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Final Technical Report:

Support for Students, Postdoctoral Fellows and Trainees who have been accepted to present at the Terachem 2014 Symposium

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I. GOALS FOR THE PERIOD AND COMPARISON WITH ACTUAL ACCOMPLISHMENTS

This award under the Office of nuclear Physics, isotope Development and Production for Research and Applications Program ($20,000) was to provide bursaries for U.S. students/trainees to enable them to participate in the Terachem 2014 Symposium.

The awardees were selected by the Society of Radiopharmaceutical Sciences (SRS) education/awards committee chaired by Henry Van Brocklin. The SRS is an international, multidisciplinary professional organization dedicated to the advancement of excellence in education and research in radiochemistry and radionuclide imaging science and in the study and use of radiotracers. The SRS was formed primarily to train the next generation of radiochemistry and nuclear scientists. The SRS provides academic and intellectual support for the radiochemical and imaging sciences by:

- Supporting and sponsoring educational programs to train students and scientists in the field;
- Disseminating information concerning isotope production and separations, radiochemistry and imaging sciences by sponsoring scientific and professional publications (e.g., *Nuclear Medicine & Biology* is the official journal of the SRS), and
- Striving to improve the welfare of mankind by maintaining and advancing the highest possible standards in education, research, and the practice of radiochemistry and radionuclide imaging sciences.

The education of the next generation of radiochemists and imaging scientists is the primary mission of the SRS. Terachem-2014 Symposium, co-sponsored by the SRS, is aimed at the education and preparation of the professional radiochemists utilizing radioactive metals and imaging scientists of the future. This international symposium has made major and sustained contributions in this regard for over twenty-five years since the first Symposium was held in 1982.

It would be difficult (and perhaps impossible) for a significant number of promising young U.S. students/trainees working in radiochemistry on production and radionuclide imaging sciences

fields to attend the Terachem-2014 Symposium without financial assistance to cover travel and subsistence expenses. Thus, it was proposed that the office of nuclear sciences award $20,000 to cover the costs of up to 16 student bursaries to assist student participants in covering travel and subsistence expenses related to their study. Applications from perspective student/trainee participants were accepted and reviewed by an Awards Committee (Drs. Henry VanBrocklin, Carolyn Anderson, Sally Schwarz and Cathy Cutler) to select the awardees based on scientific merit and need. A stipend of $1,250 was provided to each awardee selected. The selection process included review of their application documents submitted to SRS, their symposium Abstract and results of peer reviewers' comments and a rating of scientific merit. To be eligible for a bursary to attend the Symposium, their Abstract had to be accepted for presentation at the Symposium. In order to increase the breadth of participants and represented institutions, only one student/trainee was chosen from a single research group.

II. REASONS WHY ESTABLISHED GOALS WERE NOT MET

All goals and objectives were met. Only 12 students met the criteria and received the $1,250 stipend. This left $5,000.00 in unspent funds. The abstract by Aranh Pen was submitted too late to make the journal but was presented at the meeting.

III. SUMMARY OF PROJECT STATUS.

The awards committee reviewed 30 applicants and deemed 12 of them worthy to receive the bursary to attend the meeting. Following is the names of the applicants: Anthony Degraffenreid form the University of Missouri; Vernal Richards from Washington University; Eric Price Memorial Sloan-Kettering Cancer Center; Sam Groverman Hunter College; Melissa Deri Hunter College; Alexander G. White University of Pittsburgh; Aranh Pen University of Michigan; Zhengtao Qin University of California, San Diego; Kim Reinig University of Missouri; N. Bandera Washington University; Sai Kiran Sharma Memorial Sloan-Kettering Cancer Center; Victoria Calzado University of Missouri.

Provided below are the abstracts that were printed in the Journal of Nuclear Medicine and Biology volume 41, Issue 7. The Terachem-2014 Symposium provided a dynamic setting for the students/trainees and other participants with opportunities to present and discuss their ground-breaking research being conducted worldwide with radioactive metals for a variety of applications of radiometals for biological, environmental and medical sciences in medicine and the biomedical sciences. This was a particularly important setting for stimulating cross-disciplinary interactions, collaborations and knowledge exchange between scientists with expertise in radiometal production, purification, targetry, molecular imaging, radiochemistry, synthesis and characterization of radiometal bioconjugates. The Symposium was held in Bressanone, Italy which is a small town located south of the Brenner Pass near the Italy-Austria border. This venue was particularly effective in facilitating high quality and personal discussions between senior scientists with expertise in radiometal chemistry, production and radiotracer design and application and students/trainees.

We appreciate the Department of Energy Nuclear Physics support of this project. It aided in work force development and training of nuclear and radiochemists in radiometal production, purification, chelation and utilization.

IV. EVENTS WITH SIGNIFICANT IMPACT ON THE PROJECT

None.

V. Abstracts of Students receiving bursaries

Anthony Degraffenreid

Session A2. Other Radiometals in Diagnosis and Therapy and New Labelling Strategies

Oral Presentations

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3,4,3-(LI-1,2-HOPO): An alternative chelator for ^{89}Zr radiopharmaceuticals

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^{89}Zr is an attractive radionuclide for antibody-based PET tracers because its 78.41 h half-life matches the biological residence time of IgG antibodies. Currently, antibodies are radiolabeled with ^{89}Zr using desferrioxamine B (DFO); however, the observed uptake of radioactivity in the bones of mice given ^{89}Zr-DFO-antibody constructs suggests *in vivo* release of $^{89}Zr^{4+}$ [1]. A better chelator for $^{89}Zr^{4+}$ could eliminate the release of the bone-seeking $^{89}Zr^{4+}$ cation *in vivo* and produce a safer PET tracer.

We investigate the efficacy of a hydroxypyridinone-based chelator – 3,4,3-(LI-1,2-HOPO) or HOPO – as an alternative to DFO. The Zr–HOPO complex was evaluated not only experimentally, *in vitro* and *in vivo*, but also theoretically. HOPO was found to rapidly and effectively form a 1:1 complex with $^{89}Zr^{4+}$ that exhibits remarkable stability and favorable *in vivo* clearance. The stability of ^{89}Zr-HOPO matched or surpassed that of ^{89}Zr–DFO in every experiment. Ultimately, HOPO has the potential to replace DFO for ^{89}Zr-based PET imaging agents.

Reference

[1] Deri MA, et al. Nucl Med Biol 2013;40:3–14.

http://dx.doi.org/10.1016/j.nucmedbio.2014.05.103

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A novel multivalent bifunctional siderophore chelator scaffold for radiolabeling with gallium-68 based on fusarinine C

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The cyclic peptide siderophore triacetylfusarinine C (TAFC) showed excellent complexing properties for ^{68}Ga resulting in high specific activity and excellent metabolic stability. We postulated that, starting from its deacetylated form fusarinine C (FSC) trimeric bioconjugates are directly accessible to develop novel ^{68}Ga-labeled targeted radiopharmaceuticals. For a proof of principle, the ferric form of FSC was coupled with an $\alpha_v\beta_3$ integrin targeting RGD sequence *via in situ* activation using HATU/HOAt and DIPEA. Subsequent removal of iron allowed radiolabeling of FSC(RGD)$_3$ with ^{68}Ga resulting in high radiochemical yield (>98%) reaching SA of up to 1 TBq/μmol. The logD value was −3.6 revealing a hydrophilic character and stability studies of [^{68}Ga]FSC-(RGD)$_3$ in different media showed a high *in vitro* stability. An IC_{50} value of 1.8 ± 0.6 nM was determined indicating a high binding affinity for $\alpha_v\beta_3$ integrins. The internalized activity was 5.3% cpm/mg protein for $\alpha_v\beta_3$ positive M21 cells which was reduced to approximately 1/25 of the reference activity via addition of c(RGDyV). Biodistribution studies in nude mice resulted in an uptake in the $\alpha_v\beta_3$ positive tumor of 4.3% ID/g 60 min after injection, compared to 1.13% in receptor negative tumours, more than 3-fold higher than the monomeric ^{68}Ga-NODAGA-RGD in the same tumour model (1.35%). [^{68}Ga]FSC-(RGD)$_3$ served as an example for the feasibility of a novel multivalent bifunctional chelator based on cyclic peptide siderophores and showed excellent targeting properties for $\alpha_v\beta_3$ integrins.

