MEETING ABSTRACTS

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ORAL PRESENTATIONS

OP03
Selective extraction of medically-related radionuclides from proton-irradiated thorium targets
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Background: Clinicians rely on nuclear medicine for the treatment of numerous diseases impacting millions of patients annually. Recently, Targeted Radiotherapy (TR) has been successfully advanced with the US FDA approval of several radionuclide-based drugs. Combinations of several types of radionuclide emissions for therapy (i.e., α, β therapeutic) could lead to even more effective treatment options. One of the limiting factors in the development of TR as a widely adopted treatment option is the availability of select radionuclides with optimum emission properties (both in volume and periodicity of delivery), which poses a challenge due to the fact that different radionuclides typically require different target materials and/or nuclear reaction pathways for their formation.

Materials and methods: We already published a successful strategy for the isolation of 225Ac from irradiated thorium targets [5]. We also published the recovery of Pa isotopes [6] from proton irradiated thorium. In this work, we propose the isolation of several other medically-related radionuclides namely 103Ru, 223/225Ra, 111Ag from the same target material.

Results: Several methods based on ion exchange chromatography and solid phase extraction show promise for the co-extraction of 103Ru and Ra isotopes from thorium irradiated targets. Anion exchange in HCl media proved to be an efficient method for the isolation of 103Ru, while a combination of cation exchange resin/citrate and DGA resin/HNO3 is suitable for Ra isotopes separation.

Discussion/conclusion: Production yields for the proposed radionuclides were evaluated by comparison of actual product yields with calculated (predicted) yields. Radiochemical strategies for co-extraction of 103Ru and 223/225Ra isotopes based on ion exchange and solid phase extraction chromatography will be discussed.

OP04
Comparison of [68Ga]FSC(succ-RGD)3 and [68Ga]NODAGA-RGD for PET imaging of αβ3 integrin expression
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Background: The arginine-glycine-aspartic (RGD) peptide sequence serves as a high-affinity antagonist of the integrin αβ3 receptor that plays an important role in tumor angiogenesis. Recently we reported [68Ga]FSC(succ-RGD)3, a trimeric RGD peptide, exhibited excellent targeting properties for αβ3 integrin expression and significant tumor uptake compared to monomeric [68Ga]NODAGA-RGD. Here we report the PET imaging properties of [68Ga]FSC(succ-RGD)3 in different xenograft tumor model and compared them with [68Ga]NODAGA-RGD.

Materials and methods: The PET imaging properties of [68Ga]FSC(succ-RGD)3 were studied in nude mice bearing M21 human melanoma xenografts and human glioblastoma U87MG xenograft tumor. A parallel PET imaging of 68GaNODAGA-RGD in same mouse bearing U87MG xenograft tumor was performed as a comparison.

Results: The static PET image of [68Ga]FSC(succ-RGD)3 in nude mice showed highly visualized tumors of M21 (positive) whereas nonvisualized tumor of M21-L (negative) tumor xenografts 1 h post injection confirming receptor-specific activity accumulation. The dynamic PET images of [68Ga]FSC(succ-RGD)3 showed rapid clearance of [68Ga]FSC(succ-RGD)3 from the circulation while the tumor remained clearly visible. A direct comparison of [68Ga]FSC(succ-RGD)3 with [68Ga]NODAGA-RGD in nude mice bearing U87MG xenograft tumor using PET/CT resulted comparable target/background ratio (tumor/kidneys ratio = 1.3 and 1.6, tumor/muscle ratio = 4.9, 5, respectively, 90 min post injection). The time activity curves from dynamic PET data showed an increase of the activity concentration of [68Ga]FSC(succ-RGD)3 in tumor firstly, then remained almost constant whereas that of [68Ga]NODAGA-RGD decreased quickly. The significant enhanced tumor uptake (3.8 vs. 1.6 % ID/g) in addition to the slower washout rate from tumor for [68Ga]FSC(succ-RGD)3 not only allows the PET
OP05

A new NPY-Y1R targeting peptide for breast cancer PET imaging

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Background: NPY-Y1 receptor (NPY-Y1R) is a promising target for breast cancer imaging. Previously, our group prepared and tested a series of truncated NPY analogs derived from BVD-15 (L-Pro56, Tyr32, Leu35)[NPY(28-36)-NH2] for 68Ga-labeling. Unfortunately the biological half-life of the most potent tracer, Lys(64Cu/DOTA)[BVD15], when injected in mouse plasma was shorter than 15 minutes. In this study, we improved the design of BVD15 in order to increase its stability in vitro and in vivo and maintain its targeting capability.

Materials and methods: Modifications of the peptide backbone, the chelator and the use of D- and non-natural amino acids were proposed to improve the peptide tracer stability. The peptides were synthesized on solid phase and conjugated to NOTA chelator. Binding studies on MCF-7 human breast cancer cells (2) were performed after each structural modification to make sure that the potency and the selectivity of the new NOTA-peptide conjugates to NPY-Y1R were maintained. Once active compounds were identified, they were radiolabeled with 64Cu for performing plasma stability, cellular uptake, internalization, and blocking studies on MCF-7 cells in order to rapidly identify the promising candidates for in vivo studies.

Results: A 64Cu/NOTA-BVD15 derivative presenting a very low Kd (9 nM) and showing a very high stability in plasma up to 20 h and in vivo for 30 minutes has been identified. Cell assays showed a constant uptake and internalization over the whole experiment. The internalized fraction after 2h was ~20%. The radiopeptide uptake was blocked in presence of an excess of unlabeled peptide.

Discussion/conclusion: We have identified a new 64Cu-labeled peptide showing a good stability and an excellent affinity to NPY-Y1R. On the basis of the cellular results, the 64Cu/NOTA-BVD15 derivative appears to have a potential for the targeting of NPY-Y1R positive tumors.

OP07

SPECT Imaging of G3β Expression by [99mTc(N)PNP43]-Bifunctional Chimeric RGD Peptide not Cross-Reacting with avβ5

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Background: Recently a new bifunctional chimeric RGD peptide (RGDDechi), comprising a cyclic Arg-Gly-Asp pentapeptide covalently bound to an echistatin domain, has been reported. In vitro and in vivo biological studies evidenced that this chimeric peptide selectively binds to avβ3 integrin and does not cross-react with avβ5. In order to obtain an optimal SPECT radiotracer, a series of [99mTc(N)PNP] labelled peptides has been prepared and their pharmacological properties investigated.

Materials and methods: RGDDechi-hCit (1) and three truncated peptide derivatives [RGDDechi1-17 (2), RGDDechi1-16 (3) and RGDDechi1-14 (4)] lacking the two, three and five C-terminal amino acids, were synthesized in solid phase by Fmoc chemistry and conjugated with a cysteine linked to the Lys1 side chain to allow the labeling with [99mTc(N)PNP(43)-syn-thr]3 [PMN3 = (CH3)2Pe(CH2)NC(H2)O(C2H5)CH2(CH2)2). In vitro stability and pharmacological properties of the corresponding compounds, 99mTc-1-4, were assessed. Challenges with an excess of glutathione and cysteine and Log P values were also investigated. Furthermore, radiolabeled peptides (99mTc-1-4) were applied to study in vivo stability and the pharmacokinetic profiles on tumor bearing mice.
Results: All 99mTc-compounds were obtained with RCY > 90%. Log P values demonstrate the hydrophobic nature of the radiolabeled peptides ranging from -2.96 to -2.12. No significant variations in RCPs of the complexes were detected in challenge experiments with an excess (10 mM) of glutathione and cysteine. In general, a high extent of radiolabeled peptides accumulate selectively in cells expressing αβ3 integrin and does not accumulate in cells expressing moderate levels of αβ5 and undetectable levels of αβ3 integrins. In agreement with in vitro findings, biodistribution studies showed that the 99mTc-3 radiolabeled chimeric peptide selectively localizes in tumor xenografts expressing αβ3 and fails to accumulate in those expressing αβ5 integrin.

Discussion/conclusion: 99mTc-labeled RGDechi, RGDechi1_17 and RGDechi1_16 chimeric peptides can be used for highly selective αβ3 expression imaging by SPECT technology. Among the tested compounds, 99mTc-T2 possesses the best distribution profile and highest localization in tumor expressing αβ3. This research was supported by MIUR through PRIN 20097FJHPZ-004 and FIRB “RINAME”2010-RBA-P114AMK, by Programma Operativo Nazionale Ricerca e Competitività PON 01_02388 and by Italian Association for Cancer Research AIRC IG 13121.

OP09
New dienophiles for the inverse-electron-demand Diels-Alder reaction and for pretargeted PET imaging

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Introduction: In cancer research, pretargeted PET imaging has emerged as an effective two-step approach that combines the affinity and selectivity of antibodies with the rapid pharmacokinetics and favorable dosimetry of smaller molecule radiolabeled with short-lived radionuclides. This approach can be based on the bioorthogonal inverse-electron-demand Diels-Alder (IEDDA) “click” reaction between tetrazines and trans-cyclooctene (TCO) derivatives. Our project aims to develop new [18F]TCO-dienophiles with high reactivity for the IEDDA reaction, improved in vivo stability and favorable pharmacokinetics. New dienophiles were synthesized using an innovative continuous-flow micro-photocatalysis process, and their reaction kinetics with a tetrazine were determined. In vivo stability studies of the most promising [18F]radiolabeled-TCO-derivative ([18F]trans-EB-70) was investigated, and its potential for pretargeted PET imaging was assessed.

Materials and Methods: Organic chemistry Fluoro-cis-cyclooctene derivatives and mesylate precursors for radiofluorination were synthesized in 5-8 steps. Structures of intermediates and final compounds were confirmed by NMR and HRMS. Photochemistry Trans-for-cis isomerization was performed using a microfluidic setup. Kinetics Reactions between dienophiles and 3,6-diphenylpyridin-2-yl)-1,2,4,5-tetrazine in MeOH at 25°C were monitored by UV-spectrophotometry at 290 nm (pseudo-first order conditions). [18F]Radiolabelling (semi-automated) Nucleophilic substitution of mesylate trans-EB-77 using [18F]K222 was achieved in MeCN at 90°C for 15 min. [18F]trans-EB-70 was purified by HPLC. In vivo stability ([18F]trans-EB-70 was evaluated in healthy NMRI mice, by ex vivo biodistribution (2, 10, 30, 60 min p.i.). In vivo pre-gating: Prostate tumor slices (LNCaP and PC-3 cells) were successively incubated with a prostate-specific membrane antigen (PSMA) inhibitor modified with 3-(trifluoromethyl)phenyl)-6-phenyl-1,2,4,5-tetrazine, and [18F]trans-EB-70. Direct incubation with the corresponding [18F]preclicked-compound, and blocking experiments (2-phosphonamidopentane-1,5-dioic acid) were performed.

Results: Fluoro-cis-cyclooctene derivatives and mesylate precursors were synthesized in 3-35% overall yields. The trans-for-cis micro-photoisomerization reached yields of 48%. Reaction kinetics of the new dienophiles are fast, with k₅ ranging from 475.6±32.8 to 1910.3±195.9 M⁻¹.s⁻¹. Radiosynthesis of [18F]trans-EB-70 was achieved in 60 min, with 12% radiochemical yield (decay-corrected), a radiochemical purity >99% (for at least 2h), and 70-188 GBq/μmol¹ specific activity. Biodistribution of [18F]trans-EB-70 in mice demonstrated absence of in vivo defluorination and a fast clearance via urinary and hepatobiliary systems. Regarding in vitro experiment, the binding on LNCaP tumor slices (expressing PSMA receptors) subjected to pretargeting was PSMA-specific and slightly inferior to the binding of [18F]preclicked-compound. No significant binding was observed in PC-3 cells (negative control).

Discussion/conclusion: We demonstrated that [18F]trans-EB-70 is a suitable dienophile for the IEDDA “click” reaction and for pretargeting applications. Therefore, [18F]trans-EB-70 will be investigated further in pretargeted PET experiments.

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OP10
New complexing agent for Al18F-labelling of heat-sensitive biomolecules: Synthesis and preclinical evaluation of Al18F-RESCA1-HAS

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Introduction: The Al18F-labelling strategy involves formation of aluminum mono(18F)fluoride (Al18F2+) which is trapped by a suitable chelator –mostly bound to a biomolecule- in aqueous medium. At this moment however, the need for elevated temperatures (100-120 °C) limits its widespread use. Therefore, we designed new restrained complexing agents (RESCAs) for use of this strategy at moderate temperature. RESCA1 is an acyclic pentadentate ligand with a N2O3 coordinating set that is able to complex (Al18F2+) efficiently at 25 °C. To evaluate the stability and kinetic inertness of the chelate in vivo, RESCA1 was conjugated to human serum albumin (HSA) and labelled with [18F]. The Al18F-labelled conjugate was monitored in vivo for 6 h p.i.

Materials and methods: HSA was reacted with RESCA1-TFP, RESCA1-HSA (7.5 mg, 110 nmol) in 750 μl sodium acetate buffer (0.1 M, pH 4.5) was added to a freshly prepared [Al(18F)2+] solution (1.4 Gbq, 50 nmol AlCl3, 12 min, RT). The product was purified using a PD-10 column and RCP was determined with SEC-HPLC. To test stability in serum, Al18F-RESCA1-HSA in 100 μl PBS was added to rat serum (900 μl), kept at 37°C and monitored up to 4 h. Ex vivo biodistribution was studied at 1 h, 3 h and 6 h p.i. of Al18F-RESCA1-HSA (2-7.5 MBq) in healthy female rats. Small-animal whole-body PET imaging was performed using a FOCUS 220 tomograph.

Results: RESCA1-HSA was obtained with a chelator-to-protein ratio of 3, estimated by ESI-TOF-HRMS analysis. Al18F-RESCA-HSA was prepared in high RCY (>70%) and purity (>95%) in < 30 min. 91% of product was still intact in rat serum after 4 h incubation. Distribution in rats showed high retention in blood with 5.42 ± 0.23% ID/g, 4.93 ± 0.22% ID/g and 3.66 ± 0.06% ID/g at 1h, 3h and 6h respectively from which the blood biological half-life was calculated to be 8.6 h. No significant increase in bone uptake was observed, indicating excellent in vivo stability of the Al18F-labelled construct.

Discussion/conclusion: We successfully labelled for the first time a heat-sensitive biomolecule via the Al18F-method in one radiolabelling step. Al18F-RESCA1-HSA showed excellent stability and favourable properties for PET blood pool imaging applications.
**OP11**
A novel versatile precursor efficient for F-18 radiolabelling via click-chemistry
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Introduction: In the last years, the Cu(II)-catalyzed Huisgen [3+2] cycloaddition between terminal alkynes and azides emerged as a powerful tool in F-18 radiolabelling of biomolecules such as peptides, because of its regioselectivity, mild aqueous organic conditions, reduced reaction times, and high yields.1 A weak point of the method is the lack of suitable commercially available, stable precursors.2 In this paper we report the synthesis and F-18 radiolabelling of a new, versatile, easy to handle, and stable azido precursor useful for click-chemistry.

Materials and methods: Reagents and solvents were purchased from Sigma-Aldrich. [18F]Fluoride was produced with an IBA Cyclone 18/9 cyclotron. Radioactive synthesis were carried out on a fully automated radiosynthesis module (GE TracerLab FX-FN Pro), and analyzed by analytical RP-HPLC with UV and radioactive detectors. Non-radioactive compounds were fully characterized by NMR, ESI-MS and IR.

Results: A series of bifunctional precursors, bearing the azido moiety and different leaving groups (e.g. tosylate, mesylate, iodo), coupled to a short polyethylene glycol chain (to improve their stability and hydrophilicity) were designed and successfully prepared following a ten-step synthetic pathway. A protection-deprotection strategy of functional groups achieved the precursors and the fluorinated reference with good yield and purity. Precursors were radiolabelled with F-18 and then coupled to propargylglycine as alkynyl counterpart. [18F]Fluoride was purified following standard procedures, and nucleophilic displacement of the iodide leaving group took place at 100°C in 20 min. The resulting labelled azide was successfully purified using a Sep-Pak tC18 cartridge (51% radiochemical yield not decay corrected, 93% radiochemical purity). The purified azide was then conjugated to propargylglycine, showing 52% conversion within 30 min at room temperature. Purification and formulation have still to be optimized.

Discussion/conclusion: A new optimized precursor useful for F-18 radiolabelling and click-chemistry was prepared. It demonstrated to be effective in radiolabelling non-protected alkynyl-modified aminocids, by a fully automated synthetic procedure. Good results, in terms of radiochemical yield and purity, were obtained from the iodo-derivative precursor. Purification and formulation of the final cycloaddition product are in progress.

**OP12**
A general applicable method to quantify unidentified UV impurities in radiopharmaceuticals
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Introduction: Radiopharmaceuticals are released for administration by a quality control procedure against pre-set specifications. One of these release specifications is the chemical purity of the drug product, determined with High Pressure Liquid Chromatography (HPLC) and UV detection. In the European Pharmacopeia (EP) hardly any specifications are given for the chemical purity of a radiopharmaceutical. When unknown impurities are present in the chromatogram, the decision if the radiopharmaceutical can be released, is very frequently based on unclear parameters like ‘no unidentified UV signals present’. There is a need for an objective specification in order to have a save and reliable release.

Aim: The purpose of the presented work is to define a generally applicable method to define tolerances for unidentified impurities in radiopharmaceuticals.

Materials and methods: A retrospective analysis was performed on HPLC analysis results of [11C]Flumazenil, [11C]PIB, [11C]Erlotinib, [11C]DPAPA713, [18F]PK209 and [18F]FES. Quantification of the carrier signal in the UV chromatogram was determined by use of calibration curves, utilizing Chromelon® 6.8. Unidentified impurities were semi-quantified utilizing the surface area in the UV chromatogram relative to the quantified carrier signal. Based on the EP. monography of [11C]Flumazenil and [18F]FET, the specification for unidentified UV impurities was determined to be 0.22 pmol/injection volume for a single unidentified impurity and 0.88 pmol/injection volume for the total of unidentified impurities. This specification was tested for over 500 batches of the radiopharmaceuticals and compared to the less specific parameter ‘no unidentified UV signals present’.

Results: In a pilot assessment we encountered in 5-10% cases unidentified UV impurities leading to rejection of the batch, based on the specification ‘no unidentified UV signals present’. Of these rejected batches 25% was also rejected with the new defined specification. Reason for this reduced number of rejections is that with the new specification the presence of unidentified impurities is evaluated objectively. The analysis of the full database is currently on-going.

Discussion/conclusion: With this method the amount of unidentified impurities can be estimated in an optimal and objective way, utilizing EP limits of [11C]Flumazenil and [18F]FET, without operator variability.

**OP13**
Development of [18F]Fluoro-C-glycosides to radiolabel peptides
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Introduction: The [18F]-labeling of peptides for PET applications has been used for many years.1 However, the sensitivity of these peptides does not allow their direct radiolabelling under harsh conditions, except few recent examples. A solution is to use a prosthetic group, an easily radiolabeled small molecule, subsequently coupled in mild condition to the peptide. In continuation of our previous work,2 we therefore propose to develop and use new C-glycoside-based prosthetic groups. The use of sugar derivatives as prosthetic group would improve bioavailability and pharmacokinetic properties of peptides.

Materials and methods: These C-glycoside derivatives should have a good leaving group thus allowing easy substitution by fluorine-18, i.e. triflate. A copper catalyzed azide alkyne cycloaddition (CuAAC) was then used for coupling these carbohydrates with a peptide. Some model peptides containing a cysteine residue as RGDC and c(RGDfC) were used for many years.1 However, the sensitivity of these peptides does not allow their direct radiolabelling under harsh conditions, except few recent examples. A solution is to use a prosthetic group, an easily radiolabeled small molecule, subsequently coupled in mild condition to the peptide. In continuation of our previous work,2 we therefore propose to develop and use new C-glycoside-based prosthetic groups. The use of sugar derivatives as prosthetic group would improve bioavailability and pharmacokinetic properties of peptides.

