells that internalize and collect it, then localize them through a machinery that interprets the provenience of the γ-rays, assigning to this signal a position in the organism, appearing as bright areas in the image. (54-55)

Regarding NENs, only high-graded tumors are able to highly internalize ^{18}F-FDG, but, as we mentioned earlier, NETs are typically well-differentiated tumors, so this tracer is not properly indicated for this purpose.

That said, ^{18}F-FDG PET is useful in those undifferentiated NENs that do not depict with SSAs, because of the SSTRs' expression lack on their membrane surface, representing a good prognostic instrument allowing a biological and functional study of the neoplasm. (56)

Therefore, unsatisfactory outcomes provided by ^{18}F-FDG PET with well-differentiated NENs encouraged the development of new SSAs, in particular those that today we call "DOTA-peptides", usually radiolabeled with ^{68}Ga (Gallium-68, the most promising radionuclide both in diagnosis approach with NENs, today considered as a gold standard in this context). (57)

DOTA-peptides provided since the beginning very promising results, stimulating further studies and improvements of this kind of radiopharmaceuticals, thanks to the higher accuracy and sensitivity of this technique, even compared to SPECT/CT and, especially, SRS.

In conclusion, it is important to remark that, even if ^{18}F-FDG is the most used radiotracer in oncology and it does not always provide sufficient results in NETs, it has still indications in higher graded and undifferentiated neuroendocrine neoplasms.

^{18}F-DOPA is another radiotracer employed in NENs nuclear imaging, this time based on a completely different metabolic pathway: it is an aromatic amino acid radiomarked with fluorine isotope, initially proposed for dopamine receptors imaging, many years ago.

Considering its mechanism, it has been suggested in the assessment of NETs, since these cells are part of the so-called APUD system (Amine Precursor Uptake and Decarboxylation), that are able to internalize this kind of molecules. ^{18}F-DOPA, in terms of pharmacodynamics, is similar to **^{123}I-MIBG**, (58) because both are tracers of catecholamine metabolism. The latter has been largely employed in pheochromocytoma, neuroblastoma and paraganglioma, because of high caption activity of these neoplasms regarding a catecholamine radiotracer like this, but also ^{18}F-DOPA is finding a clinical application (especially in paraganglioma and insulinoma, because the latter has a lower affinity to SSAs due to its low expression of SSTRs, in particular SSTR-2). ^{123}I-MIBG is also employed together with ^{131}I-MIBG in the theragnostic approach of neuroblastoma, that is to say the first radionuclide is better

indicated for a diagnostic purpose, while the second one is a therapeutic strategy.

^{11}C-HTP (^{11}C-hydroxytryptophan) is a radiotracer involved in serotonin-producing tumors, with a high affinity 5-HT receptors (5-hydroxytriptamine receptors). It has demonstrated good results in tumors overexpressing this type of membrane receptors. The major downside is the short half-life of ^{11}C, requiring an on-site cyclotron to guarantee its production, but this a privilege reserved only to few academic centers: in synthesis, the entire process of production of this radionuclide is too difficult and expensive to become a largely employed technique, even if it provides a good tumor-to-background ratio, that becomes even better if the patient is treated with a carbidopa premedication. (59)

With further studies and evidence provided, it has been demonstrated that these radiotracers (^{18}F-FDG, ^{18}F-DOPA, ^{123}I-MIBG, ^{11}C-HTP) report inferior results compared to DOTA-peptides labelled with ^{68}Ga: it is not a coincidence, in fact, that these radiopharmaceuticals have become the current gold standard in diagnosis of well-differentiated NENs provided with SSTRs (that represent, as aforementioned, the vast majority of cases of neuroendocrine tumors).

Let's dive more in depth into this topic, describing the choice of ^{68}Ga and DOTA-peptides.

^{68}Ga-DOTA-PEPTIDES

^{68}Ga provides a far higher *in vivo* radiochemical stability of chelates in comparison with radiohalogenated ones: the reason of this behavior is related to a physiological de-iodinating and de-fluorinating activity in the organism, that results in the release of the radionuclides, complicating pharmacokinetic analysis.

