Vascular endothelial growth factor (VEGF), released by tumor cells, is an important growth factor in tumor angiogenesis. Bevacizumab is designed to directly bind to VEGF extracellulary to prevent interaction with VEGF receptors. Given the drawbacks of radiolabeled monoclonal antibodies (mAbs) such as slow blood clearance and unspecific binding to normal tissues, antibody pretargeting is an approach which combines the desirable properties of high tumor uptake of antibodies with rapid pharmacokinetics and fast whole-body clearance of radioactivity.

In this study, we synthesized and characterized a biotin derivative, that contains an NSO donor atom system which can act as a tridentate chelator for the ^{99m}Tc(I)-tricarbonyl core. The ^{99m}Tc(CO)$_3$(NSO)-biotin derivative was then prepared and quality control of this complex was performed with HPLC. A streptavidin conjugate of bevacizumab (bevacizumab/SA) was also prepared, and HPLC studies were performed in order to determine binding of the ^{99m}Tc(CO)$_3$(NSO)-biotin derivative to the streptavidin–bevacizumab conjugate. Serum stability studies showed no loss of radiolabel from ^{99m}Tc(CO)$_3$(NSO)-biotin. Concerning the in vitro pretargeting assay, the binding of the effector molecule to cancer cells pretargeted with bevacizumab/SA will be further evaluated in MDA MB 231 breast cancer cells transfected with the VEGF-165 isoform.

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Characterization of ^{99m}Tc-caspofungin as fungal infection agent and assessment of potential influence of pretreatment

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Reliable clinical interpretation of scintigraphic images for the diagnosis of fungal infections is not always simple because of low specificity and sensitivity of the available radiopharmaceuticals [1]. Our group studies potential radiopharmaceuticals for infection diagnosis, in this particular case characterization of ^{99m}Tc–caspofungin–tricarbonyl complex and the influence of pretreatment with caspofungin is evaluated. ^{99m}Tc–caspofungin–tricarbonyl complex was obtained by water molecules substitution of *fac*-[Tc(I)(H$_2$O)$_3$(CO)$_3$]$^+$ precursor with caspofungin (MERCK) [2]. RCP was higher than 90%, the complex was stable for up to 20 hs post labeling. Lipophilicity was 0.56, yeast binding 11% and protein binding (65–75%). Scintigraphic images were acquired in 2 groups ($n = 3$) of Nude NIH (S)Fox1nu/nu Swiss based male mice bearing hind paw infection with *Candida albicans* (0.2 mL, 9×10^8 ufc). A preclinical image system for small animals Triumph^{TM} PET/SPECT/CT (Gamma Medica, Inc.) was used. G1: received 3 days pretreatment with caspofungin, 50 μL (0.5 mg/mL), G2: received 3 days pretreatment with 50 μL saline as control.

Scintigraphic image evaluation showed a clear distinction between healthy and injured tissues T/NT = 4 in G1 and T/NT = 3 in G2. However, difference of T/NT ratio between G1 and G2 was not statistically significant ($p < 0.05$). ^{99m}Tc–caspofungin–tricarbonyl complex is a promising detection agent of fungal infections and influence of pretreatment with caspofungin could not be proven.

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Imidazole stabilized [2 + 1] Re(I)/^{99m}Tc(I) complexes as isostructural nuclear and optical probes

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The synthesis, stability and photophysical properties of [2 + 1] Re(I)/Tc(I) complexes of bipyridine and a series of triazole and imidazole derivatives were investigated as a means of identifying complexes suitable for use in creating targeted isostructural optical/nuclear molecular imaging probes. To prepare the desired complexes, [Re(CO)$_3$(H$_2$O)$_3$]$^+$ was combined with 2,2′-bipyridine (bipy) to give [Re(CO)$_3$(bipy)Br] which in turn was converted to the desired complexes through the treatment with functionalized triazoles and imidazoles and heating the mixture to reflux overnight. The corresponding ^{99m}Tc complexes [^{99m}Tc(CO)$_3$(bipy)(L)]$^+$ (L = triazole/imidazole) were obtained by adding [^{99m}Tc(CO)$_3$(bipy)(H$_2$O)]$^+$ to a series of triazole and imidazole ligands and heating at 40 °C for 30 min. Quantitative transformation of the intermediate to the final product was confirmed by HPLC and through comparison to the authentic Re standards. Stability studies showed that the imidazole derivatives exhibited superior robustness and a half-life in plasma suitable for *in vivo* imaging [1]. The crystal structure of the fluorescent and targeted Re complex [Re(CO)$_3$(bipy)(ZA)]$^+$ where ZA = zoledronic acid, was obtained and the corresponding Tc complex prepared as a new class of bone seeking radiopharmaceutical. The synthesis, characterization and *in vivo* testing of these agents will be presented.