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Synthesis of tridentate ligand: Potential theranostic application of a radioarsenic trithiol complex

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Arsenic-72 is a 26 h half-life positron emitter (2.49 MeV) with nuclear properties useful for diagnostic imaging by positron emission tomography (PET). Arsenic-77 is a 38.8 h half-life beta emitter (683 keV) potentially useful for radiotherapy applications. Radioisotopes of arsenic have half-lives suitable for use with antibodies as targeting vectors while traditional radionuclides, ^{11}C, ^{18}F, ^{64}Cu, and ^{86}Y, do not have sufficiently long half-lives (up to 4 days) to permit chemical derivatization and in vivo localization for radioimaging or radiotherapy. Utilization of these radioisotopes requires the development of compounds with high *in vivo* stability for conjugation to the targeting probes. Direct complexation of arsenic using a trithiol chelate may provide a simplified route for the development of a linkable radioarsenic complex. A simple trithiol was synthesized and characterized by ^{1}H and ^{13}C NMR, electrospray ionization mass spectrometry (ESI-MS), and single crystal X-ray diffraction. Radiotracer studies with no-carrier added ^{77}As resulted in high radiolabeling yield (>96%); its stability was evaluated by cysteine challenge.

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Robust and efficient bifunctional chelators for ^{64}Cu to target $\alpha_v\beta_3$ integrin

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The choice of bifunctional chelator (BFC) and radioisotope is crucial for imaging of biological function. ^{64}Cu ($t_{1/2}$ = 12.7 h) is a β^+ and β^- emitter, making it useful for both imaging and radiotherapy. The accuracy of imaging with ^{64}Cu often depends on BFC and hence, the importance of BFC cannot be overemphasized in terms of Cu–BFC complex robustness in vivo.

We have previously reported propylene cross-bridged TE2A (PCB-TE2A) chelator, which showed exceptional kinetic inertness and better radiolabeling profile than widely used CB-TE2A. Recently, a new tetraazamacrocyclic chelator TE2A-Bn-NCS, with two acetic acid pendant arm for strong Cu(II) complexation and an extra NCS pendant arm for easy conjugation with biomolecule, was

Vernal Richards

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Evaluation of new target materials for cyclotron production of ^{186}Re and ^{99m}Tc
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Cyclotron production of ^{99m}Tc and ^{186}Re stands as an invaluable method to produce both radiometals. This method of preparation has become attractive to (1) combat the impending shortage of ^{99m}Tc due to aging reactors and (2) produce ^{186}Re with higher specific activities. Our proof of concept studies indicate that the use of the refractory carbides of enriched ^{100}Mo and ^{186}W, along with powdered ^{100}Mo metal, is good candidates for the cyclotron production of the aforementioned radiometals. Unlike $^{100}MoO_3$ and $^{186}WO_3$, sintered targets of the refractory carbides and metallic powder have been able to withstand the high temperatures that come with bombardment at high beam currents for extended time periods. To date, we have employed currents of up to 20 μA for 40 μAhr integrated currents for both radionuclides. Targets were amenable to the thermo-chromatographic processing method, resulting in recoveries of tens of mCi for ^{99m}Tc and mCi quantities for ^{186}Re for our proof of principle experiments. This processing method results in the facile retrieval of both $^{100}MoO_3$ and $^{186}WO_3$ in yields in excess of 90%. Through solid state reactions, these oxides were converted to the carbides of ^{100}Mo and ^{186}W, and to metallic powdered ^{100}Mo, indicating the longevity in the use of these materials.

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Poster Communications

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Large-scale cyclotron production of ^{99m}Tc
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^{99m}Tc is currently supplied as the decay product of ^{99}Mo, produced by an aging fleet of research reactors around the world. Challenges to the existing supply chain are two-fold: research reactors rely on enriched uranium and generate nuclear waste. Secondly, several reactors will cease operation in the next 2–5 years. Here we report the development of a viable, comprehensive solution to produce ^{99m}Tc in sufficient quantities to supply a large urban area using a conventional medical cyclotron.

This paper presents data on the demonstration of commercial-scale cyclotron production of ^{99m}Tc using the ^{100}Mo(p,2n)^{99m}Tc transformation. Using cyclotrons with proton energies of 16 and 18 MeV, our team has established preliminary saturation yields between 1.8 ± 0.2 and 3.4 ± 0.4 GBq/μA, producing approximately 115 and 348 GBq after a 6 hour irradiation, respectively. In addition to evaluating high-power target, target transfer and dissolution hardware performance, work underway also includes measuring both Tc and non-Tc radionuclidic impurities, clinical validation and an application for regulatory approval with a goal of implementing a cost-effective solution before the Canadian NRU reactor ceases medical isotope production in 2016.

In conclusion, we have demonstrated that direct production of ^{99m}Tc via proton bombardment of ^{100}Mo can be achieved in high yields using two different brands of medical cyclotron. Our approach has been installed in multiple existing facilities (self-shielded or vaulted), and can be scaled to a higher power cyclotron using a dedicated target station for high-current production.

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High purity ^{67}Cu using 40–50 MeV protons at the Brookhaven Linac Isotope Producer
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Copper-67 ($t_{1/2}$ = 2.58 days) is a medically important radioisotope with potential for use in imaging and target radiotherapy of disease. It is currently produced at Brookhaven Linac Isotope Producer (BLIP) using high energy protons (128–140 MeV protons). Co-production of large quantities of ^{64}Cu (10 fold higher) at these high energies requires its decay (>3 days) before ^{67}Cu is radionuclidic pure (>95%). Co-production of a range of long-lived radionuclidic contaminates requires a three column separation method. New cross section data reported by IAEA (TRS473 2011) show an energy window (40–50 MeV) where the co-production of ^{64}Cu is substantially reduced and the ^{67}Cu production rate is acceptable. Changing the proton energy for ^{67}Cu production can result in the co-production of different types and quantities of contaminating radioisotopes. Therefore a new separation process for high purity ^{67}Cu is required. This research examines the development of new targetry to degrade the high energy proton beam to ≈45 MeV and the use of novel organic solvent acid mixtures to separate the desired ^{67}Cu and simultaneously purify the expensive enriched ^{68}Zn target material for reuse.

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Development of the non-standard PET radionuclides ^{43,44g}Sc and ^{45}Ti
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INM-5: Nuklearchemie, Forschungszentrum Jülich

For progress in medical research and practice, the importance of non-standard PET radionuclides is increasing. Of those the radionuclides ^{43,44g}Sc and ^{45}Ti represent interesting examples with potential to enlarge the scope of PET tracers/studies.

For utilization of radionuclides, an exact knowledge of their relevant nuclear data and the development of radiochemical separation methods are essential. Cross sections for the production of ^{45}Ti via the ^{45}Sc(p,n)^{45}Ti reaction were measured. Utilizing cation exchange chromatography the no-carrier-added product could be isolated. The positron intensity of this radionuclide was re-investigated using a very pure ^{45}Ti source in combination with X-ray, beta- and γγ-coincidence spectrometry: an absolute emission probability of 85.7% was obtained. In order to find an optimal way for production of the potential PET-nuclides ^{43}Sc and ^{44g}Sc, which is not based upon the irradiation of enriched target materials, several reaction pathways were examined. During this work ^{43}Sc proved to be the most promising PET nuclide of scandium, and the ^{nat}Ca(α,x)^{43}Sc nuclear reaction was identified to be the most effective production route, showing a maximum

$((CH_2)_2\text{-}O)_2\text{-}(CH_2)_2\text{-}NH\text{-}CO\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CO\text{-}\beta Ala$) were synthesized and radiolabeled with ^{64}Cu at a radiochemical purity of at least 95%. All three radiolabeled GE11-conjugates were stable in buffer as well as in human blood serum. The binding properties of the radiolabeled conjugates were then evaluated *in vitro* using EGFR-rich (A431, FaDu) and EGFR-negative (MDA-MB-435s) cell preparations. However, as a result of the *in vitro* studies for all three GE11-conjugates no binding affinity could be determined. These findings may be explained by the highly hydrophobic character of the produced GE11-conjugates with accompanying tendency for aggregation.

