3p-C-NETA-TATE: A versatile somatostatin analogue for Al^{18}F-labeled and therapeutic SSTR2 targeting radiopharmaceuticals

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Radiopharmaceutical

Nuclear medicine:
Plays an important role in the diagnosis, follow-up and treatment of cancer

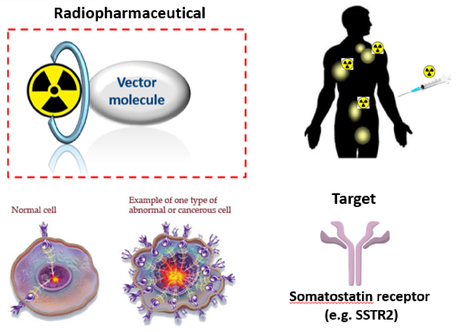
Radiopharmaceuticals consist mostly out of two parts:

1) **Vector molecule** with high affinity and selectivity for the target
   → Small organic molecule, peptide, antibody or antibody fragment

1) **Radionuclide**
   → Diagnostic radionuclide (PET or SPECT)
   → Therapeutic radionuclide (β$^-$, α or Auger-electron emitters) **= THERANOSTIC approach**

→ The radiopharmaceutical is distributed within the body by the vascular system and allows targeting of a primary tumor and all its metastases

Target:
Low expression in healthy tissue
High expression in tumour tissue
→ Somatostatin receptors (SSTRs) are highly expressed in many neuroendocrine tumors (NETs)
→ Excellent target for diagnosis and targeted radionuclide therapy (TRNT) of NETs



Background

Somatostatin-based radiopharmaceuticals (e.g. [^{68}Ga]Ga-DOTATATE and [^{177}Lu]Lu-DOTATATE) have been used to diagnose, monitor, and treat neuroendocrine tumor patients with great success [1]. However, widespread implementation of [^{68}Ga]Ga-DOTA-SSA PET in clinical practice is often hampered by practical, regulatory and economic factors related to ^{68}Ge/^{68}Ga-generators, such as the high cost, limited availability, low activity yield per elution and regulatory and reimbursement barriers. The Al^{18}F-method combines the advantages of a chelator-based radiolabeling method with the imaging and logistical advantages of fluorine-18. [^{18}F]AlF-NOTA-octreotide, a promising ^{18}F-labeled somatostatin analogue and potential alternative for ^{68}Ga-DOTA-peptides, is under clinical evaluation [2].

A major drawback is that the most utilized chelator for the Al^{18}F-method, 1,4,7-triazacyclononane-N,N',N''-triacetic acid (NOTA), is not compatible with therapeutic radionuclides such as the β--emitter lutetium-177 (^{177}Lu) and the promising α-emitter bismuth-213 (^{213}Bi). Ideally, the same precursor (combination of chelator-linker-vector) can be used for production of both diagnostic and therapeutic radioprobes with very similar (e.g. Al^{18}F/^{213}Bi/^{177}Lu) pharmacokinetic properties, which is possible with the promising chelator 3p-*C*-NETA **(Figure 1)** [3], allowing accurate personalised dosimetry estimation, and radionuclide therapy of NET patients in a theranostic setting.

In contrast to external high energy photon or proton therapy, targeted radionuclide therapy (TRNT) is a systemic cancer treatment allowing targeted irradiation of a primary tumor and all its metastases, resulting in less collateral damage to normal tissues. The single α-emitting radionuclide ^{213}Bi ($T_{1/2}$ = 45.6 min, Eα = 8.4 MeV, γ = 440 keV, α-particle range = 40–80 µm, corresponding to 2-10 cell diameter) has interesting properties and might be considered as a magic bullet for TRNT [4]. Further, it can be produced on site using a ^{225}Ac/^{213}Bi generator.