The productive process of ^{68}Ga offers some undeniable advantages: it is granted by ^{68}Ge/^{68}Ga generators, without the need of onsite cyclotrons, and it is even more convenient considering that these machineries are becoming more and more available on the market, making possible a rapid spread of radiopharmaceuticals involved in PET imaging, especially in Europe. ^{68}Ge (Germanium-68, the parent radionuclide of ^{68}Ga) has a half-life of 270 days, allowing the use of the generator for 9-12 months or even a longer period of time. Due to the 68-minutes half-life of ^{68}Ga, it is possible to manipulate it for an extended period of time, in addition to the possibility of binding it to many peptides and other small molecules.

^{68}Ga is a positron-emitting radionuclide ideal for PET imaging purposes, thanks to its 89% positron emission and not relevant 3.2% of γ-ray emission. (60)

DOTA-peptides are SSAs (Somatostatin Analogues) that are constituted by a peptide (acting as a ligand for SSTRs, mimicking native somatostatin) and labelled to a radionuclide using a BFC (bifunctional chelator, a molecule that is able to bind simultaneously the peptide and a radionuclide), represented by DOTA (1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid). In conclusion, these compounds are called "DOTA-peptides" and represent the current gold standard in PET imaging regarding neuroendocrine tumors that show somatostatin receptors on their cellular surface.

Historically speaking, ^{68}Ga-DOTA-peptides gained a key role in NEN imaging thanks to their high affinity to overexpressed somatostatin receptors on neoplastic cells, especially regarding SSTR-2 (presented by 70-90% of NETs) and SSTR-1 and SSTR-5, even if

the latter have been described in minor percentage of cases. (61)

The main characteristic and advantage of these compound is that their pharmacokinetics is not influenced by the chelated radionuclide itself, making them particularly versatile in a theragnostic approach, because they can be eligible for both diagnostic and therapeutic purposes simply switching the radionuclide they bind.

According to several studies, DOTA-peptides showed similarities with OctreoScan® (or OCT) and peptides labeled with β^- emitting isotopes (such as ^{90}Y and ^{177}Lu, emitting corpuscular radiations involved in radionuclide therapy): in this way, since a neoplastic lesion captures the radiotracer either in OctreoScan® PET or in DOTA-peptides PET, it means that the neoplasm expresses receptors suitable also for a therapeutic strategy, making these techniques useful prognostic assessment instruments. As a consequential point of view, a further employment is possible with the use of DOTA-peptides as a prior evaluation to enlighten a predictable cost/effective treatment with "cold" SSAs, that are extensively used in therapy; this is made possible by their high radiochemical stability and substantial maintenance of the molecular structure, meaning their targeting function is preserved. (62)

DOTA deserves a few more words spent in its regards: 1,4,7,10-tetraazacyclododecane-N, N', N'', N'''-tetraacetic acid (or simply "DOTA") is a universal bifunctional chelator that can label several radionuclides always forming stable complexes, such as ^{111}In, ^{68}Ga, ^{64}Cu, ^{90}Y and ^{177}Lu (note: the last two isotopes are utilized in PRRT, a radiopharmaceutical-based therapy strategy, while ^{64}Cu is being studied for both diagnosis and therapy purposes).

The major advantages of ^{68}Ga-DOTA-compunds are an impressive in vivo stability, good pharmacokinetic properties and a substantial and specific uptake by somatostatin receptors; all these characteristics make this type of SSAs a very useful instrument in PET imaging in order to obtain significantly better and more accurate images, together with a better biological and functional assessment of the lesions. (63)

DOTA-peptides have been largely compared to SRS, enlightening several advantages that make the first a far better choice in nuclear imaging:

1. SRS uses mainly OctreoScan® (or ^{111}In-penteotride), meaning that a cyclotron is needed to produce this radionuclide: in contrast, as aforementioned, ^{68}Ga does not have this type of necessity, elevating it as a better choice in terms of availability and costs.
2. The entire duration of an SRS investigation consists in at least two different acquisitions: the first one is performed 4h after the administration of the radiopharmaceuticals, in addition to a second one 24h later; instead, DOTA-peptide PET only needs one acquisition 2h after the injection of the compound, defining a simpler procedure even for the patient himself.
3. The diagnostic accuracy of DOTA-peptides PET is far higher than SRS and SPECT, in addition to a lower dosimetry; thanks to its physical characteristics, DOTA-peptides offer a higher sensitivity and a better spatial resolution (PET

provides a value of 3-6 mm versus 10-15 mm of SPECT).