Results: Triflated precursors of these C-glycosides prosthetic groups and the non-radioactive references were synthesized in alpha and beta configuration. Fluoride-18 radiolabelling was optimized and the automated radiosynthesis of [18F]Fluoro-glycopeptides with some model peptides (RGDC, c(RGDfC)) was presented.
Introduction: In nuclear medicine a remarkably high demand of 68Ga-radiotracers has emerged during the last decade. For a variety of non-68Ga-containing radiotracers a microfluidic approach for their syntheses could be established, enabling an enhancement of yields due to high surface-to-volume ratios. In this proof-of-principle study, the 68Ga-radiolabeling of PSMA HBED-CC and NODAGA-RGD using a microfluidic approach was evaluated. Furthermore, adding Tween 20 (a surfactant suitable for biomedical applications) and its impact on the radiochemical yield was investigated.

Materials and methods: The syntheses of 68Ga-PSMA HBED-CC and 68Ga-NODAGA-RGD (both precursors from ABX) were performed using an Advion Nanotek LF microfluidic device. The system incorporates a flow-through reactor that consists of a silica capillary (l=2 m, Ø100 μm, V=15.6 μL). 68Ga3+ was obtained from a 68Ge/68Ga-generator (3.7 GBq; Obninsk) according to a fractionized protocol. The precursors and 68Ga3+ were loaded in two storage loops and distinct volumes thereof were pushed through the reactor with different flowrates (30, 50, 80 μL/min) at different temperatures (25, 50, 80, 100, 120, 150°C) using NaOAc and HEPEs for pH adjustment, respectively. Additionally, the influence of Tween 20 as a surfactant, to reduce known adsorption effects in microfluidic tubing, was investigated.

Results: Accomplished experiments revealed feasibility of 68Ga-labeling of PSMA HBED-CC and NODAGA-RGD using a microfluidic device. All temperatures and flowrates resulted in mean radiochemical yields in a range of 20–55%. Syntheses at higher temperatures and flowrates proved to be more efficient. For instance, HEPEs buffered syntheses at 100°C and flowrate of 80 μL/min yielded 68Ga-PSMA HBED-CC in 42.6 ± 22.1% (n=10) and 68Ga-NODAGA-RGD in 49.7 ± 32.5% (n=7). Applying the same parameters, Tween 20 could strikingly improve the yield of 68Ga-PSMA HBED-CC to 70.8 ± 16.8% (n=13).

Discussion/conclusion: This study provides the proof-of-principle of 68Ga-labeling in a microfluidic “flow-through” system. The application of Tween 20 led to drastically increased yields of 68Ga-PSMA HBED-CC due to its surfactant nature. As a result, this microfluidic approach will be pursued to increase availability of 68Ga-radiopharmaceuticals according to the dose-on-demand principle.

Discussion/conclusion: The synthesis and radiosynthesis of 6-18F/11C-fluoro-C-glycosides displaying a three carbon arm terminated with an azide group were optimized, CuAAC of fluoro-C-glycosides with RGD derivative peptides gave 18F/11C-fluoro-glycopeptides in good yields.

OP16
Surprising reactivity of astatine in the nucleophilic substitution of aryliodonium salts: application to the radiolabeling of antibodies
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Introduction: Aryliodonium salts have recently emerged as versatile precursors for the synthesis of 18F-radiolabeled compounds for PET imaging. However, little is known about the applicability of these reagents for labeling with the heaviest radiohalogens iodine and astatine, both useful for nuclear imaging and/or therapy. In this study, we aimed at probing the reactivity of radioiodine (125I) and astatide (211At) towards diaryliodonium salts in order to assess their usefulness for radiolabeling biomolecules of interest in nuclear medicine.

Materials and methods: First, parameters of radio-iodination and astationation reaction (solvent, temperature, duration and counter-ion of iodonium) were studied on model compounds. Bifunctional iodonium salts were then designed, allowing the synthesis of [125I]-SIB and [211At]-SAB, two prosthetic groups widely used for radioiodination and astationation of biomolecules. Both [125I]-SIB and [211At]-SAB were conjugated to the multiple myeloma targeting mAb 97E.4 (anti-CD138). Conjugation yields and resulting immunoreactivity were compared with the conventional arylstannane chemistry approach.

Results: Initial reaction parameters studies highlighted a striking difference of reactivity between radio-iodide and astatide that could not be anticipated from the trends observed within the halogen series. Not only the astationation reaction was highly efficient at much lower temperature than iodination, but it appeared also solvent and counter-ion independent (not iodination). Thermochemical studies highlighted a large difference of activation energy in acetonitrile between both halogens with Ea = 23.5 kcal/mol and 17.2 kcal/mol for radio-iodination and astationation, respectively. Quantum chemical calculations support the hypothesis that astationation occurs via a monomeric iodonium complex whereas iodide occurs via a dimeric complex which requires more energy for the reaction to proceed. This explains the large reactivity difference observed. Radiolabeling of an antibody with specifically designed iodonium salts outperformed conventional arylstannane chemistry approaches in terms of global efficiency (radiochemical yields >90%, conjugation yields ≈ 75%, and simpler purification: no HPLC needed) with excellent preservation of immunoreactivity of the IgG with both radionuclides and less concerns regarding the toxicity of precursors and side products.

Discussion/conclusion: In comparison with the conventional arylstannane approach, aryliodonium salts appear as more efficient precursors for preparation of radio-iodinated and astatinated radionuclidic iodonium complexes whereas iodide occurs via a dimeric complex which requires more energy for the reaction to proceed. This explains the large reactivity difference observed. Radiolabeling of an antibody with specifically designed iodonium salts outperformed conventional arylstannane chemistry approaches in terms of global efficiency (radiochemical yields >90%, conjugation yields ≈ 75%, and simpler purification: no HPLC needed) with excellent preservation of immunoreactivity of the IgG with both radionuclides and less concerns regarding the toxicity of precursors and side products.
compounds. Furthermore, they allow simpler purification procedures (easy transfer to automation), with the additional advantage of a much lower toxicity, which is of primary importance for human use. Most of all, the unexpected reactivity of astatine we unveiled highlights that a lot is still to be discovered about the chemistry of this radionuclide which remains to date largely unexplored.

**OP17**

64Cu-NOTA-pertuzumab F(ab’)2 fragments, a second-generation probe for PET imaging of the response of HER2-positive breast cancer to trastuzumab (Herceptin)

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Introduction: SPECT/CT imaging with 111In-BzDTPA-pertuzumab detected trastuzumab (Herceptin)-mediated HER2 downregulation in human breast cancer xenografts in mice and was correlated with a good response to treatment. This agent is now being studied in a Phase I/II clinical trial sponsored by OIKR (PETRA; ClinicalTrials.gov identifier: NCT01805908). Our objective was to develop and characterize a second-generation positron-emitting analogue for PET/CT imaging. 64Cu-NOTA-pertuzumab F(ab’)2 to provide greater sensitivity, more accurate radiotracer quantitation and a lower radiation absorbed dose.

Materials and methods: To determine the optimal dose, mice with subcutaneous HER2-overexpressing tumours were injected with 5, 50, 100 or 200 μg of 64Cu-NOTA-F(ab’)2 (2.2±0.6 MBq) and sacrificed at 24 h p.i. for biodistribution. To determine the normal tissue distribution, pharmacokinetics and radiation dosimetry of 64Cu-NOTA-F(ab’)2 non-tumour-bearing Balb/c mice were administered 50 μg of 64Cu-NOTA-F(ab’)2 (2.9±0.3 MBq) and sacrificed at select time points. Three groups of mice bearing HER2-overexpressing tumours were injected with 64Cu-NOTA-F(ab’)2 (50 μg; 10±0.4 MBq) with or without administration of pertuzumab (1 mg) 24 h before, or with 64Cu-NOTA-F(ab’)2 prepared from non-specific human IgG (50 μg; 8.2±1.9 MBq) to demonstrate specificity. Mice were imaged with PET/CT at 24 and 48 h p.i. and sacrificed for biodistribution.

Results: The 50 μg dose of 64Cu-NOTA-F(ab’)2 showed the highest tumour to blood ratio, followed by the 100, 5 and 200 μg doses (18.2±7.2, 15.4±0.8, 14.0±5.0, and 10.9±1.8, respectively). In non-tumour bearing mice, only the kidney retained significant radiotracer uptake at 24 h p.i. (49.1±5.9 %ID/g). Initial estimates for τ1α and τ1β were 1.2 h and 6.0 h, respectively. The projected total body radiation absorbed dose to humans was 0.02 mSv/MBq, half that estimated for 111In-BzDTPA-pertuzumab. Tumour to normal tissue contrast of PET/CT images appeared similar between the 24 and 48 h p.i. imaging time points. Tumour accumulation of 64Cu-NOTA-F(ab’)2 was significantly higher in mice administered 64Cu-NOTA-F(ab’)2 without pertuzumab blocking (8.4±3.4 %ID/g) relative to mice pre-injected with 1 mg of pertuzumab (3.9±0.5 %ID/g; P<0.05), and mice administered 64Cu-NOTA-F(ab’)2 prepared from non-specific human IgG (2.7±0.5 %ID/g; P<0.05).

Discussion/conclusion: 64Cu-NOTA-F(ab’)2 is HER2 specific and can visualize HER2-overexpressing tumours at 24 or 48 h p.i. with PET/CT imaging. The lower projected radiation absorbed doses for 64Cu-NOTA-F(ab’)2 compared to 111In-BzDTPA-pertuzumab may make this a more attractive imaging agent to detect trastuzumab-mediated HER2 downregulation in breast cancer. Supported by grants from the OIKR Smarter Imaging and High Impact Clinical Trials (HICT) Programs.

**OP18**

Development of radiohalogenated analogues of a avb6-specific peptide for high LET particle emitter targeted radionuclide therapy of cancer

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Introduction: Targeted radionuclide therapy (TRT) of solid tumors has a limited efficacy mainly because these tumors have high radio-resistance and take up limited amounts of radiolabeled vectors. Strategies to overcome this include the use of small peptides combined with radionuclides that emit highly cytotoxic particles, namely high linear energy transfer (LET) particles such as alpha particles and Auger electrons. We have chosen to target the epithelial-specific integrin αvβ6, which is weak or absent on normal tissues but is upregulated on many cancers where it is strongly associated with reduced survival. One targeting vector is a 20 mer peptide, A20FMDV2 which we have previously used to image αvβ6-positive tumours with SPECT (In-111) or PET (F-18, Ga-68). The peptide showed extremely high kidney retention when radiolabelled with radiometals, but this was not seen with the F-18 analogue. Due to anticipated dose-limiting toxicity in kidney for radiometal-based TRT, we have developed a peptide suitable for radiohalogenation with the Auger electron emitter I-125 and the alpha particle emitter At-211.

Materials and methods: The 20 mer high affinity αvβ6-targeting peptide, A20FMDV2 used in previous work has been derivatised to contain a trimethylstannyl benzamide moiety so that it can be radiolabelled with I-125 and At-211. The peptide has been radiolabelled with I-125 and At-211 and radioligand binding and internalization assays have been carried out in cells that overexpress αvβ6 and compared with that of the 111In-DTPA-A20FMDV2 studied previously. Clonogenic assays have been carried out on both the non-radiolabelled peptide and the radiolabelled analogue in both αvβ6-positive and negative cells to determine the effect of high LET irradiation.

Results: Radiolabelling efficiency with I-125 was >91 % and the analogue showed high binding affinity and rapid internalisation. Clonogenic assays showed that the non-labelled peptide alone was able to inhibit cell growth and that this effect was not seen on αvβ6-negative cells. This effect was enhanced when radiolabelled with a high LET particle emitter.

Discussion/conclusion: The αvβ6-specific peptide when radiolabelled with a high-LET particle emitter shows promise as an agent for targeted radionuclide therapy.

**OP19**

Ligand Specific Efficiency (LSE) as a guide in tracer optimization

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Introduction: Successful radiotracers result from a favorable combination of target density, ligand affinity, nonspecific binding and permeation. All these parameters can be measured independently and the interplay between some of them is well known: for instance, Bmax/Kd > 10. Importantly, the required affinity of a tracer is also correlated to nonspecific binding: An increased affinity is beneficial
only if the nonspecific binding remains constant. This work aimed at identifying an index taking into account the relationship between these two parameters, to guide optimization from the early stage of tracer development projects.

**Materials and methods:** Similarly to the Ligand Efficiency (LE) index, we explored the usefulness of the Ligand Specific Efficiency index (LSE), which we defined as the ratio between affinity (expressed as e.g. pIC50 or pKd), and the logarithmic value of the experimental non-specific binding measurement. CHI/IAM.\\(^{\text{[3]}}\)

LSE provides a measure of affinity, normalized to non-specific binding. It shows how efficient the ligand is at binding to the desired target, compared to all other non-specific binding partners.

**Results:** A series of well-described PET tracers was evaluated to set the LSE threshold. This analysis showed that an LSE > 5 and preferably LSE > 5.4 is required for a successful tracer. This concept was applied to the development of a prostacyclin receptor (IPR) tracer. Our chemical starting point was Ro1138452, which we selected based on encouraging overall properties, including a high affinity for IPR (Kd = 0.23 nM). In contrast, its CHI/IAM value of 5.8 clearly indicated a high tendency for non-specific binding. Despite such high non-specific binding, Ro1138452 has a LSE value of 5.1, which is close to the minimum value that would be expected for a successful PET tracer. This raised hope that some improvement in binding specificity would allow the use of a close derivative for imaging purposes. The use of LSE during the IPR tracer optimization will be presented.

**Discussion/conclusion:** LSE is based on a rather intuitive concept, in the sense that a good PET tracer candidate should have the optimum balance of affinity and binding specificity. It is a convenient index to evaluate and compare molecules based on measured, rather than in silico values, and is applicable independently of target and chemotype. It is a useful index from the beginning of a project, facilitates the selection of the most promising scaffold and guides their optimization.

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**OP24**

Influence of the fluorescent dye on the tumor targeting properties of dual-labeled HBED-CC based PSMA inhibitors

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**Introduction:** Image-guided cancer surgery using fluorescence imaging has high clinical impact and already shows potential to improve the outcome of oncological surgery (1). As first clinical experiences with the ²⁶⁶Ga-labeled PSMA-targeting inhibitor Glu-urea-Lys-Ahx-HBED-CC (PSMA-11) demonstrated high and specific tracer uptake in prostate cancer lesions a fluorescence dye conjugate of PSMA-11 might represent a promising bimodal tracer. The combination of preoperative staging by means of PET/CT and PET/MRI, followed by image-guided surgery will further improve the accuracy of detecting PSMA-positive tumor lesions by merging the strengths of both techniques. Therefore, various fluorescent dyes were conjugated to the inhibitor PSMA-11 to determine the impact of the dye conjugation on the ligand’s in vitro and in vivo characteristics.

**Materials and methods:** The optical-dye labeled tracer PSMA-HBED-CC-FITC, PSMA-HBED-CC-AlexaFluor488 and PSMA-HBED-CC-IRDye800CW were synthesized based on PSMA-11. The binding properties were analyzed in a competitive cell binding assay followed by internalization experiments in human PSMA expressing LNCaP cells. Biodistribution studies were performed in LNCaP tumor-bearing mice (BALB/c nu/nu) to determine specific tumor uptake and pharmacokinetic properties.

**Results:** Comparative cell binding experiments revealed a high affinity to PSMA expressing cell lines for all conjugates, which is in line with the values obtained with the reference ²⁶⁶Ga-PSMA-11. The radiolabeled fluorescent-dye conjugates showed specific cell uptake and were effectively internalized into the PSMA expressing cell line LNCaP. First in vivo results indicated slightly varying pharmacokinetic properties depending on the fluorescent dye. The FITC- and AlexaFluor488-conjugates revealed a higher tumor uptake compared to ²⁶⁶Ga-PSMA-11, while a minor, but still satisfying uptake was detected for the IRDye800CW-conjugate.

**Discussion/conclusion:** Conjugation of a fluorescent dye to the well-established imaging agent PSMA-11 showed rather minor dye-dependent impact on cell binding properties, tumor uptake and the pharmacokinetic characteristics. In order to further improve the biodistribution profile of the IRDye800CW-conjugate, structural optimization will be done. These first preclinical results emphasize the potential of a dual-labeled PSMA inhibitor to serve as a multimodal imaging agent, enabling sensitive pre-, intra- and post-therapeutic identification of metastases with one and the same molecule.
OP25

[18F]MEL050 as a melanin PET tracer: fully automated radiosynthesis and evaluation for the detection of pigmented melanoma in mice pulmonary metastases

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Introduction: Melanoma is a highly malignant cutaneous tumor of melanin-producing cells. Early detection of melanoma is the best way to reduce mortality. Several radiolabeled imaging probes have been evaluated for melanoma imaging. MELO50 is a synthetic benzamide-derived molecule that specifically binds to melanin with high affinity. Our aim was to implement a fully automated radiosynthesis of [18F]MELO50, including HPLC purification and formulation, using for the first time, the AllInOne radiosynthesis platform (Travis, An., Belgium), and to validate this PET radiotracer in vivo in a mouse model of melanoma.

Materials and methods: [18F]MELO50 was synthesized using a one-step bromine-for-fluorine nucleophilic heteroaromatic substitution. Briefly, [18F]MELO50 was prepared from a bromo-precursor, using no-carrier-added 18F-KF-Kryptofix 222 (dimethylformamide, 150°C, 6 min), followed by preparative HPLC purification (C18 column (300x7.8 mm, 7 μm), isocratic elution with acetonitrile/20 mM ammonium bicarbonate (20:80, v/v), 3.0 ml/min) and cartridge-reformulation (Sep-Pak® Plus C18 environmental) of the collected fraction. Radiochemical and chemical purity, stability and specific activity measurements were monitored using analytical HPLC. Chemical identity of the labeled compound [18F]MELO50 was assessed by co-injection with non-radioactive standard MELO50. Experimental model of pulmonary metastatic melanoma was obtained by IV injection of 816-F10 cells in NMRI mice. Mice (n=8) were imaged 15 days after inoculation, using INVEON microPET/CT device (Siemens), after IV injection of 0.36 ±0.04 MBq/g of [18F]MELO50. Dynamic and static acquisitions were acquired from time of injection to 2h after tracer injection. The maximum percentage of [18F]MELO50 Injected Dose per g of lung tissue (%ID/g) Max was determined using ROIs manually drawn on 1h-post injection PET images, and correlated to ex vivo findings.

Results: The fully automated radiosynthesis of [18F]MELO50 required an overall radiosynthesis time of 60 min, with an end-of-synthesis yield of 20-26% (n=12). Isocratic semi-preparative HPLC allowed efficient separation of [18F]MELO50 from the reaction mixture. The radiotracer was consistently produced with radiochemical purity higher than 99%. The specific activity was maintained at RCP>98% over 6 h. PET/CT images retrieved known biodistribution of [18F]MELO50 in mice, and allowed clear visualization of <1mm lung tumours with [18F]MELO50 %ID/g Max of 4.7±2.6%.

Discussion/conclusion: We successfully implemented the radiosynthesis of [18F]MELO50 using the AllInOne radiosynthesis platform, including HPLC separation and formulation. In vivo PET/CT validation of the radiotracer was obtained in a mouse model of metastatic pigmented melanoma, showing high specific [18F]MELO50 uptake in sub-millimetric lung tumours.

OP26

Design and Preclinical Evaluation of Novel Radiofluorinated PSMA Targeting Ligands Based on PSMA-617

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Introduction: Urea-based inhibitors of the prostate-specific membrane antigen (PSMA) are well known and promising candidates for the diagnosis (1, 2) and therapy of prostate cancer. The aim of the project was the development of F-18 labeled PSMA ligands based on the theranostic compound PSMA-617. The compounds evaluated during the preliminary experiments showed a high uptake in non-target organs caused by their relatively high lipophilicity. Therefore we currently reduce this lipophilicity by the addition of charged amino acids to the linker region of our PSMA inhibitors (3).