Objectives

In this study we evaluated 3p-*C*-NETA-TATE as theranostic precursor for NET imaging and therapy, and present first results of the preclinical evaluation of the diagnostic compound [^{18}F]AlF-3p-*C*-NETA-TATE [5] and therapeutic ligands [^{213}Bi]Bi-3p-*C*-NETA-TATE and [^{177}Lu]Lu-3p-*C*-NETA-TATE.

Materials and Methods

3p-*C*-NETA-TATE was synthesized using standard solid/liquid-phase peptide synthesis and purified using HPLC. [^{18}F]AlF-3p-*C*-NETA-TATE was synthesized in an automated AllinOne® synthesis module. 3p-*C*-NETA-TATE (10 µM) was radiolabeled manually with ^{177}Lu (NaOAc, 0.1 M, pH 4.1, 12 min) or ^{213}Bi (Tris-HCl, 4 M, pH 8.5, 7 min) at 40 or 95 °C. The *in vitro* stability of the corresponding radiocomplexes was evaluated in formulation buffer, PBS and human serum at 37 °C using radioHPLC.

In vitro cell binding and internalization was performed for [^{18}F]AlF-3p-*C*-NETA-TATE, [^{213}Bi]Bi-3p-*C*-NETA-TATE (185 kBq/well; A_m: 1.23 GBq/µmol) and [^{177}Lu]Lu-3p-*C*-NETA-TATE (53 kBq/well; 176.66 A_m: GBq/µmol) using SSTR2 expressing cells (QGP1.SSTR2 and/or BON1.SSTR2) [4] and the pharmacokinetics of [^{18}F]AlF-3p-*C*-NETA-TATE were evaluated in healthy rats and in xenograft (QGP1.SSTR2) bearing mice using µPET/MRI and µPET/CT, respectively. [^{18}F]AlF-NOTA-Octreotide was used as benchmark. Cell viability and clonogenic assays were performed with [^{213}Bi]Bi-3p-*C*-NETA-TATE and [^{177}Lu]Lu-3p-*C*-NETA-TATE using BON.SSTR2 cells.

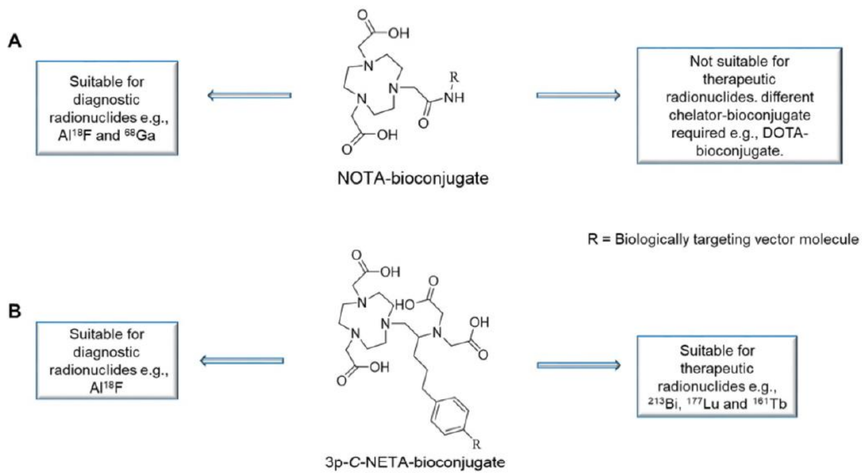


Figure 1. Versatility of 3p-C-NETA in contrast to NOTA. (A): NOTA is only suitable for radiolabeling of diagnostic radionuclides (Al^{18}F and ^{68}Ga). **(B):** In contrast, 3p-C-NETA has potential for both diagnostic (Al^{18}F) and therapeutic radionuclides (^{213}Bi, ^{177}Lu, and ^{161}Tb), making it a true theranostic chelator.