Reference

[1] Li Z, Zhao R, Wu X, et al. FASEB J 2005;19:1978–85.

http://dx.doi.org/10.1016/j.nucmedbio.2014.05.035

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Structural investigation of $^{nat/67/68}Ga$ HBED-CC complexes

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Introduction: With the availability of Ge/Ga generators, ^{68}Ga is becoming an isotope of choice for developing PET agents without a cyclotron. Cyclic chelators such as DOTA, NOTA or acyclic chelators, such as *N,N'*-bis[2-hydroxy-5-(carboxyethyl)benzyl] ethylenediamine-*N,N'*-diacetic acid (HBED-CC), are often used to generate functional chelating ligands for binding Ga to bioactive organic molecules primarily because they can complex[^{68}Ga]Ga^{3+} in solution at a much faster rate than other ligands. It is expected that Ga^{3+}HBED-CC may form structural isomers (enantiomers and stereo-isomers). We tested this hypothesis under different reaction conditions in solution.
Methods: After formation of complexes, $^{nat/67/68}Ga^{3+}$-HBED-CC, **I**, and $^{nat/67/68}Ga^{3+}$-Glu-NH-CO-NH-Lys(Ahx)-HBED-CC, **II** (a PSMA targeting tumor imaging agent), HPLC and LC-MS analyses were carried out.
Results: Both ligands formed $^{nat/67/68}Ga$ complexes as described in the literature [1]. However, UV/radio-HPLC and LC-MS analyses revealed (at least) two gallium complexes in the HPLC profiles; but ^{nat}Ga-complex/LC-MS revealed that these two peaks had the same molecular weight [$M + H^+$] (calc for **I**: 599.1156, found 599.1108 and 599.1076; calc for **II**: 1013.3271, found 1013.3259 and 1013.3261) indicating that structural isomers were obtained.
Conclusions: It appears that Ga^{3+}HBED-CC and Ga^{3+}Glu-NH-CO-NH-Lys(Ahx)-HBED-CC consisted of more than one $^{nat/67/68}Ga$ species with an identical and correct molecular weight. The exact chemical nature of these complexes (chiral and stereo-isomers) may be important for their biological properties and require additional investigation.

Reference

[1] Eder M, Schäfer M, Bauder-Wüst U, Hull W-E, Wängler C, Mier W, et al. Bioconjug Chem 2012;23:688–97.

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H_4octapa vs H_4C3octapa: The difference of a single carbon atom

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The ligands H_4C3octapa and *p*-SCN-Bn-H_4C3octapa were synthesized for the first time. These new ligands were compared with the previously published ligands H_4octapa and *p*-SCN-Bn-H_4octapa, to determine whether addition of a single carbon atom to the backbone of these ligand scaffolds would effect metal/radiometal chelation and stability. The In^{3+} and Lu^{3+} complexes of H_4C3octapa were synthesized, studied by NMR spectroscopy, DFT structure analysis, potentiometric titrations, and compared to the analogous H_4octapa complexes. The ^{1}H NMR spectra of [In(C3octapa)]$^-$ and [Lu(C3octapa)]$^-$ were substantially different from the analogous H_4octapa complexes by VT-NMR and 2D COSY/HSQC-NMR experiments. Evaluation of DFT structures revealed different bond lengths and geometries. The bifunctional ligands *p*-SCN-Bn-H_4C3octapa and *p*-SCN-Bn-H_4octapa were conjugated to the antibody trastuzumab, radiolabeled with ^{111}In and ^{177}Lu, and their radiochemical yields and serum stability were directly compared. The H_4C3octapa-trastuzumab conjugates displayed inferior radiochemistry properties to H_4octapa-trastuzumab. In a 5 day stability challenge in blood serum ^{111}In-octapa- and ^{111}In-C3octapa-trastuzumab were determined to be ~91% and ~24% stable, respectively, and ^{177}Lu-octapa- and ^{177}Lu-C3octapa-trastuzumab to be ~89% and ~4% stable, respectively. This work demonstrates that changing even a single carbon in a ligand can result in remarkable differences in radiochemical stability.

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Binding properties of radiolabeled cetuximab conjugates

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The monoclonal antibody cetuximab (C225) binds with high affinity to the epidermal growth factor receptor (EGFR), which is a major molecular target for treatment of different types of cancer. Radiolabeled C225 has been proven to be appropriate for cancer imaging and treatment. This study comprises an affinity comparison of different C225 conjugates incorporating *p*-SCN-Bn-NOTA (**1**), *p*-SCN-Bn-dipicolyl-TACN (**2**) and *p*-SCN-Bn-CHX-A"-DTPA (**3**). Evaluation of the K_i values using homogenates of A431 cells (EGFR^{high}/Her2^{high} expression) revealed minimal loss of affinity for these conjugates compared to unchanged C225. Saturation assays have been applied to compare the binding properties of ([^{64}Cu]Cu-**1**)$_2$-C225, ([^{64}Cu]Cu-**2**)$_2$-C225, ([^{90}Y]Y-**3**)$_2$-C225 and ([^{111}In]In-**3**)$_2$-C225 on homogenates of different cancer cell lines. The labeled conjugates were found to bind with high specificity and affinity to both the A431 and FaDu (EGFR^{medium}/Her2^{low} expression) cells, however, the affinity for the FaDu was higher than for the A431 cells. The affinity of ([^{64}Cu]Cu-**1**)$_2$-C225 and ([^{64}Cu]Cu-**2**)$_2$-C225 for both EGFR expressing cell lines was somewhat higher than that displayed by ([^{90}Y]Y-**3**)$_4$-C225 and ([^{111}In]In-**3**)$_3$-C225. No specific binding was observed in the case of the EGFR-negative MDA-MB-453S cells. Moreover, immunoreactive fractions of more than 80% were determined, indicating that the conjugates are promising candidates for further *in vivo* evaluation.

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Abstracts for Symposium on Technetium and Other Radiometals in Chemistry and Medicine (TERACHEM 2014), September 10–13, 2014

Session A1. Technetium and Rhenium in Coordination Chemistry

Oral Presentations

1

Organometallic technetium chemistry; past, present and future

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Routinely applied, metallodrugs are coordination compounds and rarely of organometallic nature, Cardiolite® being the most mentionable exception. Over the last decade, bioorganometallic chemistry grew to a prosperous field to which Tc and Re chemistry contributed essential results [1]. The "aquo-ion" $[^{99m}Tc(OH_2)_3(CO)_3]^+$ opened a realm of chemistry, fundamental and imaging oriented and in line with the concepts of bioorganometallic chemistry of stable elements. Contributions of many groups, in support of this statement, will be highlighted. Beyond pure imaging with ^{99m}Tc acting "just" as tag, new chemistry and concepts are required. One strategy, induced by cyclopentadienyl chemistry with ^{99m}Tc, points to a theranostic approach [2]. ^{99m}Tc complexes for imaging are complemented by (cold) rhenium homologues; both designed according to pharmaceutically applied lead structures [3]. Despite $[(Cp\text{-}R)^{99m}Tc(CO)_3]$ being full organometallic compounds, their syntheses are "shake and bake". Theranostics could be a future opportunity, not only inspiring fundamental $^{99(m)}Tc$ research but also keeping it alive. Basic and applied research in future must parallel each other. To give a glance at both, new results from different organometallic fields such as further η^6-coordinating ligands will round up the presentation.

References

[1] Morais GR, et al. Organometallics 2012;31:5693–714.
[2] Kelkar SS, et al. Bioconjug Chem 2011;22:1879–903.
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2

Synthesis of *fac*-$\{^{99(m)}TcO_3\}^+$ complexes: Activation of $[^{99(m)}TcO_4]^-$ by phosphonium cations

Henrik Braband, Michael Benz, Roger Alberto

University of Zurich

The reaction of high-valent *fac*-$\{^{99m}TcO_3\}^+$ complexes with alkenes via (3 + 2)-cycloaddition is an innovative approach for the synthesis of radioconjugates [1-3]. Recent developments, based on the interaction of phosphonium salts with the robust $[^{99(m)}TcO_4]^-$ anion in neutral water, led to a simple procedure for the synthesis of $[^{99m}TcO_3(tacnR)]^+$ type complexes (tacnR = 1,4,7-triazacyclononane or derivatives). Due to this new approach *fac*-$\{^{99m}TcO_3\}^+$ complexes are now available in high yields and purity for stereoselective labeling of biomolecules. The potential of the new bioconjugation strategy has been demonstrated by labeling of a series of different vectors (pharmacophores, non-natural amino acids, and carbohydrates) [4]. Furthermore, the labeling via (3 + 2)-cycloaddition has been established as a novel procedure for the labeling of silica based particles, which will help to gain more detailed *in vivo* data of silica (nano)particles by non-invasive radioimaging, in the future.

References

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[4] Wuillemin MA, et al. Dalton Trans 2014;43:4260.