4. From a biological perspective, DOTA-compounds show a ten times higher affinity to somatostatin receptors, if compared with OctreoScan®.
5. A peculiar capability of PET is precisely measuring the extent of uptake of the radiopharmaceutical in a defined region of interest (ROI), using the measurement of SUV_{max} (Standardized Uptake Value), a useful parameter both in therapeutic response and prognostic assessment.

DOTA-peptides are not equal each one to the other: three of them are the most used and studied, currently, in clinical applications. They are not interchangeable, in fact they differ in terms of receptor subtype affinity, but regarding their radiochemical structure and radionuclide labeling capabilities, there is no substantial difference. After all, when a certain DOTA-peptide is employed with a patient to diagnose a NEN, it is good practice utilizing the same one also in follow-up monitoring, avoiding even the slightest difference in images results (potentially influencing the assessment of the disease and misleading the physician).

Following, we present a schematic overview to contextualize them:

a. ^{68}Ga-DOTA-**NOC** (^{68}Ga-DOTA,1-Nal3-octreotide): this is the most promising radiotracer in NENs' PET imaging, because of its wide and high affinity to several SSTR subtypes, in particular to SSTR-2, SSTR-3 and SSTR-5. If we compare it with SRS (that utilizes ^{111}In-Pentetreotide, which shows affinity for only SSTR-2), it is evident how PET overperforms scintigraphy.

b. ^{68}Ga-DOTA-**TOC** (^{68}Ga-DOTA-Tyr3-octreotide): this peptide provides the higher binding affinity to SSTR-5.

c. ^{68}Ga -DOTA-**TATE** (^{68}Ga-DOTA-Tyr3-octreotate): it shows the highest level of affinity to SSTR-2 among all other DOTA-peptides, displaying a binding capability to this specific receptor subtype ten times better than the latter.

New chelators are also being studied: one of them, NODAGA has been proposed as an alternative chelator in ^{68}Ga-peptides, because of its better image quality that is able to provide. NODAGA offers a higher tumor-to-background ratio, in addition to better performance in labeling radionuclides. After all, more studies are needed to allow NODAGA taking place in the clinical scenario, so by now the main protagonists remain DOTA-peptides.

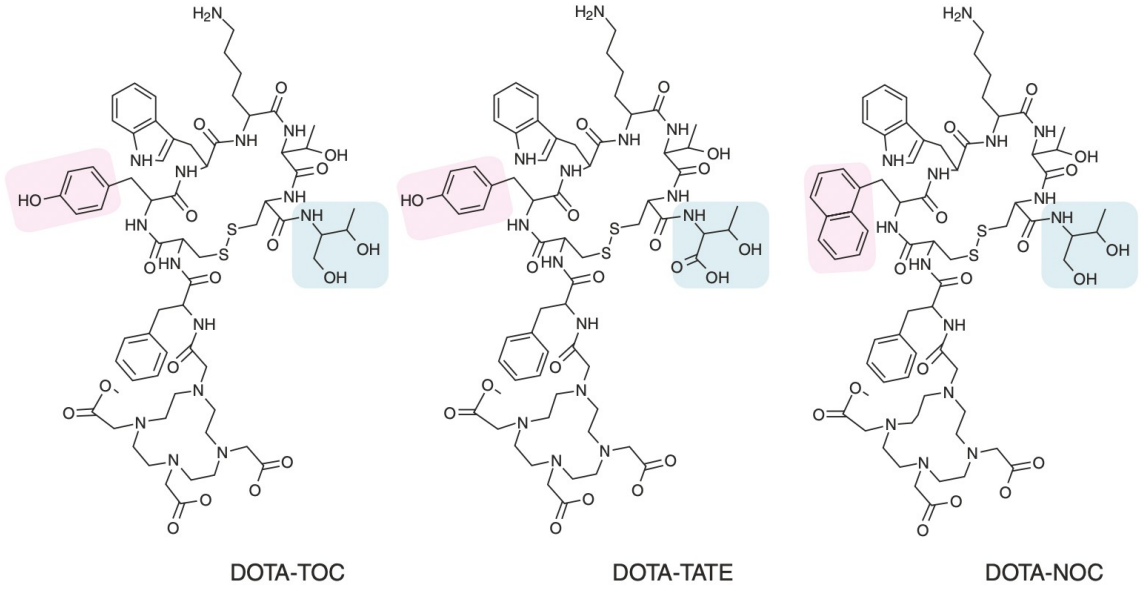


Figura 2 DOTA-peptides: chemical structure and differences

^{18}F-FDG PET/CT AND OTHER TRACERS: STILL USEFUL INSTRUMENT IN NENs?