Results

[^{18}F]AlF 3p-*C*-NETA-TATE was obtained in good RCY (56 ± 10%) and >98% radiochemical purity. [^{18}F]AlF-3p-*C*-NETA-TATE displayed excellent *in vitro* stability with >95% intact tracer after 4 hours in all tested conditions. High SSTR2 specific cell binding and internalization (18.4 ± 2.1 % of which 78.3 ± 2.1 % is internalized) was observed after 60 min incubation (**Figure 2**). Finally, [^{18}F]AlF-3p-*C*-NETA-TATE showed excellent pharmacokinetic properties (rats and mice) and tumor accumulation (SUV_{mean}60 min:2.7 ± 1.1), which was comparable as for [^{18}F]AlF-NOTA-Octreotide (SUV_{mean} 60 min:3.2 ± 0.76). We were also able to block uptake in SSTR2 expressing organs and in tumors (>90%) by coinjection of 2.5 mg/kg octreotide acetate, indicating SSTR2 specific uptake (**Figure 3 and 4**).

3p-*C*-NETA-TATE efficiently sequestered ^{177}Lu (RCC >95%) and ^{213}Bi (RCC >90%) at 12 and 7 min respectively at 40 °C (**Figure 5**). [^{177}Lu]Lu-3p-*C*-NETA-TATE showed excellent *in vitro* stability in both PBS and mouse serum (>90% intact complex at day 3). Starting with 94.3% radiochemical purity, [^{213}Bi]Bi-3p-*C*-NETA-TATE demonstrated good stability (>90% intact radiocomplex) after 5 h in both PBS and human serum. High SSTR2 specific cell binding and internalization (11.4 ± 0.7 % of which 62.1% is internalized) was observed after 60 min incubation for [^{177}Lu]Lu-3p-*C*-NETA-TATE whereas only 3.3 ± 0.5% cell binding (of which 39.2% is internalized) was observed for [^{213}Bi]Bi-3p-*C*-NETA-TATE, probably due to blocking effects because of low apparent molar activity. >99% blocking after co-incubation with 100 µM octreotide was observed for both tracers at 60 min (**Figure 6**). Reduced viability of BON-1.SSTR2 was observed after 48 h incubation with [^{213}Bi]Bi-3p-*C*-NETA-TATE (9.9 ± 0.9% viability at 0.1 MBq activity). Also, only 0.1 MBq activity of [^{213}Bi]Bi-3p-*C*-NETA-TATE was required to achieve cell surviving fraction of 0.1 ($SF_{0.1}$).

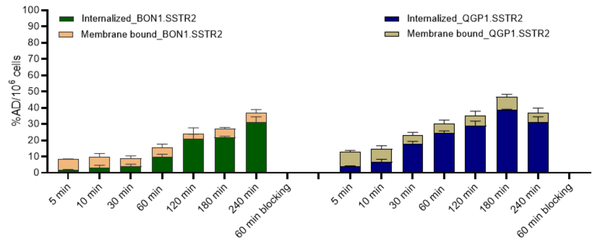


***Figure 2**. Cell binding and internalization of [^{18}F]AlF-3p-C-NETA-TATE on BON1.SSTR2 and QGP1.SSTR2 cell lines. Adherent cells were incubated with the radioligand of interest (n = 3, 180-190 kBq/well) for 5, 10, 30, 60, 120, 180 and 240 min at 37 °C in the presence or absence of octreotide acetate (100 µM, n = 3). Total bound fraction is the sum of membrane bound + internalized fraction.*