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3

Imaging carbon nanotube-mediated drug delivery with ^{99m}Tc and ^{111}In

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Carbon nanotubes (CNT) are potentially versatile drug delivery platforms due to their large aspect ratio which allows for amplification of drug and imaging effects. Furthermore, this nanomaterial exhibits distinctive fibrillar pharmacology. When covalently functionalized, CNT can accommodate a numerous amount of different molecular components designed for theranostic purposes. It was demonstrated that antibodies, peptides, oligonucleotides, and other drugs can be appended covalently and non-covalently to CNT. The goal of this project is to co-image drug and CNT delivery wherein the CNT mediates delivery *in vivo*. Radiochemical methods to bind ^{99m}Tc to CNT using succinimidyl 6-hydrazinium nicotinate hydrochloride and a tricine co-ligand, rapidly and straightforwardly, are being

0969-8051

Melissa Deri

Session A2. Other Radiometals in Diagnosis and Therapy and New Labelling Strategies

Oral Presentations

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3,4,3-(LI-1,2-HOPO): An alternative chelator for ^{89}Zr radiopharmaceuticals

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^{89}Zr is an attractive radionuclide for antibody-based PET tracers because its 78.41 h half-life matches the biological residence time of IgG antibodies. Currently, antibodies are radiolabeled with ^{89}Zr using desferrioxamine B (DFO); however, the observed uptake of radioactivity in the bones of mice given ^{89}Zr-DFO-antibody constructs suggests *in vivo* release of $^{89}Zr^{4+}$ [1]. A better chelator for $^{89}Zr^{4+}$ could eliminate the release of the bone-seeking $^{89}Zr^{4+}$ cation *in vivo* and produce a safer PET tracer.

We investigate the efficacy of a hydroxypyridinone-based chelator – 3,4,3-(LI-1,2-HOPO) or HOPO – as an alternative to DFO. The Zr–HOPO complex was evaluated not only experimentally, *in vitro* and *in vivo*, but also theoretically. HOPO was found to rapidly and effectively form a 1:1 complex with $^{89}Zr^{4+}$ that exhibits remarkable stability and favorable *in vivo* clearance. The stability of ^{89}Zr-HOPO matched or surpassed that of ^{89}Zr–DFO in every experiment. Ultimately, HOPO has the potential to replace DFO for ^{89}Zr-based PET imaging agents.

Reference

[1] Deri MA, et al. Nucl Med Biol 2013;40:3–14.

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A novel multivalent bifunctional siderophore chelator scaffold for radiolabeling with gallium-68 based on fusarinine C

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The cyclic peptide siderophore triacetylfusarinine C (TAFC) showed excellent complexing properties for ^{68}Ga resulting in high specific activity and excellent metabolic stability. We postulated that, starting from its deacetylated form fusarinine C (FSC) trimeric bioconjugates are directly accessible to develop novel ^{68}Ga-labeled targeted radiopharmaceuticals. For a proof of principle, the ferric form of FSC was coupled with an $\alpha_v\beta_3$ integrin targeting RGD sequence *via in situ* activation using HATU/HOAt and DIPEA. Subsequent removal of iron allowed radiolabeling of FSC(RGD)$_3$ with ^{68}Ga resulting in high radiochemical yield (>98%) reaching SA of up to 1 TBq/μmol. The logD value was −3.6 revealing a hydrophilic character and stability studies of [^{68}Ga]FSC-(RGD)$_3$ in different media showed a high *in vitro* stability. An IC_{50} value of 1.8 ± 0.6 nM was determined indicating a high binding affinity for $\alpha_v\beta_3$ integrins. The internalized activity was 5.3% cpm/mg protein for $\alpha_v\beta_3$ positive M21 cells which was reduced to approximately 1/25 of the reference activity via addition of c(RGDyV). Biodistribution studies in nude mice resulted in an uptake in the $\alpha_v\beta_3$ positive tumor of 4.3% ID/g 60 min after injection, compared to 1.13% in receptor negative tumours, more than 3-fold higher than the monomeric ^{68}Ga-NODAGA-RGD in the same tumour model (1.35%). [^{68}Ga]FSC-(RGD)$_3$ served as an example for the feasibility of a novel multivalent bifunctional chelator based on cyclic peptide siderophores and showed excellent targeting properties for $\alpha_v\beta_3$ integrins.

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Synthesis of tridentate ligand: Potential theranostic application of a radioarsenic trithiol complex

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Arsenic-72 is a 26 h half-life positron emitter (2.49 MeV) with nuclear properties useful for diagnostic imaging by positron emission tomography (PET). Arsenic-77 is a 38.8 h half-life beta emitter (683 keV) potentially useful for radiotherapy applications. Radioisotopes of arsenic have half-lives suitable for use with antibodies as targeting vectors while traditional radionuclides, ^{11}C, ^{18}F, ^{64}Cu, and ^{86}Y, do not have sufficiently long half-lives (up to 4 days) to permit chemical derivatization and in vivo localization for radioimaging or radiotherapy. Utilization of these radioisotopes requires the development of compounds with high *in vivo* stability for conjugation to the targeting probes. Direct complexation of arsenic using a trithiol chelate may provide a simplified route for the development of a linkable radioarsenic complex. A simple trithiol was synthesized and characterized by ^{1}H and ^{13}C NMR, electrospray ionization mass spectrometry (ESI-MS), and single crystal X-ray diffraction. Radiotracer studies with no-carrier added ^{77}As resulted in high radiolabeling yield (>96%); its stability was evaluated by cysteine challenge.

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Robust and efficient bifunctional chelators for ^{64}Cu to target $\alpha_v\beta_3$ integrin

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The choice of bifunctional chelator (BFC) and radioisotope is crucial for imaging of biological function. ^{64}Cu ($t_{1/2}$ = 12.7 h) is a β^+ and β^- emitter, making it useful for both imaging and radiotherapy. The accuracy of imaging with ^{64}Cu often depends on BFC and hence, the importance of BFC cannot be overemphasized in terms of Cu–BFC complex robustness in vivo.

We have previously reported propylene cross-bridged TE2A (PCB-TE2A) chelator, which showed exceptional kinetic inertness and better radiolabeling profile than widely used CB-TE2A. Recently, a new tetraazamacrocyclic chelator TE2A-Bn-NCS, with two acetic acid pendant arm for strong Cu(II) complexation and an extra NCS pendant arm for easy conjugation with biomolecule, was

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PCB-TE2A-NCS: A cross bridged BFC for ^{64}Cu-based radiopharmaceuticals

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Bifunctional chelator (BFC) is a key component in developing ^{64}Cu-based imaging agents. For the successful development of ^{64}Cu-based imaging agents, the BFC is required to make facile and strong conjugation with biomolecule as well as to hold radiometal ions firmly in physiological conditions.

Previously, we have reported that propylene cross-bridged TE2A (PCB-TE2A) could form more stable Cu complex than ECB-TE2A and could be utilized as a BFC in the same way as ECB-TE2A. However, upon conjugation with biomolecule, one of its acetic acid pendant arms would be consumed and would not be available for strong coordination bonding to Cu(II) ion. In addition to this, the overall charge of the Cu-BFC-conjugate will also change from neutral to +1, which leads to retarded body clearance.

Here, we report a new designed propylene cross-bridged cyclam derivative (PCB-TE2A-NCS), in which an extra functional group (NCS) for easy conjugation was introduced on the propylene cross-bridge to keep both carboxylate groups for strong coordination to Cu(II) ion. Our synthetic strategy was proven to be highly efficient in terms of overall yield and total synthesis time compared with previously reported cross-bridged BFCs. PCB-TE2A-NCS was also conjugated with a cyclic RGD peptide through the NCS functionality and radiolabeled with ^{64}Cu ions. The in vivo targeting affinity of ^{64}Cu-PCB-TE2A-NCS-c(RGDyK) was examined in nude mice bearing U87MG xenografts.

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Peptide conjugates for EGFR-targeting

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We have synthesized ^{64}Cu-labelled peptide conjugates based on a 1,4,7-triazacyclononane (TACN) framework that may be applied for in vivo PET imaging. A peptide sequence (LARLLT, "D4") was used to target the epidermal growth factor receptor (EGFR). Overexpression and mutations of this cell-surface receptor are involved in carcinogenesis and progression of many human cancers.