Materials and methods: The PSMA binding motif Glu-NH-CO-NH-Lys was synthesized by a well-established method (4) using solid phase chemistry and subsequently an amino acid linker was built up by fmoc-based solid phase peptide synthesis (SPPS). The non-radioactive reference compounds were also prepared analogically and eventually conjugated by 6-fluoronicotinic acid. After separation from the resin and deprotection the peptidomimetics were labeled using the TFP-ester of 6-[18F]fluoronicotinic acid as prosthetic group. The Ki values of all compounds were determined by competitive binding assays on PSMA-positive LnCaP cells against 68Ga-PSMA-10 (5) using the respective cold reference compounds. Additionally, the cellular internalization of the radiofluorinated ligands was determined.

Results: All [18]F-labeled PSMA-inhibitors presented in this study showed low nanomolar affinities towards PSMA, usually paired with high internalization ratios of more than 15 %, shown in vitro. Among those PSMA-1007 showed an outstandingly high internalization ratio of about 70 % while the Ki was in the typical range (6 nM). Hence PSMA-1007 was further evaluated in vivo. The organ distribution showed a high and specific tumor uptake of 8.0±2.4 %ID/g. Finally, the PSMA-targeting potential of PSMA-1007 was further demonstrated by dynamic μPET experiments.

Discussion/conclusion: Based on our experience with PSMA-617 and preliminary results we developed a series of radiofluorinated PSMA inhibitors with high affinities and internalization ratios. Among those a promising candidate for further translation into the clinic has already been found. This candidate – namely PSMA-1007 – is currently transferred into first-in-man studies. Nevertheless further optimization of the lead compound is still ongoing.

OP27

A novel radiolabeled peptide for PET imaging of prostate cancer: 64Cu-DOTHA2-PEG-RM26

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Introduction: The Gastrin-Releasing Peptide Receptor (GRPR) is over-expressed in a wide variety of prostate cancers, hence its interest as a potential biomarker. As such, previous work used different radiola-
beled GRPR-binding peptides to specifically target tumors in vivo (1, 2). Recently, we synthesized a novel bifunctional chelator bearing hydroxamic acid arms, called DOTHA$_2$, for which our group demonstrated fast and stable complexation to $^{64}$Cu (3). The goal of this study was to develop a GRPR antagonist, D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH$_2$ (RM26), conjugated to the novel $^{11}$(Cu)-DOTHA$_2$ to visualize prostate tumor by PET imaging.

Material and methods: DOTHA$_2$-PEG-RM26 was synthesized on solid support. The inhibition constant (K$_i$) was measured on PC3 cells. Dynamic PET images were acquired during 60 minutes after bolus administration on nu/nu male mice bearing PC3 tumors. Biodistribution studies were performed at different time points, using balb/c male and nu/nu male mice bearing PC3 tumors. To assess specific binding, a cohort received 500 nmol/kg of unlabeled peptide 15 minutes prior tracer injection for both PET and dissection studies.

Results: The K$_i$ of Cu-DOTHA$_2$-PEG-RM26 was in the low nanomolar range (0.68 nM). DOTHA$_2$-PEG-RM26 complexed $^{64}$Cu with fast kinet-
ics at room temperature. The radiopeptide showed high stability, low residual activity in various tissues and fast clearance in normal mice. Small animal blocking experiments showed a significant uptake drop compared to tracer by biodistribution in the GRPR-rich pancreas for both balb/c and nu/nu mice (respectively p< 0.05 and p < 0.01 at 30 min). A significant uptake decrease from 5.3±0.8%ID/g to 3.4 ±1.4%ID/g was also observed in PC3 tumors when unlabeled peptide was added in biodistribution experiments at 30 minutes, whereas a significantly reduced tumor uptake was also assessed by PET imaging from 20 to 60 minutes (p<0.05) post-injection.

Discussion/conclusion: The use of $^{11}$(Cu)-DOTHA$_2$-PEG-RM26 is prom-
ising for visualizing prostate tumors. These preliminary data suggest that DOTHA$_2$ can be used to develop many other peptide- and protein-derived PET tracers.

OP29

Biodistribution of $^{[18F]}$Amylovis®, a new radiotracetr PET imaging of $\beta$-amyloid plaques

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Aim: $^{[18F]}$-2-(3-fluoropropyl)-6-methoxynaphthalene ($^{[18F]}$Amylovis®) is a new naphthalene-derivative for detecting $\beta$-amyloid plaques in Alzheimer’s disease. The aim of the study is the assessment of the animal biodistribution of this new radiotracer.

Material and methods: $^{[18F]}$Amylovis® was synthesized by nucleo-
philic substitution of the tosyl group of the precursor. Thirty five healthy male Balb/C mice of 10-12 weeks were divided into 6 groups of 5 animals each and injected with similar doses of $^{[18F]}$Amylovis® through a lateral tail vein. Blood samples were collected and the ani-
mals were sacrificed at 5, 15, 30, 45, 70 and 180 minutes. Organs of interest were removed and washed with saline. Radioactivity of blood, plasma, urine, faeces, brain, cerebellum, heart, liver, stomach, spleen, bowel, colon, left kidney, muscle, bone and tail was measured in a well counter. To assess protein binding, plasma samples were di-
luted with acetone and centrifuged at 4000 g. Pellets of proteins and supernatants were separated and their radioactivity measured in a well counter. RadioTLC analysis of plasma were performed for the same purpose in silicagel 60 and mobile phase of acetone/tetrater (95/5). 20μL of each supernatant was analysed by HPLC-RP using a C18 column and acetone/tetrater (75/25) as mobile phase to identify plasma metabolites. Pharmacokinetic parameters (AUC, $t_{1/2}$, $\text{C}_{\text{max}}$, $\text{Cl}$, $V_{ss}$) were calculated using non-compartmental analysis (NCA). Dynamic PET/CT images of healthy and transgenic APPSwe/ PS1dE9 mice were acquired for 2.5 h after i.v. administration. Immunohistochemistry of control and transgenic mice brains were performed to identify $\beta$-amyloid plaques.

Results: $^{[18F]}$Amylovis® crossed blood brain barrier. PET/CT images showed significant differences between healthy and transgenic mice, expressed in Cortex to cerebellum SUV ratio, with maximum differ-
ence at 30 minutes. Postmortem studies of immunohistochemistry showed also differences in healthy vs transgenic mice (amyloid posi-
tive). Plasma $T_{1/2}$ of 37 min. No significant protein binding was ob-
served. Renal and hepatic pathways were the main excretion routes. Some amount of in vivo degradation metabolites appeared in blood from 1 h post-administration.

Conclusion: $^{[18F]}$Amylovis® could be a promising PET radiotracer for amyloid plaques visualization.

OP30

Synthesis and preclinical evaluation of $^{[11C]}$-BA1 PET tracer for the imaging of CSF-1R

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Introduction: Colony stimulating factor-1R (CSF-1R) is also called as Feline McDonough Sarcoma (FMS), is a type-III kinase recep-
tor. FMS widely expressed and considered to regulate develop-
ment, maintenance and functioning of mononuclear phagocyte lineage such as monocytes, macrophages, dendritic cells, langer-
hans cells, microglia and osteoclasts1. Over expression of FMS have been implicated in number of disease states including can-
cer, rheumatoid arthritis, Crohn’s disease and Bone disorders. FMS also known to play a key role in microglia differentiation and activation and assuming that it was key mediator in neu-
roninflammatory process.

Materials and methods: All the chemicals and reagents used in the experiment of CSF-1R ($^{[11C]}$-BA1) was purified by using semi-preparative-reversed-phase high pressure liquid chromatog-
raphy (RP-HPLC). The peak corresponding the reference com-
pound will be collected and checked for the purity using analytical RP-HPLC. Baseline biodistribution study was performed at 2, 10, 30 and 60 min. respectively. Blocking experiment (10 mg/kg cold compound) was performed at 30 min time point in healthy female adult mice n=3 and compared with vehicle treated batch. In vitro autoradiography experiments were carried on rat brain slices by incubating tracer and cold blocking solu-
tion. MicroPET imaging studies were performed on a Focus$^TM$ 220 microPET scanner with female rats.

Results: Reference and precursor molecules were synthesized with comparable purities and yields reported in the literature, the radiochemical yield (Alkylation yield with $^{[11C]}$(CH3) 60 % and radiochemical purity of 98 % and specific activity (n=5) 247.3 GBq/μmol. Biodistribution study shows higher tracer up-
take into the brain % ID 4 at 2 min time point, main route of excretion via renal and hepatobiliary circulation. High lung up-
take was observed with % ID 14 at 2 min. Blocking with cold compound did not observe any blocking effect in brain but we
observed blocking in peripheral organs like liver and spleen, also observed slight blocking in pancreas and kidneys. We did not observed any blocking with in vitro autoradiography experiments. Baseline microPET scans suggests good uptake of tracer into brain with SUV 1.2 but with blocking we did not observed any blocking effect despite we observe higher uptake in brain with SUV 2.

Discussion/conclusion: We successfully synthesized, \[^{11}\text{C}\]BAI. In preclinical evaluation we did not observe any significant blocking effect in the brain, we are currently looking for other high affinity molecules for CSF-1R.

OP31
In vivo imaging of the MCHR1 in the ventricular system via \[^{18}\text{F}\]FE@SNAP
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Introduction: The melanin concentrating hormone receptor 1 (MCHR1) is predominately expressed in the lateral hypothalamus and is playing a key role in energy homeostasis and obesity. Recently, it has been shown that the MCHR1 is expressed in the ependymal cells of the 3rd ventricle where it is involved in the regulation of cilia beat frequency. This beating facilitates cerebrospinal fluid circulation, which is crucial for brain function, as defects in ventricular cilia result in hydrocephalus. Our aim was to investigate the potential of the MCHR1 ligand \[^{18}\text{F}\]FE@SNAP \(^2\) for PET-imaging of the MCHR1 in the ventricular system.

Materials and methods: In vivo experiments were conducted in naïve male Sprague Dawley rats. For small-animal PET/CT/MR experiments, rats were anesthetized with isoflurane. 25min after \[^{18}\text{F}\]FE@SNAP iv injection (47.8±1MBq; SA: 19.7±6GBq/μmol), vehicle (baseline group, n=3) or 15mg/kg SNAP-7941 (blocking group, n=3) were administered through the tail vein. 75min after tracer injection, the rats were sacrificed. Radioactivity concentrations in brain were calculated and expressed as SUVs. In another tracer injection, the rats were sacrificed. Radioactivity concentrations in brain were calculated and expressed as SUVs.

Results: PET/CT/MR experiments: the SUV in the ventricular system was 0.73±0.11 for the baseline group and 0.34±0.07 for the blocking group, which represents a significant reduction. Ex vivo autoradiography showed a distinct uptake in the ventricular, which was significantly reduced in the blocking group.

Conclusion: \[^{18}\text{F}\]FE@SNAP evinced specific uptake in the ventricular system of naïve rats regardless their state of consciousness and is therefore a suitable imaging agent for cilia beat function.

OP32
Synthesis of the first carbon-11 labelled P2Y12 receptor antagonist for imaging the anti-inflammatory phenotype of activated microglia
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Introduction: Activated microglia are a hallmark of neuroinflammation (NI), which in turn is associated with neurodegenerative diseases. The P2Y\(_{12}\) receptor (P2Y\(_{12}\)R) is upregulated in the anti-inflammatory phenotype of activated microglia, and is not expressed on macrophages and other brain cells. Therefore, P2Y\(_{12}\)R could be an interesting new target for PET imaging of microglial activation in NI. Recently, a series of P2Y\(_{12}\)R antagonists with high binding affinity was reported. Based on this series, the purpose of the present study was to label urea derivative 5 (IC\(_{50}\) = 6 nM) with carbon-11.

Materials and methods: The synthesis of sulfonylurea 5, as reference, and azetidine amine 3 and sulfonyl azide 6, as precursors for the radiosynthesis of \[^{11}\text{C}\] is depicted below. Carbon-11-labelling was performed via a rhodium(II)-mediated [\(^{11}\text{C}\)]CO carbonylation reaction \(^[4,5]\). \[^{11}\text{C}\] was evaluated using in vitro autoradiography of healthy mouse brains and ex vivo biodistribution and radiometabolite analyses in healthy male Wistar rats.

Results: Compound 5 and precursors 3 and 6 were successfully synthesised. \[^{11}\text{C}\] was obtained in a radiochemical yield of 10±2 % (corrected for decay, calculated from \[^{11}\text{C}\]C02 (n=6)), and high (radio)chemical purity (≥98%) and specific activity (79±32 GBq/μmol) were achieved. In vitro autoradiography studies of the healthy mouse brain, \[^{11}\text{C}\] could be blocked (81%) with ticagrelor, indicating specific binding to P2Y12R. However, rapid metabolism in rat plasma was observed with only 30±4% (n=3) of \[^{11}\text{C}\] left at 45 min p.i. In addition, ex vivo biodistribution revealed that \[^{11}\text{C}\] did not enter the rat brain.

Discussion/conclusion: \[^{11}\text{C}\] is not suitable for in vivo studies, but can still be used in vitro to validate P2Y\(_{12}\)R as a target for imaging the anti-inflammatory phenotype of activated microglia.

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OP33
Radiosynthesis of a selective HDAC6 inhibitor [\(^{11}\text{C}\)K8631 and in vitro and ex vivo evaluation
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OP34
Improving metabolic stability of fluorine-18 labelled verapamil analogues

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Introduction: (R)-[11C]verapamil is widely used as a PET tracer for investigating P-glycoprotein (P-gp) function in patients with epilepsy, Alzheimer’s disease and other neurodegenerative diseases [1]. Recently, we have developed the fluorine-18 analogues (R)-N-[18F]fluoroethynlorverapamil (1) and (R)-O-[18F]fluoroethynlorverapamil (2), potentially enabling P-gp studies in centres without an on-site cyclotron. These analogues showed specific P-gp substrate behaviour, but metabolic stability was poor. The purpose of the present study was to assess whether deuterated analogues would have better metabolic stability.

Materials and methods: To a dried 19F/K232/K2CO3 complex, 2-bromoethyl-d3-tosylate in DMF was added and reacted for 10 min at 90°C to obtain 2-bromo-[18F]fluoroethanol-d3. This was distilled at 100°C through an AgOTf column at 200°C into a cooled (0°C) vial containing 1.5 mg of (R)-normethyl verapamil (1a) and 3 mg of K2CO3 in ACN. While stirring, this reaction mixture was heated at 120°C for 15 min and purified by HPLC, resulting in 1b. Tracer 2b, 3b and 4b were obtained by direct fluorination of precursors 2a, 3a and 4a and only 2b required final Boc-deprotection with TFA. 1b and 2b were administered to Wistar rats, and the level of labelled metabolites was measured in blood plasma and brain samples.

Discussion/conclusion: We successfully radiolabeled and evaluated a P-gp PET tracer to visualize the dynamics of HDAC6 in normal and disease states. Recently, Lu and coworkers synthesized [11C]KB631, a highly potent (IC50=1.4 nM) and selective (3700-fold selectivity against HDAC1) PET tracer for HDAC6 imaging in the brain, which showed similar pharmacokinetic properties as tubastatin A [3]. However, [11C]KB631 showed low brain bioavailability [4]. In this regard we opted to use this tracer as a more peripheral imaging agent. The radiosynthesis was redesigned and preliminary results were obtained with a biodistribution study and autoradiography experiments in brain and tumor slices.

Materials and methods: [11C]KB631 was synthesized through methylation of the corresponding precursor (300 μg) with [11C]-MeI in anhydrous DMSO (250 μL) at 100°C for 4 min. The biodistribution was studied in NMRI-mice at 2, 10, 30 and 60 min (n = 3/time point) post injection (p.i.). Organs and tissues were harvested and radioactivity was counted in a gamma-counter. Autoradiography studies were carried out with [11C]KB631 on wild-type Wistar rat brain tissue and PC3/LNCaP prostate tumor slices. Slices were incubated with 18.5 MBq (0.5 μL) (brain) or 18.5 MBq/250 μL (tumor) of tracer with/without 100 μM of cold authentic reference compound (KB631) or non-structural related pan-HDAC inhibitor Suberoylanilide hydroxamic acid (SAHA) [5].

Results: Based on prep HPLC integration, the methylation yield was 55 % with a radiochemical purity of 97 % and a specific activity of 48 GBq/μmol. Biodistribution studies indicated low brain uptake (<0.1 %ID at 2, 10, 30 and 60 min) and renal and hepato-biliary excretion. Autoradiography experiments showed regional binding. Binding in brain/tumor slices was highly placeable in the presence of 100 μM non-labeled reference KB631 (up to 90% for brain, PC3 and LNCaP) or 100 μM SAHA (73% brain, 39% PC3 and 59% LNCaP).

Discussion/conclusion: We successfully radiolabeled and evaluated a potential carbon-11 labeled radiotracer for in vitro and ex vivo visualization of HDAC6. However, biodistribution studies indicated low brain uptake, peripheral potency still needs to be further examined. Autoradiography studies showed regional and replaceable binding. Furthermore, radiometabolite studies followed by μPET on a mice tumor model will be performed.

Materials and methods: To a dried 19F/K232/K2CO3 complex, 2-bromoethyl-d3-tosylate in DMF was added and reacted for 10 min at 90°C to obtain 2-bromo-[18F]fluoroethanol-d3. This was distilled at 100°C through an AgOTf column at 200°C into a cooled (0°C) vial containing 1.5 mg of (R)-normethyl verapamil (1a) and 3 mg of K2CO3 in ACN. While stirring, this reaction mixture was heated at 120°C for 15 min and purified by HPLC, resulting in 1b. Tracer 2b, 3b and 4b were obtained by direct fluorination of precursors 2a, 3a and 4a and only 2b required final Boc-deprotection with TFA. 1b and 2b were administered to Wistar rats, and the level of labelled metabolites was measured in blood plasma and brain samples.

Results: 1b, 2b, 3b and 4b were obtained in a radiochemical yield of 3, 6, 5 and 10%, respectively, a purity >98% and a specific activity >80 GBq·μmol⁻¹. Results of the metabolite analysis are presented. The deuterated analogues showed improved metabolic stability compared with the non-deuterated compounds.

Discussion/conclusion: Labelling of tracers 1b-4b was successful. Although, both 1b and 2b showed significantly increased metabolic stability, total intact tracer levels at 15 min are still lower than desired. The resulting effect of increased metabolic stability for PET imaging will be evaluated in P-gp KO mice. To determine the effect of a deuterated N-methyl group, tracer 3b and 4b were designed and still need to be evaluated for metabolic stability.

OP36
Development of a novel PET tracer for the activin receptor-like kinase 5

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Introduction: Pulmonary arterial hypertension (PAH) is a disease in which pulmonary arterial obstruction increases vascular resistance, leading to right ventricular failure [1]. Inhibition of transforming growth factor-β (TGF-β) signalling via activin-receptor-like kinase 5 (ALK5) prevents progression and development of pulmonary hypertension [2]. To further understand the role of ALK5 in PAH, the purpose of this study was to synthesize a carbon-11 labeled ALK5 tracer (IC50 = 5.5 nM) [3] and to assess its potential as a positron emission tomography (PET) ligand for measuring ALK5 expression and activity in vivo.

Materials and methods: The [11C]ALK5 tracer was synthesized by a carboxylation reaction. The radiolabeling was carried out by heating [11C]CO2, the precursor molecule, isobutyl iodide and BEMP in DMSO for 10 minutes at 75°C. The tracer was evaluated using biodistribution and metabolite studies in Wistar rats (n=4 at each point at time 15 and 60 min). In addition, specific binding was assessed using autoradiography on ALK5 expressing MDA-MB-231 tumor sections.