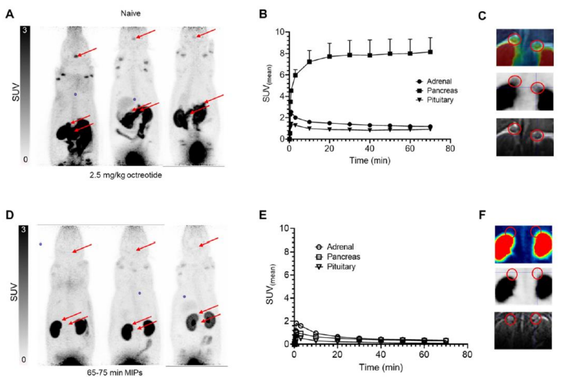


Figure 3. *In vivo* biodistribution of [18F]AlF-3p-C-NETA-TATE in rats using control and blocking conditions (2.5mg/kg octreotide). A,D) Maximum intensity projections (PET) at 65-75 minutes post-injection, with SSTR2-expressing organs (pituitary and adrenal glands, pancreas) highlighted by arrows in representative naïve (top) and blocked (bottom) animals. **B,E)** Time activity curves (TACs) of SSTR2-expressing organs in naïve (top) and blocked (bottom) animals (n=3), demonstrating high and blockable SSTR2 uptake. **C,F)** Coronal slices indicating delineation of adrenal glands on MRI sequence subsequently applied to PET data to generate TAC. (upper): PET-MRI fusion, (middle): PET (lower): MRI (PET images from 65-75 min timepoint).

References

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Conclusions

3p-*C*-NETA-TATE is an excellent and versatile agent that can be used for both targeted radionuclide therapy (^{177}Lu, ^{213}Bi) and diagnostic applications (Al^{18}F) and has advantages as an alternative to the DOTA-SSA analogues in current clinical use. [^{18}F]AlF-3p-*C*-NETA-TATE and [^{213}Bi]Bi-3p-*C*-NETA-TATE/[^{177}Lu]Lu-3p-*C*-NETA-TATE will be further evaluated as potential theranostic pairs in SSTR2 expressing tumor mice.

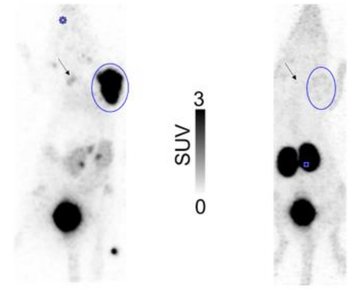


***Figure 4**. µPET imaging of [^{18}F]AlF-3p-C-NETA-TATE in QGP1.SSTR2 tumor bearing mice. MIP image (65–75 min p.i.): (left) [^{18}F]AlF-3p-C-NETA-TATE; (right) [^{18}F]AlF-3p-C-NETA-TATE + 5 mg/kg octreotide. Blue circle and black arrow indicate tumor and thymus region respectively.*

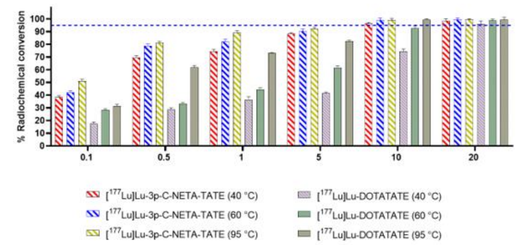
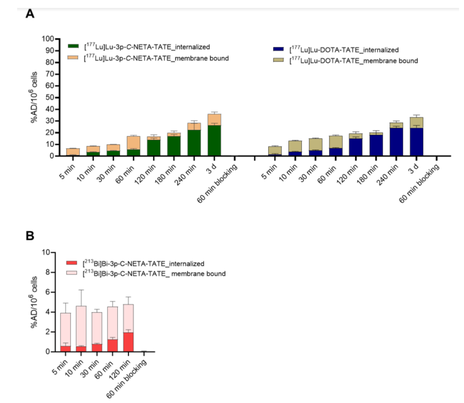


***Figure 5.** Radiochemical conversions (RCC) of [^{177}Lu]Lu-3p-C-NETA-TATE and [^{177}Lu]Lu-DOTA-TATE. 3p-C-NETA-TATE (0.1-20 µM) or DOTA-TATE (0.1-20 µM) was labeled with 2-5 MBq of [^{177}Lu]$LuCl_3$ (0.4 M NaOAc, pH 4.1) for 15 min at 40, 60 or 95 °C. Blue line is inserted to indicate a yield of 95%;*



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Acknowledgements and contact

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