Four different linker groups were introduced to influence solubility and lipophilicity. The TACN-peptide conjugates are obtained in high yields after purification by RP-HPLC. Radiolabelling with ^{64}Cu(II) was rapidly achieved under mild conditions (pH = 5.5; 22 °C). The receptor binding abilities of the labelled conjugates have been evaluated using immunoprecipitation and by determination of the dissociation constants, revealing only weak interactions (K_d >100 nM) compared to its native ligand epidermal growth factor (EGF; $K_d = 0.04 \pm 0.002$ nM). However, it was not determined if the "D4" peptide alone could target the EGFR.

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Polyacrylamide nanogel systems with ^{64}Cu chelating cross-linkers for PET imaging

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In recent years, increasing attention has been given to nanotechnologies within the fields of molecular imaging combined with drug delivery. We recently reported the design and synthesis of metal-chelating crosslinkers enabling formulation of polyacrylamide hydrogels (nanogels) for magnetic resonance imaging (MRI); however, a major drawback of this imaging modality is lack of sensitivity. To overcome this limitation, nanogels were formulated containing DTPA, DOTA, and NOTA metal chelators for ^{64}Cu-based PET imaging. ^{64}Cu transchelation studies against EDTA showed that NOTA containing nanogels have superior stability in comparison with DOTA and DTPA after 24 h (NOTA = 90%, DOTA = 61%, DTPA = 0%). We then chose to compare in vivo PET imaging performance of DOTA and NOTA chelator-containing nanogels as a platform for passive tumor targeting of mouse mammary carcinoma xenografts via the enhanced permeability and retention (EPR) effect. Both NOTA and DOTA nanogel systems showed greater contrast in tumor tissue in comparison to free ^{64}Cu after 24 and 48 h post probe injection. More significantly, NOTA nanogels showed higher uptake in the tumors at 48 h in comparison to DOTA systems (NOTA Tumor/Muscle = 9.0, DOTA Tumor/Muscle = 5.7) suggesting that the enhanced stability of the ^{64}Cu-NOTA complex results in a more promising nanogel formulation. These data show that NOTA-based nanogels show promise for future studies utilizing targeted nanogels for theranostics combining PET, MRI, and drug delivery.

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The site-specific radiometallation of antibodies on the heavy chain glycans

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Immunoconjugates labeled with radiometals have emerged as critical tools in nuclear medicine. However, the lack of site-specificity in traditional methods for the attachment of chelators can lead to poorly-defined constructs and impair immunoreactivity. To circumvent these issues, we have developed a chemoenzymatic system for the site-specific radiolabeling of antibodies on the heavy chains. The methodology consists of four steps: (1) the removal of terminal galactose residues on the antibody heavy chain using β-1,4-galactosidase; (2) the incorporation of azide-modified galactosamine residues using a substrate-promiscuous galactosyltransferase; (3) the strain-promoted click conjugation of chelator-modified dibenzocyclooctynes to the azide-modified sugars; and (4) the radiolabeling of the resulting antibody construct.

We have successfully validated this system in a variety of settings, including the construction of a ^{89}Zr-labeled variant of the PSMA-targeted antibody J591 and the synthesis of ^{89}Zr- and ^{177}Lu-labeled constructs based on the colorectal cancer-specific antibody huA33. We have also expanded beyond radiometals to create immunoconjugates site-specifically labeled with radiohalogens, fluorophores, or a combination of reporters for multimodal imaging. Ultimately, we believe that this methodology could have a transformational impact on the development of diagnostic and therapeutic immunoconjugates for both the laboratory and the clinic.

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Aranh Pen

Harvesting radioisotopes at a Projectile Fragmentation Facility

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A remotely-operated liquid water target system for harvesting radioisotopes at the National Superconducting Cyclotron Laboratory (NSCL) was designed and constructed as the initial step in proof-of-principle experiments to harvest useful radioisotopes from the Facility for Rare Isotope Beams (FRIB). FRIB will be a new national user facility for nuclear science to be completed in 2020 at which radioisotopes can be collected synergistically from the water in cooling-loops for the primary fragmentation target. To develop the radiochemical expertise required to harvest long-lived radioisotopes of interest in this environment, the water target system was constructed and has been successfully used to collect beams of ^{24}Na and ^{67}Cu ions produced at the NSCL. Initial experiments included collection of an analyzed ^{24}Na test beam and collection and extraction of analyzed ^{67}Cu, a radioisotope with medical applications. The collected radioisotopes were characterized using low-background gamma spectroscopy at both Hope College and Washington University. The analyzed ^{67}Cu, counted offline, indicated that over 80% of the beam produced at the focal plane was delivered into the water cell. Analysis of the extraction technique indicated over 95% of the delivered copper isotope was successfully removed from the water, and made available for antibody labeling. The final test run, where ^{67}Cu was delivered with a higher rate of unanalyzed beam, contained a cocktail of contaminant beam particles that had to be radiochemically separated from the isotope of interest. The unanalyzed ^{67}Cu collection, with a beam rate ~100 times more than the analyzed ^{67}Cu collection, required circulation of the water in the target during beam collection to minimize localized heating. Preliminary results from each of these experiments will be presented.

Zhengtao Qin

Radiolabelled peptides with beta negative emitters are used in peptide receptor radionuclide therapy (PRRT) for the treatment of various tumours. Radiolabelled analogues of cholecystokinin/gastrin family showed promising results for PRRT in tumour expressing CCK2 receptors.

The aim of this work was to obtain two minigastrin analogues (DOTA-cMG(Nle) and DOTA-cMG(Met)) radiolabelled with 177-Luthetium and compare if the amino acid substitution affect the internalisation properties in A431 cells expressing CCK2R receptors.

Radiolabelling of DOTA-cMG(Nle) and DOTA-cMG(Met) with $^{177}LuCl_3$ locally produced (S.A. 18.97 mCi/μg of ^{176}Lu) was performed incubating 1 h at 80 °C, pH 5.7. The final specific activity for both radiopharmaceuticals was 0.067 mCi/μg of peptide. Internalisation assays were performed in receptor expressing cell line A431 (CCK2+) and A431 (CCK2−) as negative control.

Results: Radiochemical purity (Sep-Pak C18) were 93.8 and 99.6% for ^{177}Lu-DOTA-cMG(Nle) and ^{177}Lu-DOTA-cMG(Met), respectively, and retention times (HPLC-RP) were 11,1 and 11,5 min, respectively.

Both compounds showed a high internalization rate, 19.3 and 17.3% of cell bound measured/mg protein for ^{177}Lu-DOTA-cMG(Nle) and ^{177}Lu-DOTA-cMG(Met), respectively, after 2 h incubation. The blocked control with human gastrin I showed less than 1% for both radiopeptides, indicating receptor specific internalisation.

These results are similar to those reported by other researchers (COST Action BM0607) with ^{111}In-DOTA-cMG(Met) (20% A/mg protein).

In conclusion, according to these results, internalization properties aren't affected by the amino acid substitution of methionine for norleucine.

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Effects of Gly^{11}/DAla^{11}-replacement in GRPR-antagonist based radioligands

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Radiometallated bombesin (BBN) analogs deliver diagnostic or therapeutic radiation specifically to GRPR-positive tumors, such as prostate and breast cancer. Based on the reported higher bioavailability of DAla^{11}-substituted BBN analogs, we developed two new GRPR-antagonists, SB3 = DOTA-PABZA-DIG-[DPhe^{6},Leu-NHEt^{13}]BBN(6–13) and SB4 = |DAla^{11}|SB3. We were interested to reveal potential advantages of this single Gly^{11}/DAla^{11}-replacement by comparing the biological profiles of the two analogs and their ^{111}In-radioligands. Competition binding assays in PC-3 cell membranes against |^{125}I-Tyr^{4}|BBN showed that Gly^{11}/DAla^{11}-replacement deteriorated GRPR-affinity (IC_{50}s, SB3: 4.6 ± 0.3 nM, SB4: 11.2 ± 1.2 nM). During incubation in PC-3 cells (1 h/37 °C) radioligands did not internalize, as expected for radioantagonists, but remained bound on the cell surface (|^{111}In|SB3 12.7% and |^{111}In|SB4 1.2%). Radiopeptides were injected in mice and blood collected 5 min post-injection (pi) was analyzed by RP-HPLC to assess in vivo degradation. The DAla^{11}-analog |^{111}In|SB4 showed higher in vivo stability (77% intact) than |^{111}In|SB3 (55% intact). During biodistribution in SCID mice bearing PC-3 xenografts at 4 h pi unmodified |^{111}In|SB3 showed significantly higher uptake in the GRPR-positive tumors (15.3 ± 2.2% ID/g) compared to DAla^{11}-substituted |^{111}In|SB4 (3.1 ± 1.1% ID/g). In conclusion, replacement of Gly^{11} by DAla^{11} improved in vivo stability, however, at the cost of receptor-affinity, and eventually translated into unfavourably lower tumor uptake of |^{111}In|SB4 in animal models.