Results: The [11C]ALK5 tracer was synthesized with a yield of 18±6 %, a specific activity of 116±31 GBq·μmol⁻¹ and a purity of ≥ 95 %. The tracer showed a normal biodistribution ex vivo and a moderate stability in vivo. The autoradiograms presented binding of the tracer to the tumor sections. Pretreatment of the tumor sections with ALK5 blocking agents (EW-7197, SB-431542 and the ALK5 inhibitor) decreased the binding of the tracer significantly.

Conclusion: The ALK5 tracer was synthesized successfully, and initial in vitro and ex vivo studies indicate its potential as a putative tracer of ALK5, warranting further in vivo evaluation.

Acknowledgment: CVON is acknowledged for funding of this project and the BV Cyclotron VU for providing [11C]CO2.
Introduction: Radiolabelled antibodies and peptides hold promise for molecular radiotherapy but are often limited by low payload resulting in delivery of inadequate amounts of radioactivity to tumour tissue and, therefore, modest therapeutic effect. We have developed PEGylated epidermal growth factor (EGF)-gold nanoparticles (NP) with a high indium-111 ([111In] payload ([111In-EGF-Au-PEG NPs) as a prototypic NP-based theranostic radiopharmaceutical.

Materials and methods: EGF-Au-PEG NPs were prepared via an interaction between gold and the disulphide bonds of EGF followed by PEGylation by mPEG-thiol (MW: 800, 2000, 6000) and characterized by SEC-HPLC, DLS and zeta potential. The targeting property of NPs with various PEG MWS was investigated by confocal imaging showing exposure of MDA-MB-468 (1.3 x 10^6 EGFR/cell) and MDA-MB-231/H2N (10^5 EGFR/cell) cells to Cy3-EGF-Au-PEG NPs. [111In-EGF-Au-PEG (MW: 6000) and [111In-EGF-Au NPs were chosen for SPECT imaging and biodistribution studies using MDA-MB-468 xenograft-bearing mice.

Results: Successful PEGylation was confirmed by DLS and zeta potential measurements, showing the hydrodynamic sizes of NPs were 18.5, 19.4, 24.8 and 32.5 nm; the zeta potentials in water were -15, -14 and -9 mV for EGF-Au and EGF-Au-PEG with MWs of 800, 2000 and 6000, respectively. SEC-HPLC showed that the retention time of EGF-Au-PEG NPs was shorter than EGF-Au NPs as PEGylation resulted in larger NPs. Confocal imaging demonstrated that both EGF-Au and EGF-Au-PEG NPs were efficiently bound and internalised by MDA-MB-468 cells. In vivo SPECT studies in mice bearing MDA-MB-468 xenografts revealed high tumour uptake in animals that received [111In-EGF-Au-PEG (MW: 6000) compared to [111In-EGF-Au. The tumour/muscle radioactivity ratios for [111In-EGF-Au-PEG and [111In-EGF-Au were 7.2 and 2.5.

Conclusion: An [111In]-labelled EGF-Au-PEG nanosystem was developed. It enabled targeted delivery of a high [111In] payload to an EGFR-positive cancer model that can be potentially exploited for molecularly targeted radiotherapy.

Introduction: Malignant melanoma is the most lethal form of skin cancer and is the most commonly diagnosed malignancy among young adults with an increasing incidence. Hence, the development of new melanoma-specific pharmaceutical for diagnosis and/or therapy is a subject of great interest in Oxford research. The purpose of this study was to examine the effect of cyclization on the biological profile of [99mTc(N)(NP3)]-labeled α-MSH peptide analogs (NP3 = N,N-bis(dimethoxypropylphosphinoethyl)methoxyethylene). Method: A lactam bridge-cyclized H-Cys-Ahx-[βAla-Nle-Asp-His-D-Phe-Arg-Trp-Glu-NH2] (NAP—NS2) and the corresponding linear H-Cys-Ahx-[βAla-Nle-Asp-His-D-Phe-Arg-Trp-Glu-NH2] (NAP—NS1) peptide were synthesized, characterized by ESI-MS spectroscopy and their MC1R binding affinity were determined in B16/F10 melanoma cells. In vitro stability and pharmacological parameters of [99mTc(N)(NP3)] and [99mTc(N)(NP—NS2)(NP3)] were assessed. Challenges with an excess of glutathione and cysteine and Log P values were also investigated. Furthermore, 1 and 2 were applied to study in vivo stability and the pharmacokinetic profiles on healthy rats.

Results: 1 and 2 were obtained in high yield (RCY > 90%). Log P values demonstrate the hydrophilic nature of the radiolabelled peptide: -1.43 for 1; -2.087 for 2. No significant variations in RCPs of both the complexes were observed in challenge experiments with an excess (10 mM) of glutathione and cysteine. A high in vitro stability was observed for both complexes after incubation in human and rat sera as well as in rat liver homogenate; a fast degradation of 2 was detected in kidneys homogenate. 1 retains a high receptor affinity (Kd = 7.1 ± 0.5 nM). Biodistribution data of 1 display a favorable pharmacokinetic profiles characterized by a fast blood clearance and elimination from normal tissues. A rapid excretion via the renal pathway was observed according to the high hydrophilic character of the radio-conjugate. The effect of the cyclization on the pharmacokinetic profile of 2 was reflected in a reduction of the blood clearance and of the elimination from the other organs, in particular, from excretory organs such as liver and kidneys.

Conclusion: Compared with the linear peptide, cyclization negatively affects the biological properties of NAP—NS2 peptide by reducing its binding affinity for MC1R (Kd: 0.9±0.3 nM for NAP—NS1; 7.1±2.4 nM for NAP—NS2) and decreasing the overall excretion rate of the corresponding [99mTc(N)(NP3)]-labeled peptide from the body as well as its stability. Thus only the linear [99mTc(N)(NP3)]-labeled peptide is suitable for further investigations in tumor bearing animals. This research was supported by MIUR through PRIN 2009F7JHPZ-004 and FIRB “RINAME”2010-8BA114AMK.
the tracer was found in the formulated solution. The single dose toxicity study showed that the compound was well tolerated in animals. This was confirmed by the clinical study where no severe side effects were observed. High metabolic stability of $^{68}$GaNODAGA-RGD was found based on the analysis of blood and urine samples. The static scans showed rapid tracer elimination from the body with low background activity in almost all organs. The calculated effective dose was $21.5\pm5.4$ $\mu$Sv/MBq. Unfortunately, the investigated tumors did not show increased tracer accumulation indicating no or low integrin $\alpha_v\beta_3$ expression.

Conclusion: This study revealed that $^{68}$GaNODAGA-RGD can be easily produced under GMP conditions and met the requirements for the clinical use. The phase I clinical study with patients bearing HCC did not allow identification of the lesions but demonstrated rapid elimination from the body, high metabolic stability and low radiation burden. The low tracer accumulation in the tumor might be related to low receptor expression, thus further studies are needed to verify the integrin $\alpha_v\beta_3$ imaging properties.

**OP45**

Setting up a GMP production of a new radiopharmaceutical Forsbach, Sarita 1, Bergman Jörgen 1, Kivell Rikka 2
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Introduction: Good Manufacturing Practice (GMP) sets strict requirements for the manufacturing conditions, synthesis and quality of radiopharmaceuticals. Robust production and quality control methods for radiopharmaceuticals are essential for diverse and effective clinical utilization of Positron Emission Tomography (PET). This presentation describes the set up procedure of a new radiopharmaceutical in Turku PET Centre (TPC). All issues other than directly related to GMP such as toxicology, labeling chemistry, analytical methods or documentation for the authorities (i.e. IMPD) are not discussed here.

Materials and Methods: Quality standards and product specification are expressed in EU (Annex 15 Qualification and Validation, revision into operation 1 Oct 2015) and European Pharmacopoeia (8th Ed.). The setup of a new radiopharmaceutical at TPC requires existence of the following: Established quality system, Documentation system, Competent personnel, Classified clean rooms, Qualified equipment, System for material management. Initially the specifications for critical materials and primary packing materials of the new radiopharmaceutical must be defined. At TPC specifications for new radiopharmaceutical are: Appearance, Identification, Radioactivity, Radionuclidic identity, Radionuclidic purity, Radiochemical purity, Chemical purity, Residual solvents, Content of ethanol, pH, Sterility, Bacterial endotoxins, Shelf life.

Results: After ensuring that the production of a new radiopharmaceutical is robust and repeatable and all analytical methods including sterility and endotoxin tests are validated, the process can be validated according to a written validation plan. In addition bioburden (number of bacteria living on the drug solution before sterilization) must be determined. The aseptic process of operators must also be confirmed by performing media fills (the performance of an aseptic manufacturing procedure using a sterile microbiological growth medium in place of the drug solution). At TPC the process validation includes three consecutive process validation batches which shall fulfill all specifications for the given radiopharmaceutical. This also qualifies the operator for production. Additional batches must be done in order to qualify more operators. Finally, process validation and documentation is compiled. Process validation report, method description for preparation and quality control and master batch record are written.

Discussion/conclusion: After all documentation has been accepted by QA, the new radiopharmaceutical is ready for clinical production and all changes to the process must be performed via change control process.

**OP44**

Implementation of a GMP-grade radiopharmacy facility in Maastricht Ivo Pooters 1, Maartje Lotz 2, Roel Wiert 1, Felix Mottaghy 1,2, Matthias Bauwens 1,3
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Introduction: In the Netherlands, a modified “light” version of the European GMP, i.e., GMP-z for the (kit-) production of registered radiopharmaceuticals for individual patients in hospital pharmacies is in effect. However, GMP-z does not allow to synthesize radiopharmaceuticals for individual patients in hospital pharmacies. Several restrictions applied: radiothe European GMP applies. At the MUMC, a growing interest in per-
Introduction: The combination of different imaging modalities has received increasing interest over the past decade, as it enables to overcome the limitations of a single imaging modality and ensures enhanced interpretation of diseases and abnormalities in vivo. Dual modality PET/MRI imaging agents, such as radiolabeled magnetic nanoparticles, are promising candidates for a number of diagnostic and therapeutic applications (i.e. MRI-magnetic hyperthermia and radiotherapy). The aim of the present study is to evaluate the efficacy of \(^{68}\)Ga labeled \(\text{Fe}_3\text{O}_4\) superparamagnetic iron oxide nanoparticles (SPIONs) coated with DPD phosphate, as potential PET/MRI imaging agents.

Materials and methods: SPIONs coated with biocompatible DPD-phosphate were radiolabeled with positron-emitting Gallium-68 to quantify the accumulation of the nanoparticles in vivo. In vitro stability studies, in PBS, saline and human serum, were performed to evaluate their aqueous solubility in vivo. In vivo biodistribution study was performed in 9 healthy mice at 30, 60 and 120 min post-injection.

Results: \(^{68}\)Ga-\(\text{Fe}_3\text{O}_4\)-DPD SPIONs presented high radiolabeling yield (95%) and proved stable in vitro. The in vivo study exhibited significant liver and spleen uptake at all examined time points in healthy mice, whereas minor fractions attained in other organs. A small fraction of radiolabeled nanoparticles presented in bones is indicative of high affinity of phosphate to bone tissue. The biodistribution profiles between \(^{68}\)Ga-\(\text{Fe}_3\text{O}_4\)-DPD SPIONs and free \(^{68}\)Ga-acetate were also compared, indicating different pharmacokinetic behavior for \(^{68}\)Ga-acetate, with no target tissue and excretion via the kidneys.

Conclusion: \(^{68}\)Ga-\(\text{Fe}_3\text{O}_4\)-DPD SPIONs demonstrated high radiolabeling efficiency and in vitro stability and satisfactory in vivo behavior. Cytotoxicity studies to explore the potential toxic effects of the nanoparticles, as well as biodistribution studies in tumor models, are in progress.

Results: I. v. injection of \(\text{Fe}^{3+}\) containing salts of hydroxy acids blocked the metal-binding capability of transferrin serum and others and allowed decreasing gallium-68 radioactivity in blood significantly and increasing accumulation in inflammation (3-5 time). It allowed receiving more informative PET-images of inflammation early (for 30-60 min after injection). Pharmacokinetic parameters proved it.

Discussion/conclusion. There was no statistically significant difference between \(^{68}\)Ga-citrate accumulation for different inflammation model because PET imaging is indication of pathological processes and isn’t their identification.

POSTER PRESENTATIONS

PP01

Installation and validation of 11C-methionine synthesis

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Introduction: In the installation of methionine as our latest tracer we chose the Eckert and Ziegler Modular-Lab PharmTracer as production module, utilizing the so called «wet-method» for producing methyl iodide. After complete installation in the hot cell, a simplified version of Ph.Eur (Ph Eur monograph 1617) release criteria was used, and the product was found to pass. HPLC showed excellent radio purity and the installation was completed.

Materials and methods: Homocysteine thiolactone, iodic acid, 0.1M lithium aluminum hydrid in THF, potassium dihydrogen phosphate were used in the C11-Met synthesis, Methods used are described in Ph.Eur, with the exception of chiral HPLC, in which an Astec Chirobiotic T column with 70:30:0.02, MeOH:-H\(_2\)O:HOOCOH eluent was employed.

Results: During later analyses for residual solvents in connection with a GC-PQ, THF was found present in the product, 3-4 times above recommended level. The HPLC method in the Ph.Eur is 10 minutes, however, by increasing acquisition time, a radio peak appeared at about 18 minutes in the chromatogram. This peak was about 10-15% of total radio signal, meaning the required radio purity limit in Ph.Eur was not met. When analysing for enantiomeric purity, which was not initially done, about 12% of the \(d\)-enantiomer was found, not meeting the Ph.Eur limit. Increasing the flow of helium during the azetropic distillation decreased residual solvent, and subsequently the ICH residual solvent limits was met. The radio peak in the HPLC was found to be methyl iodide (Mel) which is adsorbed and co-eluted from the C-18 Sep-Pak cartridges. Eckert and Ziegler proposed a re-arrangement of the module tubing and a new synthesis template program enabling direct helium flushing of the C-18 Sep-Pak. By the use of this new configuration, removing excess Mel was achieved and the radio purity limits described by the Ph.Eur monograph was finally met. Different protocols are called for in the initial phase dissolving the precursor in ethanol and NaOH solution, however, tweaking these had little effect of the enantiomeric purity. Different batches of precursor was later analysed for enantiomeric purity. Large discrepancies were found between batches which directly influenced the enantiomeric ratio of the final product.

Conclusion: Great care should be taken in the installment of new synthesis systems, and all available tests should be run before system installation is approved. The Ph.Eur. methods may not always be sufficient to prove radio or chemical purity, even if a monograph exist.
Introduction: The interest in 68Ga-tracers has been growing strongly over the last years, mainly due to new developments in prostate cancer imaging and therapy. In collaboration with IBA, we have established an automated synthesis of 68Ga-labelled peptides including 68Ga-DOTA-TATE-NOC, 68Ga-PSMA-11 and 68Ga-PSMA-617 on Synthera®.

Materials and methods: During the tests, an IBA Synthera® and the new Synthera® Extension module were used. 68Ga was obtained initially from an old iGG 100 generator in 5 mCi. The final tests were carried out in combination with a new GalliaPharm 68Ge/68Ga generator loaded with 50 mCi. A modified standard nucloephilic IFP™ (IFP™ FDG) was designed for the synthesis process. Synthera® Extensions were used for the elution of the generator. The eluate was loaded on a Chromaxf 5S-H+ cartridge and the hydrochloric acid waste - which contains most of the 68GeCl4 - was transferred into the waste container. The activity was eluted from the cation exchange cartridge using a solution of 5 M sodium chloride in 0.1 N hydrochloric acid and transferred directly into the reaction vessel which was pre-loaded with precursor in 1.5 M HEPES buffer. Labelling was performed using a Sep-Pak® Light C18 cartridge. The product was eluted with a 1:1 mixture of ethanol/water and dispensed into the final vial through a sterile filter. Further dilution was performed with 0.9% saline by passing through the sterile filter. The peptides were synthesized in a GMP-compliant qualified area at ABX. For the DOTA-peptides, stock solutions were prepared (1 mg/ml) and freeze-dried. For PSMA-11, vials with 10 μg of precursor were used.

Results: With 50 μg of DOTA-TATE, DOTA-NOC and PSMA-617, the final radiolabelled products were obtained in >60% corrected yield. For PSMA-11, only 10 μg of precursor were used. The radiochemical purity was >98% in all cases. The Ph. Eur. spot test for HEPES was performed and showed HEPES < 200 μg/V with V being 12 to 14 ml. The pH of the final solution was 5 to 5.5. Ethanol content was < 10%.

Discussion/conclusion: We have developed a dedicated disposable IFP cassette for the IBA Synthera® and Synthera® Extension modules, which delivers all common 68Ga-tracers in high yield. The use of dedicated single-use Gallium-68 IFP™ allows for production of 18F-FDG on the same module with no cross-contamination.

Methods: The Cyclone 18 twin from IBA is a fixed energy cyclotron using 18 MeV protons in which H particles are accelerated and converted into protons (H+). Bombardment with protons of the special designed target filled to 25 bar takes place, initiating the nuclear reaction: 15N(p,n)15O. 15O2H2O was produced by conversion of the 15O2 using a new designed IBA chemistry module and placed in a shielded class A hood located in the GMP laboratory. During preparation, 15O gas flows from the cyclotron into the IBA chemistry module and is mixed with hydrogen gas and passed through a palladium column. The produced 15O2H2O is collected in a sterile 0.9% saline solution to obtain a final product suitable for patient administration. Validation of the production process included: (1) assessment of practical yields, (2) check on pharmaceutical requirements and (3) reproducibility of performance of both cyclotron and water module.

Results: According to our knowledge, this is the first time that GMP production of 15O labeled water using IBA 18 MeV proton cyclotron is described. The method was validated and met all pharmacopoeia specifications, and was subsequently approved for patient studies. The practical production yield of 15O labeled water using this method was ranged between 1300-1700 MBq measured in the syringe. This method is suitable for multiple batches within a time frame of 8 minutes. After validation more than 50 patient runs were performed with a reliability of > 99%.

Conclusion: A new production method for 15O-labeled water using Cyclone 18 and dedicated chemistry module from IBA was described and qualified according to GMP regulations. It was demonstrated to be suitable for clinical use.
**Results:** We observed that the radionucleating efficiencies of SubPc and Zn(II)Pc were significantly high (91.9±3.1% and 97.2±1.4%, respectively). The optimization conditions were determined as pH 9, 1 mg amount of iodogen and 30 minutes of reaction time for SubPc; pH 7, 1 mg amount of iodogen and 45 minutes of reaction time for Zn(II)Pc. In vitro cell line studies are in progress.

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**PP06**

**Radio-U(H)PLC – the Search on the Optimal Flow Cell for the γ-Detector**

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**EJNMMI Radiopharmacy and Chemistry 2016, 1(Suppl 1):PP06**

**Introduction:** Radio-HPLC is routinely used for analysis and quality control of radiopharmaceuticals. The introduction of U(H)PLC enabled decreased detection levels, improved resolution and significant shorter analysis times; crucial parameters for radiotracer analysis. Despite these benefits only a few examples of radio-U(H)PLC have been reported; one reason may be the absence of a suitable γ-detector with a flow cell which is compatible to UPLC conditions. We used a commercial γ-detector in combination with different flow cell inserts and report on their effect on the sensitivity and resolution of the γ-signal.

**Materials and methods:** An ACQUITY UPLC H-Class System (Waters) was used with a GABI Star γ-detector (Raytest) equipped with a 2 x 2" pinhole scintillation crystal in combination with three different flow cells, UPLC was performed on an ACQUITY HSS C18 column (2.1 x 100 mm, 1.8 μm) with a flow rate of 0.65 mL/min and a 3 min gradient of 30/70 acetonitrile/0.1% TFA. The sigma-1 receptor imaging probe $[^{18}F]$fluspidine served as analyte with 2 μL injection volume, containing 8 – 400 kBq of $[^{18}F]$fluspidine.