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14

Labelling PAMAM dendrimers with Tc-99m via HYNIC

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Dendrimers are branched macromolecules with a well-defined structure, very low polydispersity and high functionality. Poly (amidoamine) (PAMAM) dendrimers are the most studied class of dendrimers for biomedical purposes. In the present study, PAMAM G4 dendrimer conjugated with hydrazinonicotinamide (HYNIC), an efficient bifunctional chelator, was characterized and optimized. The conjugated dendrimer was labeled with ^{99m}Tc using tricine coligand and the stability of the labeled complex was evaluated. Biodistributions were performed administrating Tricine-^{99m}Tc-HYNIC-dendrimer to normal C57 black mice. Animal studies were carried out in compliance with the national laws related to the ethics during animal experimentation. The structure of the derivatized dendrimer was confirmed by ^{1}H-NMR and ^{13}C-NMR spectra and MALDI-TOF mass spectrometry. Radiolabeling was accomplished in high yield (99%) and remained stable in 24 h (98%). The L-cysteine challenges studies showed that at 1 mM concentration of L-cysteine, only insignificant decomposition of the complex occur over 3 h incubation (96%). Biodistribution studies

showed hepatic but principally renal clearance. In conclusion, macromolecules like PAMAM G4 dendrimers could be labeled with ^{99m}Tc via HYNIC with very good efficiency and with high stability, showing their potential for molecular imaging.

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^{188}Re radiotherapy facilitated by virus expressing the human sodium iodide symporter

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Rhenium-188 emits a beta with a maximum energy of 2.12 MeV, and can be easily and relatively inexpensively obtained from portable ^{188}W/^{188}Re generators as sodium perrhenate ($Na^{188}ReO_4$). This makes rhenium an attractive option for use in radio-cancer therapies. The human sodium iodide symporter (hNIS) transports NaI across cell membranes, but it has been shown that hNIS can also transport perrhenate (ReO_4^-). hNIS, which is expressed primarily in the thyroid glands, has facilitated radiotherapy for thyroid cancer for over sixty years. Oncolytic viruses expressing hNIS have been shown to induce hNIS expression in numerous different types of cancer cells. Multiple tumor types were treated with recombinant oncolytic vaccinia virus (VACV) expressing hNIS followed by treatment with $Na^{188}ReO_4$. Infected cancer cells showed a significant *in vitro* increase in radio uptake as compared to controls. *In vivo*, human tumors generated on the flanks of nude mice were treated with VACV followed by sodium $Na^{188}ReO_4$. Treated mice demonstrated significant inhibition of tumor growth and prolonged survival when compared to controls. ^{188}Re exhibits a 15% gamma emission at 155 keV, which facilitated SPECT/CT imaging of VACV infected tumors *in vivo* and demonstrated significant radio uptake when compared to controls.

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Determination of optimized conditions for ^{99m}Tc-labeled rifampicin preparation for tuberculosis imaging applications

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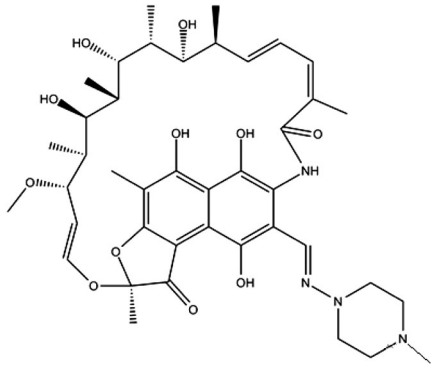
Developing new infection imaging agents is a mandate in the detection of resistant species in the clinic due to the mortality of various new strains of bacteria including *Mycobacterium tuberculosis*. Various conditions were optimized for the rapid and efficient labeling of rifampicin antibiotic with Tc-99m for ultimate use in infection imaging. Radiochemical purities were checked by ITLC using methyl ethyl ketone, normal saline on Whatman No. 1 paper. Time, temperature, ligand concentration, stannous ion amount, pH etc. were determined in the radiolabeling process and the best conditions were room temperature, pH 7, 20 micrograms of stannous chloride for 1 mg of rifampicin solid and 20 mCi of freshly milked technetium-99m pertechnetate. The complex demonstrated satisfactory stability in presence of human serum and final formulations for 6 hours. Further investigations on biological studies are ongoing.



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Novel ^{99m}Tc-labelled estrogen derivative as potential agent for estrogen receptors imaging

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With the objective to develop a potential radiopharmaceutical for estrogen receptors imaging we have prepared and evaluated an ethinylestradiol derivative labelled with ^{99m}Tc.

Ethinylestradiol was derivatized by reaction with *N*-Boc-azidoalanine for 4 days at room temperature. The product was deprotected with an excess of trifluoroacetic acid for 1 h at 85 °C. Structure was confirmed by spectroscopic techniques.

^{99m}Tc-labelling was achieved by mixing neutralized *fac*[^{99m}Tc$(CO)_3(H_2O)_3]^+$ precursor (40–50 mCi, 0.5 mL) with the derivatized ligand (4 mg) and incubating for 30 minutes at 75 °C. The labelled product had a radiochemical purity above 90% (by HPLC), which remained unchanged for at least 6 hours. The complex was stable in human plasma at 37 °C for at least 2 hours. Lipophilicity expressed as logP (partition coefficient between octanol and phosphate buffer pH = 7.4) was 1.43 ± 0.04. Plasma protein binding was 76.4 ± 0.6%. Biodistribution in normal rats at 30, 60 and 120 minutes post-injection showed high liver uptake (40.8 ± 2.4%). Excretion occurred mainly by the hepatobiliary tract. Uptake in other organs was negligible.

In vitro uptake by MCF7 cells in culture was 0.98 ± 0.02% and 30% of the uptake was blocked by preincubation with a 10 M excess of the estrogen receptors antagonist tamoxifen.

These results are promising for a potential oncological radiopharmaceutical.

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