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Radiolabelling and preliminary biological evaluation of NOTA-cRGD dimer labelled with Ga-68

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The $\alpha_v\beta_3$ integrin receptor, expressed on tumor cell membranes can be preferentially targeted by peptides containing the RGD sequence, resulting in a versatile cell recognition system. NOTA-SCN-Bn-E-[c(RGDyK)$_2$] was labelled with Ga-68 and tested for radiolabelling yield, purity, stability, in vitro binding and ex vivo biodistribution. The radiolabelling was performed using an automated system with inline quality control and Ga-68 eluate from a tin oxide based Ge-68/Ga-68 generator, purified and concentrated to 400 MBq in 0.1 mL water on an anionic exchanger resin. 40 nanomoles of peptide was labeled and purified, RCP >98%. Elution, concentration, labeling and purification procedures took less than 25 min. The ex vivo biodistribution of ^{68}Ga-NOTA-SCN-Bn-E-[c(RGDyK)$_2$] was tested in tumor bearing animal models (melanoma, AR42J, Walker, Guerrin) showing high and stable tumor uptake up to 11.6%ID/g in melanoma. The blood clearance is fast, the renal elimination is more rapid than in the case of ^{68}Ga-DOTA-E-[c(RGDfK)$_2$], while higher tumor to background ratios were observed. Real-time quantification of biomolecular interactions was also performed: the binding to receptors expressed on tumor cells surface was achieved in the first 3 min of incubation and remains stable for 30 min. In conclusion ^{68}Ga-NOTA-SCN-Bn-E-[c(RGDyK)$_2$] shows promising characteristics as radiotracer for PET imaging.

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A tri-modal tilmanocept for sentinel lymph node mapping

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Introduction: We report a novel method to prepare ^{68}Ga and ^{99m}Tc dual-labeled IRDye800CW-tilmanocept, a sentinel lymph node (SLN) targeting agent, for tri-modal molecular imaging.

Methods: Solutions of NaAc, $^{68}GaCl_3$ and $Na^{99m}TcO_4$ were added successively to an "instant kit" containing lyophilized *IRDye800CW*-tilmanocept (8 nmol), $SnCl_2$ (0.16 mg), trehalose (20 mg) and ascorbic acid (0.5 mg). After a 30-min incubation, the pH was adjusted to 7 with PBS. No purification was performed. Radiochemical purity was measured by HPLC and radio-ITLC techniques. A dose (0.11 nmol, 30 μCi ^{68}Ga, 700 μCi ^{99m}Tc) was injected to the footpad of 4 mice. Popliteal SLNs were imaged by *VistaDR* microPET at 0.5 h and *OptiMX* systems at 0.5, 24, 48, 72 h, then excised and assayed for ^{99m}Tc.

Results: Radiochemical and fluorescent purity exceeded 98%. Popliteal SLN was identified both on PET and fluorescence images at 0.5 h. Fluorescence remained strong at 72 h, when ~1% of the injected dose resided in the SLN.

Conclusions: Tri-modal tilmanocept provides quantitative pre-

describe the synthesis, characterization and biological evaluation of novel complexes of the type *fac*-[Re/^{99m}Tc(CO)$_3$(κ^3-L)]. **Re/^{99m}Tc1**, **Re/^{99m}Tc2**, **Re/^{99m}Tc3**. Ligands **L1–L3** were synthesized by incorporating a different tridentate chelator on the bone-seeking pharmacophore 1-(3-aminopropylamino)ethane-1,1-diyldiphosphonic acid, di-(2-picolyl) amine in **L1**, iminodiacetic acid in **L2** and 2-picolylamine-N-acetic acid in **L3**. **Re1.1–Re1.3** complexes were synthesized and characterized spectroscopically. **^{99m}Tc1.1–^{99m}Tc1.3** were prepared in high yield and were identified by comparative RP-HPLC studies using **Re1.1–Re1.3** as references. They were stable *in vitro* over 24 h and showed high hydroxyapatite binding (**^{99m}Tc1.1** 90%, **^{99m}Tc1.2** 85% and **^{99m}Tc1.3** 68% in 5 mg/ml HA at 1 h). Biodistribution studies in mice revealed that all ^{99m}Tc complexes exhibited fast blood/tissue clearance and high bone uptake (19.44 ± 1.44 for **^{99m}Tc1.1**, 12.19 ± 1.77 for **^{99m}Tc1.2** and 13.79 ± 0.68 for **^{99m}Tc1.3** % I.D./g at 1 h post injection) comparable to ^{99m}Tc-MDP (15.63 ± 0.65% ID/g at 1 h p.i.). The promising properties of these compounds are encouraging for further evaluation that will determine their potential as bone imaging agents.

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New ^{99m}Tc-radioimmunoconjugates for pancreatic carcinoma detection

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Pancreas carcinoma is responsible for more than 30% of tumor-related death because it is notoriously difficult to diagnose; thus, new diagnostic approaches are imperatively needed. Recently, prostate stem cell antigen (PSCA) and mesothelin demonstrated high expression and wide distribution in pancreatic cancer, but not in normal pancreas. This research aims to develop new radioimmunoconjugates (RICs) for pancreatic cancer detection based on monoclonal antibodies (mAb) to PSCA and mesothelin, which are heavily overexpressed in this tumor histotype. Either mAbs labeled with fluorophore exhibited high recognition capacity for Ag^+ tumor-cells both *in vitro* and *in vivo*, as assessed by cytometry analysis and optical imaging. ^{99m}Tc-radioimmunoconjugates were obtained with high labelling efficiency (98%), by reduction of both mAbs with 2-mercaptoethanol and incubation with $^{99m}TcO_4^-$ in presence of a weak competition ligand. Stability tests performed in saline solution and human serum demonstrated that RICs are stable for at least 48 h.

Both mAbs were radiolabeled by direct method with a high radiochemical efficiency and stability, and could be used for pancreatic cancer detection.

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Development and preliminary biological study of radiopharmaceutical for radiosynovectomy labelled by ^{188}Re

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Radiosynovectomy is a therapy used to relieve pain and inflammation from rheumatoid arthritis and related diseases. Progression of the disease leads to the destruction of the joint or loss of function. In this study the ^{188}Re-Sn suspension was synthesized and characterized according to its physico-chemical properties and biological behavior in rabbits.

In this study ^{188}Re is the chosen radionuclide as it is readily available on routine basis from the ^{188}W/^{188}Re generator (Obninsk, Russia). The suspension was prepared in two stages. The first step is recovery of rhenium-188 in an acidic medium, the second – the pH adjustment is carried out with phosphate buffer solution. To stabilize the suspension, a solution of polysorbate 80 was added. The radiochemical purity was determined by TLC on silica gel in acetone. The particle sizes were determined by laser scattering method on NICOMP™ 380 ZLS. All animal experiments were conducted in compliance with the animal protection laws and with the ethical principles and guidelines for scientific animal trials. The scintigraphic images were acquired with a SPECT/CT (Philips) at 24 and 72 h after intraarticular administration of suspension to rabbits.

We successfully synthesized ^{188}Re-Sn suspension, which is stable for more than 3 days. The optimal conditions for effective labeling (>95%) were found. About 90% of particles in the synthesized suspension were lower than 9.3 μm. The scintigraphic images of the rabbits, at 24 h and 72 h post administration, indicate relevant activity only in the knee.

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Synthesis of novel rhenium and technetium N_2O_2 schiff base complexes

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Technetium-99m Schiff base complexes, more commonly known as the "Q-series", have been considered for use as single photon emission computed tomography (SPECT) imaging agents. Rhenium is often a structural analogue to technetium and has isotopes ideal for radiotherapy and imaging, therefore Re- and Tc-Schiff base chemistry has been investigated.