**Results:** For best resolution and avoiding an excessive back-pressure to the PDA cell (<70 bar) the capillary between PDA and γ-detector should be as short as possible. This was realized by placing the γ-detector in 30 cm distance to the PDA-detector on a special table. Three different flow cells were applied:

- *a) Raytest standard flow cell (5 μL), b) Raytest 2" capillary holder equipped with a UPLC capillary 0.004" ID (9/5.12 NL), c) self-build lead insert with a 5 mm hole and UPLC capillary (2 NL).*

By injecting 400 kBq of $[^{18}F]$fluspidine and using the different flow cells, γ-signals having a peak width between 0.13 and 0.20 mm and a peak intensity 750 - 4400 cps were obtained. The capillary holder (5 NL) and the self-build insert (2 NL) showed a comparable peak width, but differentiated sensitivity. To determine the minimum detectable level samples between 170 kBq and 8 kBq of $[^{18}F]$fluspidine were injected; with 15 kBq of $[^{18}F]$fluspidine a good signal to background ratio of 6:1 was achieved.

**Conclusion:** By performing radio-U(H)PLC with a GABI Star γ-detector equipped with a Raytest 2" capillary holder (5 NL), γ-signals with good sensitivity and peak width can be obtained. In addition, the self-build flow cell insert (2 NL) gave the highest sensitivity and the best signal to background ratio.

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**PP07**

**Radiolabeling, characterization & biodistribution study of cysteine and its derivatives with Tc99m**

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**Introduction:** Several studies are available, which highlight the use of Schiff based as ligands for radio-labeling, but very little work has been reported with Mercapto compounds. It is desirable to include such compounds in the design of radiopharmaceuticals due to their importance in the biological system.

**Materials and methods:** Equimolar L – cysteine and salicylaldehyde in distilled water and ethanol were heated to form a white
colored ligand. The product formed was analyzed using FT – IR, thermo gravimetric analysis and elemental analysis. Radiolabeling of the ligand was performed using 99mTc from 99Mo generator. SnCl2.H2O was used as a reducing agent. Radio TLC was performed using 5 plates, 0.9% NaCl and aceton as the mobile phases. Geiger Muller counter detected the counts on the 5 plates. After quality control the radiolabeled drug was injected IV into the animal ear. Scanning was performed under the gamma camera.

Results: Ligand synthesis was verified from IR indicating the presence of the closed ring structure Thiozolidine ring. IR showed absence of chromophore group hence the ligand was colorless. DSC peaks indicated the reaction type as endothermic. Ligand appeared to be stable in DMSO. Effective radiolabeling was achieved using lyophilized tin chloride pyrophosphate cold kit in NaCl (0.9%). Optimization of pH, temperature and radioactivity was done using DOE. Maximum radiolabeling was achieved at pH 5. Animal study was performed for bio distribution of the radio-pharmaceutical. 99mTc – ligand uptake was seen in the soft organs immediately after injection. The areas showing higher radiotracer uptake were kidney (20 %), liver (35 %), bones (15%) and brain (10 %). Delayed images showed the radiotracer retention in the soft organs as well as in the spine of the animals. Comparative study was performed using 99mTc – MDP and 99mTc PHYTATE.

Conclusion: Sialylaldicysteine is efficiently labeled with 99mTc and is suitable in simultaneous scanning of liver, kidney and brain. Due to uptake in all soft organs in dynamic flow followed with delay images, radiotracer retention in the brain is worth consideration. This indicates the ability of drug in crossing the blood brain barrier. This study also highlights the importance of sulfur containing ligands in brain scanning.

PP08
Radiolabelling of poly (lactic-co-glycolic acid) (PLGA) nanoparticles with 99mTc
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Introduction: Radiolabelled nanoparticles have gained a wide-spread application in the diagnosis and therapy of several diseases (inflammation/infection, cancer, and others). PLGA Nanoparticles (NPs) prepared by solvent emulsion evaporation method [1] have shown more suitable characteristics with regard to those produced by nanoprecipitation: better size, a higher homogeneity and higher encapsulation efficiency. The aim of the present work is to optimize the radiolabelling of PLGA nanoparticles with 99mTc.

Materials and methods: PLGA nanoparticles were prepared using the nanoprecipitation method [1]. Then, 5 mg of lyophilized NPs were dispersed in 1 mL 0.9% NaCl and stored in a vacuum vials. Different amounts of stannous chloride dehydrate in aqueous solution (30, 20, 4, 2, 1 and 0.1 μg) were added. Then, was added 74 MBq (2 mCi) of 99mTc in 1 mL of 0.9% NaCl. Finally, the suspension was incubated for 10 min. 99mTc-NPs suspensions were analyzed by thin layer chromatography (TLC) with silica gel strips (10 x2.5 cm). With 0.9% NaCl as the mobile phase, free pertechnetate ran with the front, meanwhile particles stayed in the start. Using a solution of pyridine: acetic acid: water (3: 5: 15), radiocolloids remained at start, NPs migrated with Rf = 0.3 and free pertechnetate moved with Rf = 0.7-0.8 [2].

Results: Using 1 μg of SnCl2.H2O as a reducing agent, PLGA nanoparticles (without surface modification) were labeled with 99mTc with a yield ≥ 90 %. PLGA nanoparticles size ranged from 160-180 nm in diameter with an electric surface of -36 mV. After radiolabelling, particles increased in size up to a diameter ≈ 380 nm.

Conclusions: Results obtained, indicate that the procedures of nanoparticle synthesis, radiolabelling and quality control were reproducible and right to design a biodegradable nanosystem suitable for in vivo theranosis.

PP09 Development of [18F]PD-410 as a non-peptidic PET radiotracer for gastrin releasing peptide receptors
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Introduction: The Gastrin Releasing Peptide (GRP) receptor has high expression on prostate cancer cells. Consequently, bombesin, a 14-amino acid peptide that binds with high affinity to GRP receptors, has been widely studied for the development of radio-peptides for application in prostate cancer imaging. To overcome some limitations of radiolabelled peptides, we developed a small molecule non-peptidic radiotracer targeting GRP receptors. Small molecules offer various advantages over peptides since they can be suitably designed to modulate potency, selectivity, lipophilicity, and cell permeability and do not suffer from poor tissue penetration, poor serum stability, and quick elimination. The aim of the present study is to develop a non-peptidic fluorine-18 PET radioligand with antagonist properties, for visualization of GRP receptors. PD-410 ([3-(1H-indol-3-yl)-N-[(5-2-fluoroethoxy)pyridin-2-yl]cyclohexylmethyl]-2-methyl-2-[3-(4-nitrophenyl)ureido]propionamide) was selected as potent compound for [18F]-radiolabelling (Ki was 38 ± 2 nM).

Methods: [18F]PD410 was synthesized by reaction of [18F]fluorooethyl tosylate with the corresponding phenol precursor in presence of NaH followed by HPLC-purification. Cell experiments were carried out with PC3 cells to determine cell uptake, efflux and in vitro binding affinity.

Results: In contrast to other [18F]fluoroethylation reactions, RCM was low (≤5%) because of substantial defluorination. The uptake of [18F]PD410 in PC3 prostate cancer cells gradually increased within 60 minutes of incubation, thereafter reaching a plateau. The maximum cellular associated radioactivity was found to be 70%. Non-specific binding accounted for 40%. The efflux kinetics of [18F]PD410 showed rapid dissociation of the tracer, remaining only 6% of the tracer within 120 min. This radioactivity decreased exponentially with a half-life of 13 minutes. In vitro binding affinity of [18F]PD410 was plotted as sigmoid curves for the displacement of [18F]PD410 as a function of increasing concentrations of the GRP receptor specific inhibitor Glu[AcA-BN(7-14)]. The IC50 value was 0.10 nM for Glu[AcA-BN(7-14)].

Conclusions: [18F]PD410 displayed high affinity for GRP receptor and the in vitro results warrant further in vivo PET-studies.
**PP10**

An improved nucleophilic synthesis of 2-(3,4-dimethoxyphenyl)-6-[18F]fluoroethoxy benzothiazole (18F FEDMBT), potential diagnostic agent for breast cancer imaging by PET

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**Introduction:** Substituted benzoazoles (BT) are a class of compounds with high affinity and selectivity to ary hydrocarbon receptor (AhR), a receptor often expressed in breast cancer tissue. Among them 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (PMX 610) exhibited very potent (GI50 < 0.1 nM) antitumor properties in vitro in human breast cancer cell lines. Based on that scaffold we recently suggested [18F]fluorothiolated analogue of PMX 610, the 2-(3,4-dimethoxyphenyl)-6-[18F]fluoroethoxybenzothiazole ([18F]FEDMBT). Preliminary studies showed high homogeneity of the radiotracer in MCF-7, MDA-MB468 and MDA-MB231 breast cancer cells. Here we report an improved synthesis and purification of [18F]FEDMBT for preclinical trials.

**Materials and methods:** The extent of [18F]-fluorination of labeling precursor was followed via radioTLC (EtAchexane 1:2). The HPLC purification was performed using Waters X18 B325 250 x 10 mm column, 0.05M NH4OAc:EtOH, 50:50, 3 ml/min, UV 254 nm. For SPE purification 1.2 mL of aqueous 25% EtOH was added to the reaction mixture (5 mg of precursor, 0.6 mL of DMF) to prevent precipitation formation. The resulting solution was passed through the Sep-Pak C18 Plus Long cartridge. The cartridge was then rinsed with 2 mL of 50% EtOH allowing for complete removal of the precursor. The product, [18F]FEDMBT, was recovered by successive elution of the cartridge with 2 and 1 mL of 55% aq. EtOH.

**Results** The [18F]FEDMBT was prepared via direct nucleophilic fluorination of the corresponding tosyl precursor 1. Under optimal reaction conditions (kryptofix 2.2.2, DMF, 140°C, 5 min) a high 18F-incorporation rate of 80-85% was achieved. The HPLC purification afforded the product in >99% radiochemical purity and RCY of 33% (decay corrected). Isolated product fraction was mixed with phosphate buffered saline to adjust the pH and ethanol concentration, without final re-formulation step. Fractionated SPE purification afforded product with >99% RCP and 60% RCY (decay corrected). While SPE technique allowed for complete removal of precursor, a minor chemical impurity (not identified) remained in the final formulation. Work is being undertaken now to resolve this issue.

**Discussion/conclusion:** An optimized synthesis of [18F]FEDMBT afforded product with radiochemical purity and high yield. It can be easy automated using TraceLab FX-N Pro platform. The suggested “HPLC free” SPE purification can be applied in the production of [18F]FEDMBT for ongoing in vitro cell line experiments and preclinical trials. This study was supported by RFBR grant 15-54-52062/15 and MOST 104-2923-B-010-001-MY2.

**PP11**

Internal radiation dose assessment of radiopharmaceuticals prepared with accelerator-produced 99mTc

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**EJNMMI Radiopharmacy and Chemistry, 2016, 1(Suppl 1):PP11**

**Introduction:** 99mTc is the radionuclide most widely used in diagnostic nuclear medicine. It is available from 99Mo/99mTc generators, where 99Mo is obtained by a fission reaction in nuclear reactors. Direct reactions using proton beams (e.g. 100Mo(p,2n)99mTc) are a reliable and relatively cost-effective method to fulfill the shortage of this isotope given the imminent closure of the existing old reactors. However, the results of LARAMED project from the LNL-INFN showed that the extracted solution of 99mTc from the proton-bombarde 100Mo-enriched target contains small quantities of several technetium radioisotopes (93Tc, 94Tc, 95Tc, 95mTc and 96Tc) [1]. The aim of this work was to estimate the total contribution of technetium radioisotopes to the patient radiation absorbed dose after administration of four radiopharmaceuticals prepared with technetium-99m obtained from the 100Mo(p,2n)99mTc reaction.

**Methods:** The internal radiation absorbed doses of pertechnetate, sestamibi, hexamethylpropyleneamine oxime (HMPAO), and di-sodium etidronate (HEDP) radiopharmaceuticals, were assessed considering both technetium-99m prepared from the 100Mo(p,2n)99mTc reaction as well as from generators. Time-integrated activity in the main source organs [Ã(rs,TD)] for each radioisotope was calculated using the radiopharmaceutical biokinetic models published by the International Commission on Radiological Protection (Publication 53 and 80, ICRP). OLINDA/EXM 1.1 software was applied for dose calculations using an adult male phantom as a program input and the actual patient (not identified) remained in the final formulation. Work is being undertaken now to resolve this issue.

**Discussion/conclusion:** An optimized synthesis of [18F]FEDMBT afforded product with radiochemical purity and high yield. It can be easy automated using TraceLab FX-N Pro platform. The suggested “HPLC free” SPE purification can be applied in the production of [18F]FEDMBT for ongoing in vitro cell line experiments and preclinical trials. This study was supported by RFBR grant 15-54-52062/15 and MOST 104-2923-B-010-001-MY2.

**PP12**

A specialized five-compartmental model software for pharmacokinetic parameters calculation

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**EJNMMI Radiopharmacy and Chemistry, 2016, 1(Suppl 1):PP12**

**Introduction:** The use of pharmacokinetic modeling in preclinical nuclear medicine is very limited although it is well known that such models could be valuable tools to study radiopharmaceutical-properties. Nowadays, the preclinical evaluation of new radiopharmaceuticals is still focused only on biodistribution studies, mainly because of the lack of currently available user-friendly specialized programs to assist researchers in calculating pharmacokinetic parameters.

**Methods:** A dedicated spreadsheet software to calculate pharmacokinetic parameters such as tracer-biological half-life (T1/2), mean residence time (MRT) for each compartment, as well as the fraction of the tracer leaving the compartments per unit time (transfer rate
constant, TRC), has been developed. The menu-driven spreadsheet software is based on five-Compartment Kinetic Model (CoKMo). CoKMo was used to study pharmacokinetic parameters of 11 99mTc-nitrido complexes, reported as potential myocardial perfusion-imaging agents (MPIAs), of general formula (99mTcN(DTC-Ln)(PNP)+=DTC-Ln= alicyclic dithiocarbamates; PNP= diphosphinoamine)2, and the results were compared with those of 99mTc-Seastamibi and 99mTc-Tetrofosmin. Bio-distribution studies for all MPIAs have been performed in Sprague-Dawley rats (n = 312) to determine organ uptake.

Results: MPIAs time-activity curves have been obtained using CoKMo for 5 compartments, by interpolation of organ activity values measured at different time points. Curves have been integrated to calculate T1/2 and MRT in the studied organs of all MPIAs. Subsequently software employs a module with implemented optimization procedure, dedicated to calculate the values of the TRCs. Finally, CoKMo calculations were validated by comparison with two different software: Olinda/Exm and SAAM II. 99mTcN-(PNP)+ complexes showed a faster blood and liver clearance as compared to 99mTc-Seastamibi and 99mTc-Tetrofosmin, in agreement with some previous reported studies. However, it was found that when CoKMo pharmacokinetic data were used, a wide quantitative comparison between all MPIAs was possible, which in fact, was not possible using only the biodistribution data. The analysis of the results showed that 99mTcN-PDTC3 turned out to be the best candidate for translation into clinical practice due to its rapid and high heart uptake, and fast liver and lung clearance, which enabled an early visualization of myocardial tissue.

Conclusion: CoKMo proved to be an easy to use and flexible specialized software to study the kinetic of a great number of pharmaceutic materials labeled with an unlimited number of radionuclides. Therefore, it can be a useful tool for an extensive preclinical characterization of new radiopharmaceuticals.

PP13

Molecular imaging of the pharmacokinetic behavior of low molecular weight 18F-labeled PETOx in comparison to 89Zr-labeled PETOx

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EJNMMI Radiopharmacy and Chemistry 2016, 1(Suppl 1):PP13

Introduction: PEGylation is a methodology that is commonly used to improve the PK profile of radiotracers. In recent years biocompatible poly(2-alkyl-2-oxazoline)s (PAOx) have been developed. CoKMo was used to study pharmacokinetic parameters of 11 99mTc-nitrido complexes, reported as potential myocardial perfusion-imaging agents (MPIAs), of general formula (99mTcN(DTC-Ln)(PNP)+=DTC-Ln= alicyclic dithiocarbamates; PNP= diphosphinoamine)2, and the results were compared with those of 99mTc-Seastamibi and 99mTc-Tetrofosmin. Bio-distribution studies for all MPIAs have been performed in Sprague-Dawley rats (n = 312) to determine organ uptake.

Results: MPIAs time-activity curves have been obtained using CoKMo for 5 compartments, by interpolation of organ activity values measured at different time points. Curves have been integrated to calculate T1/2 and MRT in the studied organs of all MPIAs. Subsequently software employs a module with implemented optimization procedure, dedicated to calculate the values of the TRCs. Finally, CoKMo calculations were validated by comparison with two different software: Olinda/Exm and SAAM II. 99mTcN-(PNP)+ complexes showed a faster blood and liver clearance as compared to 99mTc-Seastamibi and 99mTc-Tetrofosmin, in agreement with some previous reported studies. However, it was found that when CoKMo pharmacokinetic data were used, a wide quantitative comparison between all MPIAs was possible, which in fact, was not possible using only the biodistribution data. The analysis of the results showed that 99mTcN-PDTC3 turned out to be the best candidate for translation into clinical practice due to its rapid and high heart uptake, and fast liver and lung clearance, which enabled an early visualization of myocardial tissue.

Conclusion: CoKMo proved to be an easy to use and flexible specialized software to study the kinetic of a great number of pharmaceutic materials labeled with an unlimited number of radionuclides. Therefore, it can be a useful tool for an extensive preclinical characterization of new radiopharmaceuticals.

PP14

Towards nucleophilic synthesis of the α-[18F]fluoropropyl-Dihydroxyphenylalanine

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Introduction: The interest towards use of 6-[18F]fluoro-L-DOPA as a PET radiotracer for the evaluation of dopaminergic system’s state and also as tumor diagnostic agent has been steadily increasing during the last decade. Considerable work is being undertaken to overcome the practical difficulties encountered in the production of 6-[18F]fluoro-L-DOPA and other ring-fluorinated amino acids via nucleophilic substitution route. Aromatic amino acids carrying mono-fluor methyl group in the α-position have also been considered as potential PET radiotracers, with nucleophilic synthesis of racemic α-[18F]fluoromethyl phenylalanine using cyclic sulfamide precursor and reported recently. Our group has suggested a route towards enantioselectively pure α-[18F]fluoromethyl-L-tyrosine via direct nucleophilic fluorination of a Nill complex. However, 18F-fluorination of the precursor bearing α-MeSO3 (meslyoxy) leaving group was inefficient, possibly due to the steric hindrance at the α-carbon of the tyrosine moiety. In continuation of that previous work we have investigated the synthesis of the α-[18F]fluoropropyl-L-DOPA via 18F-fluorination of a similar precursor.

Materials and methods: Radiofluorination of L was performed in the presence of cryptofix/K2C3O3 (acetic, 10 min, 850C, 10 mg of precursors). The extent of 18F-fluorination was followed by radioTLC (EtAc:CHCl3:HCOOH 4:1:1). After solvent removal the 18F-fluorinated intermediate was treated with 6M ac. HCl at 140°C for 10 min. The product was analyzed by radioTLC (EtAc:n-Butanol:CH3COOH 4:1:1) but where α-[18F]fluoromethyl-L-tyrosine via direct nucleophilic fluorination of a Niill complex. However, 18F-fluorination of the precursor bearing α-MeSO3 (meslyoxy) leaving group was inefficient, possibly due to the steric hindrance at the α-carbon of the tyrosine moiety. In continuation of that previous work we have investigated the synthesis of the α-[18F]fluoropropyl-L-DOPA via 18F-fluorination of a similar precursor.