We have already presented ^{18}F-FDG, but how it compares with DOTA-peptides?

We can synthetize the answer saying that DOTA-peptides overperform by far ^{18}F-FDG in most NENs cases (because they are usually well differentiated and overexpress SSTRs), but we must enlighten how ^{18}F-FDG represents the best choice in high grade and poorly differentiated tumors, thanks to their significantly increased metabolic activity (particularly glucose uptake and metabolism, needed to guarantee a higher reproduction rate for the neoplastic cells in comparison with normal tissues). (17)

Different studies have compared ^{18}F-FDG to other tracers, especially ^{68}Ga-DOTA-peptides, but also ^{18}F-DOPA and ^{123}I-MIBG (used in association with scintigraphy).

Thanks to those evidence, main *limitations* (considerable as pitfalls) of ^{18}F-FDG application in neoplasms were described during time. Essentially, they are the following:

- Well differentiated and/or slow growing tumors, such as NETs, thyroid and prostate cancer, because of the low uptake of radiolabeled glucose;
- Tumor lesions located in high physiological ^{18}F-FDG uptake areas, like high metabolic activity organs (i.e. brain, since its glucose-based metabolism) and excretion pathways (i.e. bladder);
- In presence of an inflammatory process or infection, because inflammatory cells consume a higher amount of glucose, capturing a lot of ^{18}F-FDG.

Those experiences started a new interest in radiopharmaceuticals that could have replaced ^{18}F-FDG, here are some examples of tracers already tested in a preclinical

environment, that will also come into real clinical practice:

a. ^{64}Cu-DOTA-TATE: with a low dosimetry, it showed better performance in comparison with SRS, providing excellent image results.

b. ^{18}F-FP-Gluc-TOCA: this drug is an octreotide radiolabeled analogue that showed a favorable kinetic in presence of a neoplastic rapid and high uptake, in addition to a fast plasmatic wash-out of the radiopharmaceutical itself.

c. Dopastatins: this is a new class of radiopharmaceuticals, defined as chimeric analogues, because of their hybrid binding capability with both somatostatin and dopamine receptors: they showed a high affinity to D2 receptors and SSTR-2. This type of radiotracers is particularly useful in the context of peculiar neoplasms like insulinoma, that is to say those neoplasms not providing satisfactory results in traditional somatostatin-only based nuclear imaging (as a remind, these insulinoma often cannot be depicted by OctreoScan®, ^{68}Ga-DOTA-peptides and other SSAs employed in PET imaging, since they do not overexpress SSTRs).

HYBRID SYSTEMS: HOW TRADITIONAL RADIOLOGY MEETS NUCLEAR MEDICINE

The fusion of techniques such as Computed Tomography (CT) and Positron Emission Tomography (PET) represented a deep change in nuclear medicine imaging, allowing the fusion of different data in a unique image, made possible by hybrid PET/CT machineries: in particular, since then it was possible to combine morphostructural information with functional ones. The results of this completely new approach were immediately game-changing, so these techniques, together, allowed an important step forward a better clinical outcome in neuroendocrine neoplasms and, widely speaking, also in oncology in general. (64)

^{68}Ga-DOTA-peptides PET/CT is nowadays the gold standard nuclear procedure to diagnose and generally evaluate the state of a patient with well-differentiated NENs, providing far better sensitivity and specificity in comparison with SRS and SPECT/CT, especially in small tumor dimension cases. DOTA-peptides PET/CT plays a key role in staging, re-staging, follow-up, researching unknown lesions, evaluating the state of the lesion/-s before and after therapy.

Different studies support the aforementioned superiority. (65)

The combination of PET and MRI (Magnetic Resonance Imaging) has been experimented, but today it does not cover a significant role in DOTA-peptide context. Several studies have outlined its advantages, especially in the identification of abdominal GEP lesions: for example, Beiderwllen KJ et al. conducted an in-depth analysis, showing also the weakness of this procedure in lung lesions and hyper-sclerotic skeletal metastases. (39)

As aforementioned, nowadays the best choice in functional studies of NENs patients is represented by PET/CT associated to gallium-labelled peptides, since they widely displayed its clear superiority in comparison with ^{111}In-Pentetreotide (OctreoScan® or OCT). (66)

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