Reacting $(Bu_4N)[ReOCl_4]$ with the tetradentate Schiff Base ligand α,α′-[(1,1-dimethylethylene)dinitrilo]di-*o*-cresol (sal_2ibnH_2) yields *cis*-$[Re^{V}OCl(sal_2ibn)]$, which quickly forms *trans*-$[\mu\text{-}O(Re^{V}O(sal_2ibn))_2]$ in solution. The mononuclear complex can be trapped as *cis*-$[Re^{V}O(NCS)(sal_2ibn)]$ by addition of $(Bu_4N)SCN$ to the reaction mixture. Reduction of *cis*-$[Re^{V}O(NCS)sal_2ibn]$ with triphenylphosphine gives the unique *trans*-$[Re^{III}(NCS)(PPh_3)(sal_2ibn)]$ and *trans*-$[\mu\text{-}O(Re^{IV}(NCS)(sal_2ibn))_2]$. Reaction of $(Bu_4N)[TcOCl_4]$ with sal_2ibnH_2 yields *trans*-$[\mu\text{-}O(Tc^{IV}(Cl)(sal_2ibn))_2]$. The mononuclear species, *cis*-$[Tc^{V}OCl(sal_2ibn)]$, can be trapped similar to the Re complex by the addition of $(Bu_4N)SCN$. The mononuclear NCS complexes have shown excellent stability and translation to the radiotracer level is underway.

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Synthesis of ^{99m}Tc analogue of $^{123/131}$I-mIBG for possible use in neuroendocrine tumor imaging

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other hand, molecular imaging investigations using [RGD-Glu-[^{64}Cu-NO2A]-6-Ahx-RM2] produced high-quality, high contrast images in PC-3 tumor-bearing mice at the 4 h time-point.
Conclusions: This study describes dual GRPR/PSMA- and GRPR/$\alpha_v\beta_3$-targeting radioligands and indicates [RGD-Glu-[^{64}Cu-NO2A]-6-Ahx-RM2] to be a potential candidate for translation into human patients.

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^{89}Zr labeling and preliminary evaluation of a trimeric RGD peptide based on a novel siderophore derived chelating scaffold

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Within the last years ^{89}Zr has attracted increasing attention as long lived PET radionuclide. So far the bifunctional chelating system employed for ^{89}Zr-applications is desferrioxamine B (DFO). Fusarinine C (FSC), a cyclic peptide siderophore, could be an alternative with potentially higher stability due to its cyclic structure, having complexing properties comparable to DFO. As proof of principle in this study ^{89}Zr-labelling of RGD-derivatised FSC, the optimization of analytical procedures and preliminary evaluation of this compound are reported. High radiochemical yield (>96%) for ^{89}Zr-FSC-$(RGD)_3$ could be achieved at high SA. HPLC was used for characterization of ^{89}Zr-FSC$(RGD)_3$, but was not suitable for determination of RCP. Using TLC best separation properties were achieved using ITLC-SG and CH_3COOH/H_2O (1:9) as a mobile phase. *In vitro* characterization of [^{89}Zr]FSC-$(RGD)_3$ showed comparable properties to the ^{68}Ga-counterpart with a hydrophilic character (logD value of −2.9) and low serum protein-bound activity. [^{89}Zr]FSC-$(RGD)_3$ was stable in PBS (pH 7.4), in $FeCl_3$-solution as well as in fresh human serum, in DTPA-solution at 37 °C at 24 hours a slight degradation of the ^{89}Zr-peptide was found. Cell internalized activity was 2.07% cpm/mg protein for $\alpha_v\beta_3$ positive M21 cells which could be reduced to 0.23% cpm/mg protein via addition of c(RGDyV) revealing specific receptor binding. These results indicate very promising properties of the fusarinine C scaffold as basis for multimeric targeting constructs for labelling with ^{89}Zr. Biodistribution experiments are currently ongoing.

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Enabling simultaneous imaging and treatment with the theragnostic radionuclide Tin-117 m

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The high-LET conversion electron (C.E.) emitter Sn-117 m ($t_{½}$ 14 d, γ 159 keV, 86%) shows considerable promise for the non-invasive molecular imaging and treatment of inflammatory diseases including cancer, and of atherosclerosis caused by vulnerable plaques (VP) in the coronary and carotid arteries that when ruptured cause significant cardiac events (~70%) leading to MI and sudden death. The C.E. from Sn-117 m is ideal for treating VPs, as their discrete range in tissue (~300 μm) is approximately the same as the VP thickness in human carotid and coronary arteries. We have developed and used (i) Sn-117 m electroplated coronary stents (Sn-117 m stents), and (ii) Sn-117 m-DOTA-Annexin V [TA] for evaluating the possibility of simultaneous imaging and therapy of VP with this dual-purpose (theragnostic) radionuclide.

Histochemical analysis of proliferating macrophages and smooth muscle cells in a hyper-lipidemic rabbit model, 3 d after Sn-117 m-stent implantation [4 doses: 0 (cold tin), 30, 60, and 150 μCi Sn-117 m per stent] showed that inflammatory cells in the Sn-117 m-stented segments were dramatically reduced in a dose-dependent manner. Recent studies in an Apo-E mouse VP model with systemically administered TA have also showed a significant therapeutic effect. A phase I/II clinical trial with TA, in which human carotid endarterectomy patients were dosed and imaged for VP, with histology as the comparison, has demonstrated promising results.

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Targeting Gastrin-Releasing Peptide Receptor-Positive Tumors using Yttrium-86 labeled DOTA-Bombesin(7-14) Analogs

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The gastrin-releasing peptide receptor (GRPR) is overexpressed on a variety of human cancers including breast and prostate. Bombesin (BN) is a fourteen amino acid neuropeptide that binds with high affinity to GRPR. Our laboratory was amongst the first to evaluate BN analogues radiolabelled with positron-emitting radionuclides for imaging by positron-emission tomography (PET). The goal of this study was to evaluate DOTA-linker-BN(7–14) analogues radiolabelled with ^{86}Y ($t_{½}$ = 14.7 h, β^+ = 33%, E_{avg} = 664 keV) to determine the effect of using ^{86}Y in place of ^{64}Cu on tumor and normal tissue uptake. We hypothesize that imaging using the ^{86}Y analogs would give better dosimetry estimates for their ^{90}Y therapeutic counterparts as compared to ^{64}Cu and may also reduce background signal in non-target tissues. In this study BN(7–14) analogues comprising two unique amino acid linkers (gly-ser-gly and gly-ser-ser) have been radiolabelled with ^{86}Y and evaluated for internalisation into PC-3 cells. These results show a rapid and specific uptake of both analogues over 24 h. Studies to evaluate these compounds in mice bearing PC-3 tumor xenografts by biodistribution and small animal PET imaging are ongoing.

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Synthesis and evaluation of [^{67}Ga]-AMD3100; A novel imaging agent for targeting chemokine receptor CXCR4

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In order to develop a possible CXCR4 imaging agent for oncological scintigraphy, [^{67}Ga]labeled 1,1′-[1,4-phenylenebis(methylene)] bis-1,4,8,11-tetraazacyclo-tetradecane ([^{67}Ga]-AMD3100)

of octanol/water partition coefficients suggested that [^{64}Cu]Cu-NOTA-NAP-NS1 had high hydrophilicity, and in buffer and serum it was stable after 1 h and 24 h. NAP-NS1 and NOTA-NAP-NS1 showed higher affinity than the cyclic derivatives. Linking the chelate unit at the peptide was accompanied by some loss of affinity. Saturation studies with the labeled peptide resulted in K_d values in the lower nanomolar range for [^{64}Cu]Cu-NOTA-NAP-NS1 and [^{68}Ga]Ga-NOTA-NAP-NS1, respectively. Thus, both radiolabeled peptides appear to be promising for further investigations in animal melanoma models.

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Evaluation of [^{68}Ga]-DOTA ghrelin (1–19) in LNCaP prostate carcinoma

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Ghrelin is a 28-amino acid peptide that is the endogenous ligand for the growth hormone secretagogue receptor-1a (GHSR-1a). GHSR-1a is highly expressed in prostate cancer and previous work has demonstrated that ghrelin distinguishes between healthy, benign and cancerous prostate tissue *ex vivo*. We have developed a DOTA ghrelin (1–19) analogue capable of targeting the GHSR-1a. Using orthogonal protecting groups, diaminopropanoic acid-3 and lysine-19 allowed for functionalization of the peptide with octanoic acid and DOTA respectively. A gallium standard was prepared by complexing $^{69/71}$Ga with purified DOTA-ghrelin (1–19). The IC_{50} was 9.1 nM compared to 8.1 nM for native ghrelin (1–28). Optimised radiolabelling of DOTA-ghrelin (1–19) yielded specific activities >22 GBq/μmol. *In vitro* studies using HEK293/GHSR-1a cells showed uptake of ^{68}Ga-DOTA ghrelin (1–19) that was decreased in the presence of GHSR-1a blocking agent. A one hour dynamic μPET scan showed localisation of ^{68}Ga-DOTA ghrelin (1–19) as early as 10 min in NOD/SCID mice bearing LNCaP tumours although washout was observed by one hour suggesting low *in vivo* stability. Administering blocking agent significantly reduced tumour uptake visualised at 10 min. In conclusion, we have developed a ^{68}Ga-labelled ghrelin (1–19) analogue demonstrating specificity to GHSR-1a using *in vitro* and *in vivo* prostate cancer models.