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Results: 18F-BCN-PETOx was obtained with a RCY of 4.30% (n=4, decay corrected to EOB) and a RCP of 100% after PD-10 purification. As was previously demonstrated for 99mTc-PETOx 5kDa, μPET imaging revealed rapid and complete blood clearance of 18F-BCN-PETOx 5kDa (SUV=2.42±0.16 at 10 min pi and SUV=0.27±0.08 at 1h pi). Peak liver uptake related to perfusion was reached within 1min pi (SUV=2.92±0.18) and quickly decreased (SUV=0.50±0.03 at 10 min pi and SUV=0.07±0.00 at 1h pi) indicating only minor contribution of the liver in the clearance of 18F-BCN-PETOx 5kDa. The kidneys displayed a high initial uptake (SUV=5.67±0.87 at 3.75min pi versus 7.58±2.54 at 3.75min pi for 89Zr-Df-PETOx 5kDa) but where 89Zr-Df-PETOx 5kDa was not cleared from the kidneys (SUV=2.36±0.84 at 24h pi), 18F-BCN-PETOx 5kDa showed a fast and almost complete clearance (SUV=0.27±0.08 at 1h pi and SUV=0.12±0.05 at 2h pi).

Conclusions: 18F-BCN-PETOx 5kDa is quickly cleared from the body and does not display high kidney retention. The unusual kidney retention of 89Zr-Df-PETOx 5kDa is therefore most likely related to endocytosis and lysosomal degradation of the radiolabeled polymer followed by trapping of radiometal 89Zr in the proximal tubules.
Discussion/conclusion: The nucleophilic fluorination of Ni²⁺-based labeling precursor offers potential novel route towards a new derivative of DOPA with ¹⁸F-label in the α-fluoropropyl group. Preparation of precursor employing methoxy methoxy (MOM) protective groups instead of mesyl ones, something that should resolve the deprotection problem, is currently in progress. This study was supported by SNF grant IZ73Z0_152360/1.

PP15
A convenient one-pot synthesis of [¹⁸F]clofarabine
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Introduction: [¹⁸F]Clofarabine has recently emerged as a promising radioisogand for in vivo imaging of deoxytцитidine kinase activity using PET. In the reported protocols by Shu and Wu (1, 2), 3 is prepared in a two-step process with an intermediate purification that proceeds with 10-15% radiochemical yield (RCY). We herein report a simplified procedure for the preparation of 3, in which the intermediate purification was eliminated.

Materials and methods: [¹⁸F]Fluoride was produced using a GE PET-cyclotron and dried azetropically with acetonitrile (MeCN), potassium carbonate and kryptofix (K₂.2.2). Radiofluorination was performed in MeCN at 90 oC for 20 minutes, after which solvents were removed in vacuo and deprotection was performed using 10% trifluoroacetic acid (TFA) in dichloromethane (DCM) at room temperature (rt) for 5 min. Following an additional evaporation, the crude product was dissolved in mobile phase and purified using semi-preparative HPLC (ACE, C18, 5µm, HIL 250×10 mm, eluted with MeCN: aq. NH₄OH (0.6%) 90.5:9.5 (v/v) at 6 mL/min). 3 was isolated from the eluent using a C-18 SepPak cartridge (Waters) and formulated for intravenous injection in a solution of ethanol and phosphate buffered saline (10:90).

Results: The radiochemical conversion (RC) of [¹⁸F]fluoride into 3 was between 25-30%. Higher reaction temperatures or prolonged heating did not improve the RC. Removal of MeCN from the reaction mixture prior to deprotection was found to be essential and may be ascribed to the known inhibiting properties of MeCN on trityl-group hydrolysis (3). By-product formation, sometimes observed when refluxing 2 with aqueous HCl, could be effectively eliminated by employing mild conditions for the deprotection (10% TFA in DCM, rt, 5 min) without compromising the RCY. No intermediate purification was required in the process, that produced >99% radiochemically pure 3 in 20% RCY with a specific activity >100 GBq/μmol.

Discussion/conclusion: A simplified one-pot radiosynthesis of 3 was developed in which intermediate purification was eliminated. The RCY is on par with previously reported protocols for 3 synthesis.

PP16
BODIPY-estradiol conjugates as multi-modality tumor imaging agents
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Introduction: In vivo imaging of estrogen receptor (ER) densities in human breast cancer is a potential tool to stage disease, guide treatment protocols and follow-up on treatment outcome. Among various techniques to detect ligand-ER interaction both postron emission tomography (PET) and fluorescence imaging have received ample attention.

Materials and methods: In this study we use 4,4-difluoro-4-bora-3a,4a-diazas-indacene (BODIPY) as a fluorescent probe and precursor for ¹⁸F-labeling to develop estradiol-based ligands for ER. The synthesis involves attachment of a BODIPY moiety to the C17a-position of estradiol using Sonogashira cross coupling reaction of iodo-BODIPY and various 17a-ethylaryl estradiol (EE2) derivatives. Confocal laser scanning microscopy was used to monitor cellular uptake of the EE2 conjugates after 1 h of treatment without and with estradiol to block ER. Flow cytometric analysis was performed to determine the relative fluorescence in the nuclei. The relative binding affinities to the estrogen receptors (ERα and ERβ) was assessed by a competitive radiometric assay.

Results: The Sonogashira coupling reaction of EE2 derivatives with iodo-BODIPY (1:1 molar ratio) in THF/toluene (1/2) and using PdCl₂(PPh₃)₂ as catalyst, NEt₃ as a base and Cu at room temperature gave the desired EE2-BODIPY conjugates in 45-86% yield (after purification by silica gel column chromatography). In vivo fluorescence imaging of the basic EE2-BODIPY conjugate confirmed ER-mediated uptake by the nuclei of ER-positive MCF-7/L1 cells.

Discussion and conclusion: The synthesis of a series of EE2-BODIPY conjugates was achieved via Sonogashira cross-coupling in moderate to very good isolated yields. Our in vitro nuclear cell uptake and receptor binding results warrant further studies to evaluate the potential of the EE2-BODIPY conjugates for fluorescence/PET imaging of breast cancer that overexpress ER. Fluor-18 labelling of the BODIPY moiety of the conjugate, and in vivo fluorescence/PET imaging studies, are in progress.

PP17
Easy and high yielding synthesis of ⁶⁸Ga-labelled HBED-PSMA and DOTA-PSMA by using a Modular-Lab Eazy automatic synthesizer
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Introduction: ⁶⁸Ga-labelled PSMA inhibitor analogues are one the most important class of new radiotracers under clinical observation and development for the detection of prostate cancer lesions and metastases. ⁶⁸Ga-PSMA-11 (⁶⁸Ga-PSMA-HBED-CC) has already attested its suitability in the clinical practice and ⁶⁸Ga-PSMA-617 (⁶⁸Ga-DOTA-PSMA) has recently demonstrated its potential in individual first-in-man studies gaining importance above all in view of its theranostic application as it can be labelled also with ⁹⁰Yttrium and ¹⁷⁷Lutetium. In this study the two ⁶⁸Ga-labelled radiotracers were synthesized using an automatic Modular-Lab Eazy synthesizer (Eckert & Ziegler) and the reliability of the system was validated to guarantee preparations of pharmaceutical grade.

Materials and methods: The system was assembled with a disposable cassettes and the vials were filled with commercial precursors and ready to use pharmaceutical grade reagents. After clicking the start button the following steps were performed: a 1850 MBq GalliaPharm ⁶⁸Ge/⁶⁸Ga generator (Eckert & Ziegler) was eluted with 0.1 M HCl by a peristaltic pump by passing through a cation exchange cartridge into a waste vial. The cartridge was dried and the blocked activity was eluted with 0.55 ml of a 5.5 M HCl/M NaCl solution into the reaction vial pre-filled...
with 40 ug of precursor (PSMA-11 or PSMA-617) and 2.6 ml of Sodium Acetate/HC/ETOH buffer. The reactor was heated at 110°C for 4 (PSMA-11) or 8 (PSMA-617) minutes. The mixture was diluted with 7 ml of 0.9 % NaCl solution and transferred into the final product vial by passing through a light CM cartridge and a sterilizing filter.

**Results:** Both radiopharmaceuticals were produced in high yield. Starting from an activity of 700±20 MBq the radiochemical yield were 75.6±10 and 75.2±7 % for PSMA-11 and PSMA-617, respectively (n = 5) after 15 minutes. RCP was assessed by HPLC and was always > 98 % (for 68Ga-PSMA-11 the sum of the peaks of the two isomers was considered). All the preparations were sterile and the endotoxin content was < 0.5 EU/ml.

**Discussion/conclusions:** The Modular-Lab Eazy synthesizer works with a new technology which uses a pressure distribution system to transfer the liquid instead of solenoid valves or stopcocks. A disposable, easy to assemble cassette allows the synthesis of 68Ga-PSMA-11 and 68Ga-PSMA-617 in high RCY and quality.

**PP18 Synthesis and evaluation of fusarinine C-based octadentate bifunctional chelators for zirconium-89 labelling**

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**EJNMMI Radiopharmacy and Chemistry 2016, 1(Suppl 1):PP18**

**Introduction:** 89Zr has received considerable interest as a positron emitting radionuclide for immuno-PET imaging due to the excellent nuclear and physical properties. Recently we reported that fusarinine C (FSC) is a promising alternative chelator for 89Zr-based PET imaging agents, exhibiting superior stability and kinetic inertness as compared to 89Zr-desferrioxamine B ([89Zr]DFO).[1] Here we designed FSC derivatized chelators for 89Zr labeling which were expected to on the one hand improve the stability of 89Zr-complexes by saturating the 8 coordination sphere of 89Zr and on the other hand, reduce the number of functionality making it suitable for the conjugation to monoclonal antibodies.

**Materials and methods** FSC(succ)2 and FSC(succ)3 were synthesized by FSC reacting with succinic anhydride, and FSC(succ)2AA was synthesized by FSC(succ)2 reacting with acetic anhydride. The complexation properties of FSC(succ)2AA and FSC(succ)3 with Zr4+ were studied by reacting with ZrCl4. For in vitro evaluation partition coefficient, protein binding property, serum stability as well as acid dissociation and transchelation studies of 89Zr-complexes were evaluated and compared with [89Zr]DFO and [89Zr]triacetylfusarinine C ([89Zr]TACF). The in vivo properties of [89Zr]FSC(succ)3 were further compared with [89Zr]TACF in nude mice.

**Results** FSC(succ)2AA and FSC(succ)3 were synthesized with satisfying yield. The complexation with ZrCl4 was achieved using a simple strategy resulting in high-purity [natZr]FSC(succ)2AA and [natZr]FSC(succ)3 with a 1:1 stoichiometry. Distribution coefficient of 89Zr-complexes revealed improved hydrophilic characters compared to [89Zr]TACF. All radioligands showed high stability in PBS and human serum and low protein-bound activity over a period of 7 days. Acid dissociation and transchelation studies exhibited the different in vitro stabilities following the order of [89Zr]FSC(succ)3 > [89Zr]TACF > [89Zr]FSC(succ)2AA > [89Zr]DFO. Biodistribution study of [89Zr]FSC(succ)3 exhibited a slower excretion compared to [89Zr]TACF.

**Conclusion** [89Zr]FSC(succ)3 showed best stability and inertness and [89Zr]FSC(succ)2AA predicts the potential of FSC(succ)2 as a monovalent chelator for the conjugation to targeted biomolecules in particular monoclonal antibodies.

**PP19 Fully automated production of [18F]NaF using a re-configuring FDG synthesis module**

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**EJNMMI Radiopharmacy and Chemistry 2016, 1(Suppl 1):PP19**

**Introduction:** We modified a fully automated method for [18F]NaF synthesis by re-configuring a commercial FDG synthesis module (Single synthesis module, Advanced Cyclotron Systems, Inc.).

**Materials and methods:** The Lookout program was used to sequence the steps for automated synthesis using excel spreadsheet. The [18F]fluoride solution is transferred to synthesis module. The [18F]fluoride ions are trapped in QMA. The QMA cartridge is then washed with sterile water for injection and eluted with 0.9% NaCl. The final product was passed through 0.22 μm sterile filter to sterile product vial.

**Results:** [18F]NaF was successfully produced consistently with high yield. The non-decay corrected yield after synthesis is at least 85%. Quality control of [18F]NaF is performed according to USP and EP requirements. The QC results are passed and many applications are already possible.[1] However, there is still a need for expansion of the carbon-11 chemistry toolbox. We here present unprecedented Michael addition reactions with carbon-11 labeled synths 1 – 3 as Michael donors.

**Methods:** The synthesis of [11C]Methyl acrylate 1 was performed with [11C]CO2 by fixation in a Grignard reaction with vinyl magnesium bromide (0.16M in THF) and subsequent Fisher esterification under acidic conditions. Alternatively, a palladium-mediated carboxylation reaction with [11C]CO2 in the presence of tert-butanol or trityl-amine with vinyl-halides afforded [11C]Ter-butyl acrylate 2 and [11C]Trityl-acrylamide 3. Michael addition reactions were performed with N-(diphenylmethylene)glycine tert-butyl ester 4 by adding the synths to the reaction solution using TBAF as a base in DMSO. Synthon 1 was distilled into the reaction mixture. Synthons 2 and 3 were purified by solid-phase extraction and the Michael addition was performed at 100 °C for 5 min, respectively.

**Results:** The synthesis of 1 was successful with over 70% radiochemical conversion (RCC) and >95% radiochemical purity determined by HPLC. After screening and optimization 2 was obtained in 79 ± 10% and 3 in 73 ± 5% RCC. The subsequent conjugate addition yielded [11C]-diphenylmethylene)-glutamic acid 5-methyl 1-tert butyl ester 5 in 90 ± 5%, [11C](diphenylmethylene)-glutamic acid di-tert butyl ester 6 in 93 ± 6% and [11C](diphenylmethylene)-glutamine 5-trityl 1-tert butyl ester 7 in 78 ± 3% RCC.

**PP20 Extension of the Carbon-11 Small Labeling Agents Toolbox and Conjugate Addition**

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**EJNMMI Radiopharmacy and Chemistry 2016, 1(Suppl 1):PP20**

**Introduction:** [11C]CO2 and [11C]CO are undoubtedly highly versatile radiolabeling agents with many applications. The formation of [11C]CO from [11C]CO2 has become convenient and difficult despite the vast expansion of methodology and applications.[2] Likewise, many reactions are being explored and many applications are already possible.[1] However, there is still a need for expansion of the carbon-11 chemistry toolbox. We here present unprecedented Michael addition reactions with carbon-11 labeled synthons 1 – 3 as Michael donors.

**Methods:** The synthesis of [11C]Methyl acrylate 1 was performed with [11C]CO2 by fixation in a Grignard reaction with vinyl magnesium bromide (0.16M in THF) and subsequent Fisher esterification under acidic conditions. Alternatively, a palladium-mediated carboxylation reaction with [11C]CO2 in the presence of tert-butanol or trityl-amine with vinyl-halides afforded [11C]Ter-butyl acrylate 2 and [11C]Trityl-acrylamide 3. Michael addition reactions were performed with N-(diphenylmethylene)glycine tert-butyl ester 4 by adding the synths to the reaction solution using TBAF as a base in DMSO. Synthon 1 was distilled into the reaction mixture. Synthons 2 and 3 were purified by solid-phase extraction and the Michael addition was performed at 100 °C for 5 min, respectively.

**Results:** The synthesis of 1 was successful with over 70% radiochemical conversion (RCC) and >95% radiochemical purity determined by HPLC. After screening and optimization 2 was obtained in 79 ± 10% and 3 in 73 ± 5% RCC. The subsequent conjugate addition yielded [11C]-diphenylmethylene)-glutamic acid 5-methyl 1-tert butyl ester 5 in 90 ± 5%, [11C](diphenylmethylene)-glutamic acid di-tert butyl ester 6 in 93 ± 6% and [11C](diphenylmethylene)-glutamine 5-trityl 1-tert butyl ester 7 in 78 ± 3% RCC.
Conclusion: The successful synthesis of 1 - 3 has been achieved in good radiochemical yields. Furthermore, conjugate addition to 5 - 7 is possible and we are currently working on expanding the variety of reactions with these small synthons.

Acknowledgements: RADIOMI FP7-PEOPLE-2012-ITN; [11C]COC2 provided by BV Cyclotron.

PP21
In vitro studies on BBB penetration of pramipexole encapsulated theranostic liposomes for the therapy of Parkinson’s disease
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Introduction: Brain penetration and targeting is hard due to complex structure with different barriers such as blood–brain barrier (BBB) with blood–cerebrospinal fluid (CSF) interface and CSF–blood interface. Although these tight and rigid barriers protect brain, they also prevent penetration of molecules, drugs and radiotherapeutics for diagnosis and therapy of many neurodegenerative diseases. Parkinson’s disease (PD) is assumed to be one of the most frequently observed neurodegenerative disease among geriatric disorders. PD comports motor symptoms resulting from the death of dopamine generating cells in the substantia nigra. By using the imaging modalities such as single photon emission computed tomography (SPECT), the decline in the accumulation of specific radiolabelled VEGF analogues can be managed together with therapy by evaluating therapeutic effect.

Materials and methods: Recent studies generally depend on the development of new delivery systems, theranostics, in which diagnosis and therapy can be managed together with therapy by evaluating therapeutic effect.

Materials and methods: Theranostic liposomes were formulated by polyethylene glycol (PEG) coated, nanosized, both neutral or positively charged, 99mTc labeled for SPECT imaging and pramipexole encapsulated for PD therapy. Their characterization and in vitro release kinetics were evaluated. In vitro penetration of both formulations was evaluated in a BBB cell co-culture model.

Results: Both neutral and positively charged liposomes showed proper characterization with about 10% encapsulation efficiency and around 100 nm particle sizes. All formulations fitted to the first-order release kinetics. Both formulations were found BBB permeable in cell culture studies with fluorescent images and fluorospectroscopy.

Conclusion: Promising characterization and release profiles were obtained with theranostic liposomes for both diagnosis and therapy of PD. Both neutral and positively charged formulations found BBB permeable in vitro. In vivo animal studies are continuing to obtain more accurate data. (The authors thank to Abdī ibrahīm ɪlāc for Pramipexole. This study was supported by the grant of TUBITA K, Project No: 1125244).

PP22
Factors affecting tumor uptake of 99mTc-HYNIC-VEGF165
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Introduction: Angiogenesis promotes tumor growth and metastatization and its principal effector is the vascular endothelial growth factor (VEGF) secreted by cancer cells and other components of tumor microenvironment. Nowadays, many anti-angiogenic therapies have been developed and radiolabelled VEGF analogues may provide a useful tool to non-invasively evaluate the efficacy of such drugs. Aim of the present study was to radiolabel the human VEGF-A165 analogue with 99mTc to non-invasively evaluate the efficacy of such drugs. Aim of the present study was to radiolabel the human VEGF-A165 analogue with 99mTc and evaluate the expression of VEGF in both cancer and endothelial cells in the tumor microenvironment by nuclear medicine imaging and immunohistochemistry.

Material and methods: The human VEGF-A165 analogue was radiolabelled with 99mTc using succinimidyl-6-hydrazinoninocitrate hydrochloride as a bifunctional chelator. In vitro quality controls were performed to assess its retained structure and biological activity. In vivo studies included biodistribution studies and tumor targeting experiments in athymic nude CD-1 mice bearing xenograft tumors from ARO, K1 and HT29 cell lines. Immunohistochemistry was performed on excised tumors to evaluate VEGF and VEGF receptor (VEGFR) expression in the lesion and endothelial cells.

Results: The analogue was labelled with high labelling efficiency (>95%) and high specific activity, with retained biological activity and structural integrity. Tumor targeting experiments revealed a focal uptake of radiolabelled VEGF165 in tumor xenografts with different tumor-to-background ratios. Immunohistochemical analysis tumors and xenografts revealed an inverse correlation between VEGF and uptake of the radioactive hormone. A positive correlation between radioactive VEGF165 and VEGFR1 was also observed.

Conclusion: Radiolabelled human VEGF-A165 is a promising radiotherapeutic agent to image angiogenesis and evaluate the efficacy of anti-angiogenic drugs. However, VEGF imaging may suffer from quenching effects of endogenous VEGF produced by cancer and other cells of the microenvironment.

PP23
Rhenium-188: a suitable radioisotope for targeted radiotherapy
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Introduction: Among radioisotopes for targeted therapeutic applications, 188Re is very promising, thanks to its suitable properties (β-emitter, Emax = 2.12 MeV, t1/2 = 17 h, γ-emission of 155 keV, conveniently obtained through a generator), and to the fact that it is a homologous element to 99mTc, the radioelement of choice in nuclear medicine. Nowadays, many anti-angiogenic therapies have been developed and radiolabelled VEGF analogues may provide a useful tool to non-invasively evaluate the efficacy of such drugs. Aim of the present study was to radiolabel the human VEGF-A165 analogue with 99mTc and evaluate the expression of VEGF in both cancer and endothelial cells in the tumor microenvironment by nuclear medicine imaging and immunohistochemistry.

Material and methods: The human VEGF-A165 analogue was radiolabelled with 99mTc using succimimidyl-6-hydrazinoninocitrate hydrochloride as a bifunctional chelator. In vitro quality controls were performed to assess its retained structure and biological activity. In vivo studies included biodistribution studies and tumor targeting experiments in athymic nude CD-1 mice bearing xenograft tumors from ARO, K1 and HT29 cell lines. Immunohistochemistry was performed on excised tumors to evaluate VEGF and VEGF receptor (VEGFR) expression in the lesion and endothelial cells.

Results: The analogue was labelled with high labelling efficiency (>95%) and high specific activity, with retained biological activity and structural integrity. Tumor targeting experiments revealed a focal uptake of radiolabelled VEGF165 in tumor xenografts with different tumor-to-background ratios. Immunohistochemical analysis tumors and xenografts revealed an inverse correlation between VEGF and uptake of the radioactive hormone. A positive correlation between radioactive VEGF165 and VEGFR1 was also observed.

Conclusion: Radiolabelled human VEGF-A165 is a promising radiotherapeutic agent to image angiogenesis and evaluate the efficacy of anti-angiogenic drugs. However, VEGF imaging may suffer from quenching effects of endogenous VEGF produced by cancer and other cells of the microenvironment.
Preparation of a broad palette of 68Ga radiopharmaceuticals for clinical applications
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EJNMMI Radiopharmacy and Chemistry 2016, 1(Suppl 1) PP24

Introduction: PET molecular imaging and receptor binding peptides are emerging as powerful tools for imaging. Somatostatin analogues labeled with 68Ga are the most widely used and have proven useful for the management of patients with neuroendocrine tumors. However, within the past few years several other receptor binding compounds labeled with 68Ga have shown promising results with potential clinical applications in PET centers that lack in site cyclotrons. The aim of this study was to develop a single vial kit solution for the production of 68Ga radiopharmaceuticals for clinical applications.

Materials and methods: Chemical precursors DOTA-NOC, DOTA-RGDfK dimer, DOTA-Ubiquicidin (29-41), and PSMA-11 were acquired from ABX. Gallium-68 was obtained from an ITG generator while labeling was performed in an iQS module (ITG GmbH). Stock solutions of different concentrations were prepared by dissolving the precursors in 0.25M NaOAc. Aliquots of 100 μL of the stock solutions were dispensed in Eppendorf vials and stored at -20°C. For labeling, the aliquots were diluted with 900 μL of 0.25M NaOAc, mixed with 4 ml of 68GaCl3, and incubated for 10 min. Purification was made by SPE eluting the product with 1 ml 50% EtOH. Final product was diluted to ICH in a concentration range of HEPES from 10 μg/mL to 200 μg/mL. This assay of HEPES was applied effectively in the synthesis of 68Ga-DOTANOC (EOS) in a merging operation of an ITG generator tube. After continuing the reaction vessel with a solution of 68GaCl3, and incubated for 10 min. Purification was made by SPE eluting the product with 1 ml 50% EtOH. Final product was diluted to ICH in a concentration range of HEPES from 10 μg/mL to 200 μg/mL. This assay of HEPES was applied effectively in the synthesis of 68Ga-DOTANOC (EOS). Synthesis-time Yield (uncorr.)

Table 1 (abstract PP25) See text for description

| 68Ga-DOTANOC preparation | Starting activity (68Ga-DOTANOC (EOS) | Synthesis-time | Yield (uncorr.) |
|--------------------------|--------------------------------------|----------------|---------------|
| Standard Single generator | 1.73 GBq                             | 32 min          | 50 %          |
| Experimental Dual generator | 2.56 GBq*                           | 44 min          | 43 %          |

*Theoretical starting activity of both 68Ge/68Ga-generator corrected to elution start of the second one

PP25
68Ga-peptide preparation with the use of two 68Ge/68Ga-generators
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EJNMMI Radiopharmacy and Chemistry 2016, 1(Suppl 1) PP25

Introduction: The clinical demand for 68Ga-labelled peptides is constantly rising, but the achievable radioactivity per batch is limited by the inventory of the 68Ge/68Ga generator. One possible approach to raising the radioactive yield is to merge the radioactive contents of two 68Ge/68Ga generators for one batch of 68Ga-peptide. The key to this method can be found in frame with the cationic purification (1) merging two generator eluates. Our aim was to establish this method in the synthesis of 68Ga-DOTANOC.

Material and methods: The experimental setup considered two 1.85 GBq (50 mCi) ITemba 68Ge/68Ga-generators with calibration Feb/15 and Oct/15 and a Scintomics GRP synthesis module. SC-01 peptide cassettes including the reagents were applied in the synthesis of 68Ga-DOTANOC. The automatic synthesis sequent was modified by us as followed: After the initial conditioning of the C18-SPE with ethanol, water and subsequent drying with N2 the “older” 68Ge/68Ga-generator was eluted with 7 mL 1M HCl by the motor syringe. 10 mL water was added for dilution, and the mixture was transferred over a PS-H+ SCX column (Machery-Nagel). The sequent stopped for the manual change of the generator tube. After continuing the sequent, the “newer” Generator was eluted in the same manner as described above. Once washing and drying the valve seats, 68Ga was delivered from PS-H+ into the reaction vessel with a solution of 1.4 mL 5M NaCl and 0.2 mL 6M HCl. The further labelling with 40 μg precursor in HEPES were performed as usual.

Results: We found in our preliminary experiments more the 98% of the theoretical 68Ga radioactivity after “double elution” adsorbed on the PS-H+ SCX cartridge. About 15 % of 68Ga retained on the PS-H+ SCX after transfer of the 68GaCl3 into the reaction vessel. The followed labelling of 68Ga-DOTANOC revealed an uncorrected yield of >40%.

Conclusion: The promising preliminary results with a yield of 1.1 GBq 68Ga-DOTANOC (EOS) in a merging operation of an “old” and a “new” 68Ge/68Ga generator indicates a high potential for improvement in routine preparation. Therefore, we extend our synthesis system with an additional valve actuator unit, which should handle the 68Ga elution from both generators automatically and under GMP conditions.
Preparation, in vitro and in vivo evaluation of a 99mTc(I)-Diethyl Ester (S,S)-Ethylendiamine- N,N´-DI-2-(3-Cyclohexyl) Propionic acid as a target-specific radiopharmaceutical

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Introduction: The "organometallic" complexes have been used in the development of new diagnostic as well as therapeutic radio-pharmaceuticals.1 The organometallic labeling approach has led to the creation of a precursor [M(H2O)(3)(CO)(3)+] (M = Tc, Re), which exhibits several useful characteristics. These include the small size of the core, easy preparation with aqueous-based precursor kit formulations, and readily substituted water molecules of the precursor fac-[99mTc(H2O)(3)(CO)(3)+] by a variety of functional groups.2 The aim of this study is to label diethyl ester (S,S)- ethylenediamine-N,N´-di-2-(3-cyclohexyl)propionic acid (L) with 99mTc(I)-tricarbonyl (L) as heating agents for in situ magnetic fluid hyperthermia protocols. Radiolabeled MNPs were further used for in vitro stability studies in saline and human plasma, and in vivo biodistribution studies in normal Wistar rats.

Discussion/conclusion: The 99mTc(I)-L complex revealed high radiochemical purity and stability in vitro, without any measurable decomposition. A significant difference of 99mTc(I)-L complex uptake between melanoma model and the normal mice was observed. Biodistribution studies showed high tumor uptake in B16-melanoma-bearing mice. This study indicates that 99mTc(I)-L complex may be a potential agent for melanoma detection.

Materials and methods: MNPs were synthesized by co-precipitation of ferric and ferrous salts in a basic solution. Radiolabeling was performed by mixing 0.5 mL of aqueous Fe3O4-Naked and Fe3O4-PEG600 MNPs suspension with 37 MBq 90YCl3 (0.001 mL) and incubating at room temperature on a shaker for one hour. Radiolabeled particles were then separated from free 90Y activity by precipitation with the help of permanent magnet. Radiolabeled MNPs were further used for in vitro stability studies in saline and human serum, and in vivo biodistribution studies in normal Wistar rats.
DOTA-NOC labeling is conducted as follows: a solution of 50 μg peptide in 1 mL 250 mM acetate buffer at pH 5 is added and the mixture is heated at 120°C for 5 min. The reaction bulk is then loaded on an HLB cartridge and washed with 10 mL of water. 68Ga-DOTA-NOC is then eluted with 1 mL of EtOH/water 65:35 v/v mix followed by 1 mL of saline 0.9% and collected through a 0.22 μm Millex-GV filter into a sterile vial containing 8 mL of saline 0.9%. PSMA-11 labeling is conducted as follows: a solution of 10 μg peptide in 1 mL 1.5 M acetate buffer at pH 4.5 is added and the mixture is heated at 95°C for 5 min. The reaction bulk is then loaded on a Sep-pak® Light C18 cartridge and washed with 10 mL of water. 68Ga-PSMA-11 is then eluted with 2 mL of EtOH/water 1:1 v/v mixture followed by 2 mL of phosphate buffered saline (PBS) and collected through a 0.22 μm Millex-GV filter into a sterile vial containing 6 mL of PBS.

Results: 68Ga-DOTA-NOC is produced in r <20 min with 81.5±5.2 % radiochemical yield (RCY) (decay-corrected-d.c.) and 68Ga-PSMA-11 is produced in 13 min with 97±2.5 % RCY (d.c.). Reported process times include generator elution and formulation. In both cases, final products show high radiochemical purity (TLC > 97 % and 99 % respectively).

Discussion/conclusion: Automated processes for the production of both 68Ga-DOTA-NOC and 68Ga-PSMA-11 have been successfully achieved using a commercial synthesizer and a 68Ge/68Ga generator. The labeling procedures are straightforward and efficient, thanks to the low elution volume and high purity of the generator eluate (no need for fractionation or post-elution purification).

PP30
Combining commercial production of multi-products in a GMP environment with Clinical & R&D activities
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Introduction: Monoproduciton facilities are turning to multi-product to cope with decreasing prices of [18F]-FDG (FDG) and to support R&D programs. The aim is to demonstrate that in the same facility and equipment, a busy research program can be safely integrated into commercial production.

Materials and methods: To be able to safely combine both activities a risk-assessment should be designed to identify key control points. The two azide-modified derivatives (with PEG7 and without) were then clicked with propargyl glycine to introduce a tridentate chelating unit for radiolabeling with [99mTc(III)]H2O3+ precursor. The radio-tracers, 99mTc(III)-Pra-Tz-CH2-cRGDFK and 99mTc(III)-Pra-Tz-PEG7-cRGDFK were analyzed by HPLC. Analogous rhodium complexes were synthesized for characterization of 99mTc(III)-labeled radio-tracers. Biodistribution study of the two radio-tracers was carried out in C57BL/6 mice bearing αvβ3-positive melanoma tumors. The radioactive preparation (~3.7 MBq/animal, 100 μL) was injected intravenously through the lateral tail vein. At different time intervals after injection (30, 60, and 180 min), the animals (n = 4/time point) were sacrificed and the relevant organs excised for measurement of retained activity.

Results: The two radio-tracers, 99mTc(III)-Pra-Tz-Ch2-cRGDFK and 99mTc(III)-Pra-Tz-PEG7-cRGDFK and were prepared in >90% radiochemical yield and exhibited excellent stability in saline as well as in plasma. Maximum tumor uptake was observed at 30 min p.i. for the two radio-tracers and was higher for 99mTc(III)-Pra-Tz-PEG7-cRGDFK (4.11 ± 0.51 %ID/g) as compared to that for 99mTc(III)-Pra-Tz-Ch2-cRGDFK (3.01 ± 0.77 %ID/g). Tumor accumulation of both the radio-tracers reduced to 50-60% during blocking studies with cold cRGDFK peptide, suggesting receptor-mediated uptake of radio-tracers.

Conclusion: The two neutral 99mTc(III) radio-tracers prepared exhibited receptor mediated uptake in melanoma tumor. Increased tumor uptake on introduction of PEG7 unit was compromised with slow clearance from other organs. Further modification of the peptide with a different spacer or a smaller PEG unit may lead to more favorable pharmacokinetics.

Discussion/conclusion: As a conclusion, the sites described have been functioning for several years and are able to safely combine commercial daily production while keeping a highly active R&D program with more than ten different tracers developed for pre-clinical and clinical applications.
Introduction: The shortage of 99Mo (fission) has renewed interest in alternative technetium-99m production methods either in nuclear reactor starting from 98Mo or in accelerator with 100Mo as a target material. Adsorption chromatography on alumina is not sufficient for alternative production methods due to high amount of Mo in the dissolved target solution. Several chromatographic resins were described for the separation of pertechnete from molybdate including Dowex or ABEC. Here we focused our attention on application of AnaLig Tc-02 resin for efficient recovery of 99mTc from large molybdenum excess.

Methods: To simulate a target dissolution mixture, 10 mL of sodium molybdate solution and a small amount of Na99TcO4 in NaOH or (NH4)2CO3 was delivered on the column containing 100 mg of AnaLig resin using the flow rate of 0.4 mL/min. Before 99mTc elution, the column was fed with 3 mL of sodium hydroxide or ammonium carbonate solution and subsequently with 1.5 mL of water. The 99mTc was recovered in 5 mL of water. The eluate and other eluents activities (one after introduction of Mo solution and two after rinsing columns in term with NaOH or (NH4)2CO3 solution and water) were measured with the dose calibrator Capintect CRC-55tR.

Results: Best 99mTc recovery was obtained in 2.8M NaOH or 1.5M (NH4)2CO3 solution. Under these conditions, more than 86% adsorption and elution were achieved. Most 99mTcs losses occurred during rinsing the column with water, even approaching 10% of activity and due to rinsing the column with NaOH or (NH4)2CO3 after sorption Tc-99m on Analig resin (up to 3.5%). The activity retained on the column was around 1% for solution in NaOH and about 3% for (NH4)2CO3, respectively.

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PP34

Gamma scintigraphy studies with 99mTc-amoxicillin sodium in bacterially infected and sterile inflamed rats

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Introduction: The in-time diagnosis of bacterial infection is extremely significant for the accurate treatment. Nuclear medicine scintigraphic imaging is an excellent non-invasive method of whole-body scanning, allow to determine infectious foci in all parts of the body, based on pathophysiological changes which occur earlier in the infection process. The aim of this study was to perform gamma scintigraphy studies with bacterial infected and sterile inflamed rats with newly developed radiopharmaceutical 99mTc-amoxicillin sodium (99mTc-AMOX). Method: E.coli suspension was intramuscularly injected into the right thigh muscle of rats to create infection model. Turpentine oil was intramuscularly injected into the left thigh muscle of rats to create sterile inflammation model. After the infection and sterile inflammation focus were allowed to develop for 24 hours swelling appeared and gamma scintigraphy studies were performed. 99mTc-AMOX (3.7MBq) was intravenously injected via tail vein to the rats. After administration of radiopharmaceuticals, serial static images were acquired at different time intervals (5 minutes, 1, 2, 3, 4 and 5 hours post injection).

Results: The uptake of 99mTc-AMOX following intravenous administrations was assessed on static images. The images depicted rapid distribution throughout the body and uptake in the bacterial infected and sterile inflamed thigh muscle within one hour after injection. There was a higher activity in both bacterial infected and sterile inflamed thigh muscle as compared to healthy thigh muscle. For quantitative evaluation, regions of interest were drawn around the target (infected and inflamed thigh muscle) and non-target (healthy thigh muscle) regions of the rats. The 99mTc-AMOX uptake was calculated by dividing the average counts per pixel within the region of target to the average counts per pixel within the region of non-target.

Discussion/conclusions: Based on the in vivo studies, 99mTc-AMOX has higher uptake in infected and inflamed thigh muscle than healthy muscle. The data suggest that 99mTc-AMOX remained at the infection and inflammation foci during the whole experiments, there being no statistically significant differences in the ratios during the studied period (p≥0.05).

Materials and methods: The commercially available SynChrom [18F] R&D synthesis module was readily configured for the one-step synthesis according to conditions achieved for the fluorination with non-radioactive fluoride. The radiolabelling process involved a classical [18F]fluoride nucleophilic substitution from the chlorinated precursor (Smlg) was performed at 110°C for 12 min. Crude product was purified using preparative HPLC and SPE methods.

Results: The obtained yields for a chlorine-for-[18F]fluorine substitution of 59±12% (n=5) were higher than those (45-48%) reported for the [18F]FECNT batches prepared from the N-mesyloxy precursor. The total synthesis time was 80-90 min and [18F]FECNT batches of about 2.0±0.5 GBq (n=3) with high radiochemical purity over than 99% were obtained with 32±7% overall decay-corrected yields. These yields were comparable with previously reported results for the one-step synthesis of [18F]FECNT and significantly higher than yields obtained in two-step syntheses [2]. The specific activity of the final [18F]FECNT product was 55 GBq/μmol, which is consistent with literature synthesis results (38-72 GBq/μmol) of this radiotracer and sufficient for the DAT density determination in the human PET imaging studies.

Discussion/conclusions: In this study, the new chlorinated precursor was successfully used for one-step automated radiosynthesis of [18F]FECNT. Considering these promising results as well as long-term storage stability of this precursor, reported procedure may provide a straightforward and reliable method for the production of radiotracer suitable for human use.
PP35
Preparation of 99mTc- Amoxicillin Sodium Lyophilized Kit
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Introduction: Infection detection by nuclear medicine imaging techniques based on pathophysiological changes which appear much earlier than anatomical changes in the infection process (1,2). To develop a better infection imaging which will accumulate efficiently to inflammatory foci, clear rapidly from background tissue, discriminate between bacterial infection and sterile inflammation, cost low and prepare easily we labeled amoxicillin sodium (AMOX) with 99mTc. The aim of this study is to prepare 99mTc-AMOX in a simple method with good labeling efficiency and evaluate the ready to use cold kit formulation thus making it available to the other nuclear medicine centers. The stability of 99mTc-AMOX in human serum was identified; sterility and pyrogenicity of the radiopharmaceutical was estimated.

Method: To investigate the optimum radiolabeling conditions, radiolabeling was tested with different concentrations of reducing (stannous chloride and stannous tartrate) and antioxidant (ascorbic acid) agent. Radiochemical analysis was performed with Radio Thin Layer Chromatography (RTLC) and Radio High Performance Liquid Chromatography (RHLPC) studies. Two different freeze dry kits were formulated with optimum labeling conditions and stability, sterility and pyrogenicity of the kits were performed.

Results: The effect of reducing agent concentration on the radiochemical purity was evaluated and optimum reducing agent amount found 200 μg. Under these conditions labeling efficiency was around 90%. In the presence of ascorbic acid, stability of the complex was slightly increase while labeling efficiency for early hours was not affected significantly. According to the experiments, the formation of the 99mTc-AMOX complex was very fast and reached the radiochemical purity over 95% in 15 min after radiolabeling. While keeping other reaction conditions constant and varied the pH of the reaction from 4.8 to 7.4 radiochemical purity was slightly decreased. 99mTc- AMOX was found stable during to 24 hours incubation in saline and human serum. Kits were labeled with 37MBq, 185MBq and 370 MBq 99mTc. Slightly decrease in radiochemical purity was observed with increasing of radioactivity. According to sterility test, since there was no growth in batches, kits were sterile. Also gel clot test showed that kits were pyrogen free. The freeze dry kits developed in this study were found to be stable with a shelf-life of six months when preserved at both at +5 ±3°C and +25±2°C/60 ±5% RH.