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$^{64}CuCl_2$: New theranostic agent

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$^{64}CuCl_2$ has a high tumor uptake [1]. This uptake is due to an overexpression in tumor cells of the copper transporter CTR1 [2] and probably for the increase of DNA activity replication. Our aim is to evaluate the potential of $^{64}CuCl_2$ as theranostic agent in two different lines of tumors: prostate cancer and uterine cancer. For this reason we examined two different patients in metastatic phase using case report method. These patients presented diagnosis of metastatic lesions studied with FDG PET/CT scan and CT scan. We examined in diagnostic phase these patients with $^{64}CuCl_2$ (370 MBq) and compared the pictures with FDG and CT. These patients were administered chemotherapy and were in non responder phase. We injected treatment dose of 3700 MBq and evaluated the results. We observed a significant reduction in volume of lesions and an increase in wealth general conditions. These preliminary data confirm the theory of potential role of $^{64}CuCl_2$ as a real theranostic agent of new generation.

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Preclinical application of ^{47}Sc-folate – A pilot study in tumor-bearing mice

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Purpose: The aim of this study was to investigate the use of ^{47}Sc ($T_{1/2}$ = 3.35 d) for β$^-$-radionuclide therapy ($E\beta^-_{av}$ = 162 keV) and for SPECT imaging (Eγ = 159 keV) using a DOTA-folate conjugate.
Methods: ^{47}Sc was produced via the ^{46}Ca(n,γ)^{47}Ca->^{47}Sc nuclear reaction. Separation of ^{47}Sc from the target material was performed by chromatography. ^{47}Sc-folate was investigated in vitro using folate receptor (FR)-positive KB tumor cells. The therapy study was conducted with mice bearing KB tumor xenografts. One group was treated with ^{47}Sc-folate (10 MBq) whereas control mice received only saline. SPECT/CT imaging studies were also performed.
Results: ^{47}Sc was separated from Ca twice a week. It was obtained in a solution for direct radiolabeling which yielded ^{47}Sc-folate with a radiochemical purity of >96%. In vitro, ^{47}Sc-folate showed FR-specific binding to KB tumor cells. A significant tumor growth delay and an increased median survival time (38.5 d) were observed in treated mice compared to untreated controls (25 d). The SPECT/CT scans confirmed high uptake of ^{47}Sc-folate in tumors and indicated excellent features of ^{47}Sc for imaging purposes.
Conclusion: In this pilot study we demonstrated the excellent characteristics of ^{47}Sc for therapy and for SPECT. These findings along with our previous results obtained with the PET radionuclide ^{44}Sc show great promise for the future application of the $^{44/47}$Sc matched pair concept in nuclear medicine.

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^{89}Zr immuno-PET of epithelial ovarian cancer

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[^{99m}Tc(N)PNP]-scaffold for SPECT of multidrug resistance: Early in-vitro study

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^{99m}Tc(N)-DBODC(PNP5) [DBODC = bis(N-ethoxyethyl)dithiocarbamato; PNP5 = bis(dimethoxypropylphosphinoethyl)ethoxyethylamine] is a cationic mixed-compound, originally investigated as myocardial imaging agent, identified as suitable scaffold to devise ^{99m}Tc-agents for SPECT of multidrug resistance (MDR).

To evaluate the impact of the [^{99m}Tc(N)PNP]-moiety on the tumor cell accumulation and on MDR recognition, different ^{99m}Tc(N)-DBODC(PNP5) like complexes were synthesized varying the substituents on the PNP ligand [PNPn = PNP3 bis(dimethoxypropylphosphinoethyl)-methoxyethylamine; PNP7 (bis(dimethoxyethylphosphinoethyl)-ethoxyethylamine); PNP10 (bis(dimethoxyethylphosphinoethyl)-methoxyethylamine)]. The ^{99m}Tc(N)-DBODC(PNPn) uptake was evaluated in human cancer cell lines (MCF7 and MCF7/ADR), and in the corresponding sub-lines using ^{99m}Tc-sestambi and ^{99m}Tc(N)-DBODC(PNP5) as references.

A significant increase of %cell-uptake of ^{99m}Tc(N)-DBODC(PNP7) and ^{99m}Tc(N)-DBODC(PNP10) was observed in drug-sensitive cell lines with respect to ^{99m}Tc-sestamibi and ^{99m}Tc(N)DBODC(PNP5). This amount was two and three times the %cell-uptake of the reference compounds. A reduction of the net cell uptake between drug-sensitive and drug-resistant cell lines was detected ($p < 0.001$).

Changing chemical–physical properties of [^{99m}Tc(N)PNP]-moiety significantly affects the capability of the complexes to cross the plasma membrane increasing their %cell-uptake and affinity for MDR transporters. ^{99m}Tc(N)-DBODC(PNP7) and ^{99m}Tc(N)-DBODC(PNP10) are good candidates for in-vivo exploration of MDR.

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Aptamer-HYNIC-^{99m}Tc: A molecular imaging agent of PTK7

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Aptamers are single-stranded oligonucleotides that recognize molecular targets with high affinity and specificity. The unique molecular recognition properties of aptamers are being developed to image protein tyrosine kinase-7 (PTK7), a member of the receptor tyrosine kinase family, over expressed on many cancers. In this work, a DNA aptamer against PTK7 was coupled with HYNIC(Tricine) and radiolabeled with technetium-99m. Physicochemical and biological controls were assayed in acute lymphoblastic leukemia CCRF-CEM cells. Aptamer-HYNIC-^{99m}Tc specifically bound to CCRF-CEM cells and was stable for 24 h in-vitro. Pharmacokinetic studies demonstrated rapid blood clearance. Biodistribution studies were performed with Aptamer-HYNIC-^{99m}Tc in normal Balb-c mice at 0.5 h, 2 h, 4 h and 24 h post-injection. Liver and kidney uptake values were 10.68 ± 1.43% ID/g and 12.56 ± 0.98% ID/g 2 h post injection, respectively. Uptake of radioactivity was not significant in the lungs and spleen. Urinary excretion was 45.33 ± 5.2% ID at 2 h and gradually increased to 68.39 ± 9.73% ID at 24 h. The preliminary studies of Aptamer-HYNIC-^{99m}Tc indicated good pharmacokinetic properties and warrant further investigation as a cancer molecular imaging agent.

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Thiocarbamoylbenzamidines for bioconjugation of Re and Tc

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Thiocarbamoylbenzamidines are suitable ligand systems for Re and Tc [1,2]. However, no studies regarding their suitability for bioconjugation with ^{99m}Tc have been published so far. We have designed different complexes of Re and ^{99}Tc with thiocarbamoylbenzamidines, which possess propargylic or carboxylic groups available for bioconjugation. ^{1}H-NMR studies of the click-coupling products with model molecules show the disappearance of the propargylic signal and the formation of the triazole ring as confirmed by X-Ray crystal analysis. Re, ^{99}Tc and ^{99m}Tc-bioconjugates have also been synthesized. Biodistribution studies are being undertaken.

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Biological evaluation of two glucose derivatives radiolabeled with ^{99m}Tc as potential cancer imaging agents

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The aim of this work is to develop two glucose derivatives radiolabeled with ^{99m}Tc in order to obtain ^{18}FDG analogs for SPECT. Both derivatives were designed to contain an IDA-like chelator for complexation with ^{99m}Tc attached to glucose anomeric carbon (C1) or C2. Radiolabeling with ^{99m}Tc of compounds was accomplished by direct labeling with high radiochemical purity controlled by HPLC. Complexes also probed to be highly stable in time until 5 hours of radiolabeling and hydrophilic according to LogP values. ^{18}FDG internalization studies in B16F10 murine cell line and competition