Conclusion: Simple method for radiolabeling of AMOX with 99mTc has been developed and standardized. Labeling efficiency of 99mTc-AMOX was assessed by both RTLC and RHPLC and found higher than 90%. The resulting complex was quite stable and labeling of ‘90% was maintained for up to 6 hours. Two different freeze dry kits was developed and evaluated. Based on the data obtained from this study, both products was stable for 6 months with high labeling efficiency.

PP36
Outfits of Tracerlan FXC-PRO for 11C-Labeling
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Introduction: Commercial synthesis modules for 11C-labeling are not often directly suitable for the production of different labeling agents. Modification of Tracerlab FXC-Pro synthesis module to perform both [11C]methyl iodide and [11C]carboxylation and the set-up of [11C]radiochemicals for clinical use are described.

Materials and methods: Commercial GE Tracerlab FXC-Pro synthesizer was modified to enable 11C-methylation with both [11C]methyl iodide and [11C]metomidate with both direct 11C-carboxylation with [11C]CO2. Additional magnetic valves were installed to bypass the [11C]methyl iodide recycling system for direct use of [11C]CO2 and to bypass the [11C]metomidate trilafte when using [11C]methyl iodide as such. The valves are operated by Tracerlab software. The HPLC operations were modified by installing pneumatic column selector and a syringe operated loop injector. A sterile filtration unit (SFU) was coupled with synthesizer to allow online filter integrity test. For monitoring purposes more GM tubes were also installed. [11C]PK11195 was labeled with [11C]methyl iodide while [11C]methionine and [11C]metomidate were labeled with [11C]methyl triflate. [11C]Acetate was synthesized using direct carboxylation with [11C]CO2. Semi-preparative HPLC was used for the purification of [11C]PK11195 and [11C]methionine. Solid phase extraction cartridges were used for the purification of [11C]metomidate and [11C]acetate.

Results: The average synthesis times were 30 minutes for [11C]methionine, 38 minutes for [11C]PK11195, 20 minutes for [11C]metomidate and 11 minutes for [11C]acetate. [11C]Acetate was synthesized using direct carboxylation with [11C]CO2. Semi-preparative HPLC was used for the purification of [11C]PK11195 and [11C]methionine. Solid phase extraction cartridges were used for the purification of [11C]metomidate and [11C]acetate. Kits were label ed with 37MBq, 185MBq and 370 MBq 99mTc-AMOX in human serum was identified; sterility and pyrogenicity of the kits was maintained for up to 6 hours. Two different freeze dry kits were formulated with optimum labeling conditions and stability, sterility and pyrogenicity of the kits were performed.

Conclusion: Reliable and robust routine procedures for the production of [11C]methionine, [11C]PK11195, [11C]metomidate and [11C]acetate were developed using a modified Tracerlab FXC-Pro module. The synthesis module can be easily modified and re-modified for production of many different [11C]radiochemicals.
and 170°C. Experiments were repeated in acetonitrile, dimethylsulfoxide, tert-butanol and different P1:P3 ratios (1:1 and 1:5). Products were purified by distillation and analyzed (radio-TLC, radio-HPLC). Factor-H was reacted with an azido-PEG4-carboxylate linker (azido-PEG) targeting protein amino groups. Factor-H (1.6nmol in 250μl of pH 9 PBS buffer), was reacted (4h at RT) with 48nmol of NHS-activated azido-PEG. The azido-protein was purified by ultracentrifugation, suspended in water and lyophilized. S-[18F]-FP was distilled into a vial containing 2-6nmol of azidoPEG-Factor-H, 1.4 μmol of Cu(I), 1.9μmol of TETA in 150μl of THF/H2O (2:1) and reacted at 45°C for 10'. Unreacted S-[18F]FP was removed under nitrogen flow. S-[18F]-FP-Factor-H was purified by gel filtration (RCP> 97%) and the yield (92±3% d.c.y., n=5) determined by HPLC.

Results: Best yield for S-[18F]-FP and 6-[18F]-FP (91±6% and 83±5% d.c.y, n=3) were obtained in acetonitrile (130°C), P1:P3 1:1. Reaction yield was lower in DMSO for both S-[18F]-FP and 6-[18F]-FP (68±11% and 71±19%, d.c.y, n=3) and worse in tert-butanol (33±5% and 45±2%, d.c.y, n=3). Increasing P1:P3 ratio did not influence yield and resulted in a more frequent reactor clogging.

Discussion/conclusion: We describe a microfluidic approach for the synthesis of C5 and C6 18F-alkynes and the on-line radiolabelling of peptides is a challenging stage in the process of identifying the right imaging modality.

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**PP38**
Automated 18F-flumazenil production using chemically resistant disposable cassettes
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Introduction: Production of 18F-flumazenil from 2-nitroflumazenil under GMP conditions is primarily performed with manipulators or custom built radiosynthesis modules with fixed components, as the use of N,N-dimethylformamide (DMF) at 160°C prohibits the use of commonly available disposable cassettes. Cassettes for the GE TRACERlab MXFDG synthesis modules are commercially available in different materials. ROTEM Industries and GE Healthcare use polysulfone as the main component, and it is known that this polymer is not compatible with DMF. Cassettes from ABX are reported to be more chemical resistant, with polypropylene as its main constituent.

The aim of this work was to establish a robust automated radiosynthesis procedure for 18F-flumazenil on the GE TRACERlab MXFDG, where staff previously trained on the same equipment can easily perform the radiosynthesis.

Materials and methods: Fragments of the ABX FDG cassette were exposed for 1 or 24 h to various solvents including acetonitrile and DMF at room temperature (RT) or 60°C to assess its chemical resistance. A new synthesis sequence was written for the GE TRACERlab MXFDG. A non-radioactive test run was performed with the ABX FDG cassette, and radiosynthesis was carried out with the cassette components replaced by chemically resistant manifolds (ABX).

Results: Incubation of the PP ABX FDG cassette fragments in acetonitrile at RT showed no macroscopic influence on the polymer while the fragment became soft and mouldable at 60°C. Visible changes occurred after 1 h incubation with DMF, with the polymer having completely dissolved after 24 h, regardless of temperature. To verify the implication of these results on a 1 h radiosynthesis, a test run was performed using the same cassette material and synthesis conditions without radioactivity. Parts of the manifold was found to have cracked, and a valve blockade was observed due to polymer melting. Radiosynthesis was carried out with the manifolds replaced by chemically resistant ones, and 18F-flumazenil was successfully obtained in 4 % decay corrected yield.

Conclusions: Disposable cassettes assembled with chemically resistant manifolds is a requirement for the automated synthesis of 18F-flumazenil from 2-nitroflumazenil using the GE TRACERlab MXFDG synthesis module. Neither reagent kits nor program sequences are hitherto commercially available, but GMP grade production can be accomplished if recombinant and synthetic PET imaging radiotracers be successfully carried out with modifications on existing equipment.

**PP39**
The effect of the eluent solutions (TBAHCO3, Kryptand K2.2.2) on the radiochemical yields of 18F-Fluoromethyloxime
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Introduction: [18F]Fluoromethyloxime ([18F]FOM) proves to be a unique radiotracer for the detection of prostate cancer as well as brain and lung tumors with Positron Emission Tomography (PET). The aim of this work was to improve radiochemical yields of 18F-Fluoromethyloxime ([18F]FOM) with high reproducibility. The used eluent solution plays an important role in production of [18F]FOM radiotracers. [18F]FOM is manufactured under GMP conditions using one of two eluent solutions namely Tetrahydralammonium Bicarbonate (TBAHCO3) and Kryptand K2.2.2, each time.

Method: [18F]FOM was radiosynthesized using a fully automated synthesis module TRACERlab MX. Hardware cassette and reagents kit purchased from ABX,[18F]FOM is synthesized by 18F-Fluoroalkylation of N,N-Dimethylaminoethanol (DMAE) in presence of Dimethyl Sulfoxide (DMSO) using 18F-Fluorobromomethane ([18F]FBM) as an alkylating agent.

Results: [18F]Fluoromethyloxime radiosynthesis assisted by Kryptand K2.2.2 shown irregular or poor distillation of 18F-Fluorobromomethane, which is observed by pressure trending window on TRACERlab MX during distillation step very poor gas flow through the four silica cartridges been observed which in turn leads to production of very less amounts of [18F]FOM. Tetrabutylammonium Bicarbonate (TBAHCO3) assisted 18F-FCH radiosynthesis observed with regular and continuous distillation of 18F-Fluorobromomethane through the four silica cartridges. [18F]FCH solution proved high radiochemical purities (>99%). Residual solvents analyzed using Gas Chromatography (GC), DBM & DMAE is not identified.

Conclusion: Choice of Tetrabutylammonium Bicarbonate (TBAHCO3) as eluent solution shown very consistent and improved radiochemical yields decay corrected 20-25% over Kryptand 2.2.2 as eluent solution.

**PP40**
[68Ga]Radiolabeling of short peptide that has a PET imaging potentials
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Introduction: Targeting short peptides are gaining interests in the molecular imaging field. Choosing the radioisotope to label those peptides is a challenging stage in the process of identifying the right imaging modality.

Method: All needed reagents and solvents were used with no further purifications unless it’s necessary. Peptide used was purchased from GenScript and used without further purifications. Reaction progress was monitored using both radio-analytical thin-layer chromatography
(Radio-TLC) and high pressure liquid chromatographic (Radio-HPLC). Radio-HPLC analyses were carried out on semi-preparative Phenomenex C-18 (250 mm x 10 mm). Radiolabeled peptide was detected with the eluting of 0.063% TFA in water and 0.05% TFA in MeCN at 2 ml/min applying gradient elution mode. Radiochemical yields were calculated based on the Radio-HPLC collections. Sodium Citrate Buffer (PH=7.5) was used as the Radio-TLC mobile phase.

Synthesis of DOTA- Peptide Conjugate: 9:97 E-7 mol of the intended peptide dissolved in DMSO reacts with 1.18 E-6 mol of (DOTA-NHS-ester) that was prepared in MeCN. The reaction was done in a phosphate buffer with PH=8 and heating at 90°C for 30min.

68G -labeling of DOTA-Peptide: 68G solution was added to 60 μg of conjugated DOTA-Peptide in 100 μl of HPES buffer (PH=4) and the mixture allowed to react for 30 min at 80°C.

Results: [68G-DOTA-Peptide] was analyzed on Radio-HPLC; the ration time of the product was 8.4 min and Radio-TLC checks for the collected product confirm the high radiochemical purity.

Discussion: The labeled peptide is produce in good radiochemical yields and high radiochemical purity. Initial biological works are ongoing to evaluate its imaging potential mainly on a melanoma cell line. The findings will be presented.

PP41
Is validation of radiochemical purity analysis in a public hospital in a developing country possible?
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Introduction: Pharmacopeial or manufacturer’s radiochemical purity (RCP) analysis methods are not always practical in a hospital setting, leading to modifications or substitution with quicker, simplified, or cost effective analytical procedures. This study aimed to determine whether appropriate validation procedures based on ICH Q2A and Q2B guidelines are feasible in a resource limited environment, such as most hospital radioopharmacy settings in Southern Africa.

Materials and methods: Alternative RCP test methods for Tc-99m sestamibi involving the use of Whatman 31 ET (Wh) and Schleicher and Schuell (SS) chromatography paper were used. Locally procured Macherey-Nagel (MN) aluminium oxide TLC strips were intended as control method, as strips described in the manufacturer’s instructions were unavailable. All analyses were performed on the 3 different strips in parallel, and in triplicate. In-house prepared Tc-99m sestamibi, colloid and pertechnetate were assumed to be 100% pure. Samples containing mixtures of varying concentrations of the radiochemical components were analysed. Results from tests performed by different operators were used to judge intermediate precision, and time delay between spotting and developing was used to evaluate robustness.

Results: RCP of sestamibi without added impurities was 99.8%±0.0% MN, 99.5%±0.1% for Wh and 99.3%±0.2% for SS strips. Addition of pertechnetate and colloid to Tc-99m Sestamibi after completion of kit reconstitution, resulted in values for sestamibi higher than the calculated RCP for all 3 methods. The radiochromatogram scanner’s limit of detection and limit of quantitation were determined.

Discussion and conclusion: Due to the unexpected high RCP values after addition of impurities to sestamibi, all the analytical methods lacked specificity and accuracy. The MN test method showed exceptionally high values for sestamibi due to co-elution of the free pertechnetate with sestamibi. The MN RCP test method could therefore not be used as a reference standard. All three methods met the acceptance criteria for repeatability, intermediate precision, and robustness. Validating an analytical procedure in a hospital setting is only possible once some important prerequisites are met, such as availability of specified materials for the reference procedure, reliable reference standards, and availability of HPLC for radiotherapeutics that have impurities other than pertechnetate and colloid. A template validation protocol for TLC and paper chromatography was developed.

PP42
Improved automated radiosynthesis of [18F]FEPPA
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Introduction: The translocator protein (TSPO) has been proposed as a biomarker for several conditions, including neurodegenerative disorders, cancer and non-alcoholic fatty liver disease. Hence, a number of PET ligands have been developed to image the TSPO, including the arylxocetanilide derivative [18F]FEPPA, which is currently in clinical use [1]. The synthesis of [18F]FEPPA was first reported by Wilson et al. [2] and successively fully automated [3]. Nevertheless the purification of the crude product by means of semipreparative HPLC is unsatisfactorily time consuming (retention time tr = 23 min). Therefore, the aim of this study was to establish a new set of conditions, which can reduce the retention time of the product and consequently reduce the total time of the synthesis.

Methods: [18F]FEPPA was synthesized as described elsewhere [3] with minor modifications in a Nuclear Interface synthesizer (GE Healthcare, Sweden). Briefly, the tosylate precursor dissolved in acetonitrile was added to the azetroptically dried [18F]fluoride and the reactor was heated at 90°C for 10 minutes; after cooling, the reaction was quenched with a solution 50:50 acetate buffer-acetonitrile (pH=3,6) and automatically injected onto a semipreparative HPLC Column (Merck Chromolith® RP-18e, 100×10 mm). The product fraction was collected in water and the new aqueous solution was passed through a pre-conditioned C-18 plus SepPak cartridge. Finally, the product was eluted with ethanol and formulated with saline and phosphate buffer.

Results: The new procedure yielded 3.2 ± 0.3 GBq of [18F]FEPPA (27% ± 3%, not corrected for decay) within 17 min after the azetroptic drying of the [18F]fluoride. Specific activity ranged from 430 to 600 GBq/μmol. Radiochemical purity exceeded 98%.

Conclusions: A fast and reliable procedure for the production of [18F]FEPPA was successfully implemented, drastically reducing the total time of the radiosynthesis compared to the previous published method (17 min compared to 36 min).

PP43
Synthesis and initial evaluation of A118F-RESCA1-TATE for somatostatin receptor imaging with PET
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Introduction: Indium-111 and gallium-68 labelled octreotide deriva- tives [1] are widely used in SPECT and PET imaging of somatostatin receptor expressing tumours and pancreatic dysfunction. In perspective of advantages of fluorine-18 (favourable half-life, high production capacity, low β-energy), more recently, derivatives based on
Tetraethylammonium fluoride complexation have been developed.[2] To achieve high labelling efficiency at biomolecule compatible conditions and improve radiotracer properties in vivo, we synthesized an octreotate conjugate with an acyclic trans-cyclohexane spanned pentadentate chelator: Restrained complexing agent 1, RESCA1 (1). After low-temperature \textsuperscript{18}F-competition, in vitro and in vivo characteristics of the new radiotracer were studied.

**Materials and methods:** Radiolabelling was achieved manually starting from 50–860 MBq of \textsuperscript{18}F-sodium fluoride. \textsuperscript{18}F-RESCA1-TATE (\textsuperscript{18}F) was purified by SPE and analysed with ITLC, LC-MS and RP-HPLC. Stability of the radiotracer was investigated in vivo over a period of 3 h in EtOH/Saline 1:1 (RT), PBS (37 °C) and rat plasma (37 °C). Ex vivo biodistribution data were acquired at 10 and 60 min p.i. of 1.8 MBq \textsuperscript{18}F-TATE in healthy male mice (n=4 per time point), and compared with those of \textsuperscript{68}Ga-DOTA-TATE.

**Results:** Complexation of \textsuperscript{18}F-TATE with 1 succeeded with 54-77% of product formation within 15 min at room temperature and with 85-96% after 12 min at 40 °C. 28-298 MBq of \textsuperscript{18}F-TATE were obtained with 94-98% RCP after SPE. After 3 h of incubation, the content of parent compound was 99% in formulation, 95% in PBS and 91% in plasma. Distribution of \textsuperscript{18}F-TATE in mice showed negligible bone uptake, but in comparison with \textsuperscript{68}Ga-DOTA-TATE less binding in endocrine tissues, 4 times higher uptake in kidneys, and lower excretion via the urinary pathway: 33.2%ID at 10 min and 62.2%ID at 60 min p.i., compared to 44.1%ID at 10 min and 77.9%ID at 60 min p.i., respectively.

**Discussion/conclusion:** RESCA1-TATE easily forms a chemically and biologically stable complex with \textsuperscript{18}F-TATE. The radiotracer, however, showed lower uptake in organs known for high somatostatin expression in comparison with the clinical standard. This could be caused either by in vivo binding competition with non-labelled RESCA1 conjugate, accelerated clearance or lower binding affinity of \textsuperscript{18}F-TATE. Future experiments with HPLC purified radiotracer in competition binding studies in vitro and in vivo, and investigation in a high somatostatin receptor expressing tumour mouse model will have to clarify, whether specific activity, receptor affinity and/or in vivo kinetics of \textsuperscript{18}F-TATE can explain these results.

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**PP44**

Radiolabelling and SPECT/CT imaging of different polymer-decorated zein nanoparticles for oral administration

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**Introduction and aims:** Zein is the major storage protein of maize, with a “GRAS” status, that can be easily processed to form nanoparticles. Due to its amphiphilic character, mucoadhesive properties and a relatively high resistance to the effect of digestive enzymes, the resulting nanocarriers offer great potential for oral drug delivery purposes. The aim is optimization of \textsuperscript{99m}Tc radiolabelling of different kind of zein nanoparticles (ZNPs) and the study by molecular imaging of their biodistribution in Wistar rats after oral gavage. The evaluated nanoparticles were bare zein nanoparticles (ZNP) and decorated with 3 different slippery polymers (ZNP-A, ZNP-B and ZNP-C).

**Materials and methods:** All nanoparticles were prepared by a desolvation technique. For this purpose, zein were first dissolved in an ethanol:water solution, obtained by the addition of an aqueous solution and spray-dried. NP were pre-tinned with SnCl\(_2\) and labelled with \textsuperscript{99m}Tc. Different SnCl\(_2\) concentrations ranging from 0.005 to 1.0 mg/mL were used for radiolabelling optimization. RCP was analysed by radio-TLC. For biodistribution studies 4 MBq (0.33mg NP) of \textsuperscript{99m}Tc-nanoparticles were used per animal (n=12). SPECT/CT studies were performed 1,2,4,6 and 8h post-administration. SPECT acquisition protocol was set to account for radionuclide decay. For quantitative analysis data sets were exported to PMOD software and VOIs drawn over CT images on stomach and intestine, count ratios calculated in respect to total animal counts and maximum counts normalized to the last image (8h) for each nanoparticle type.

**Results:** Radiochemical purity was >95% for ZNP-A and NPZ using [SnCl\(_2\)]=0.5 mg/mL, while 1.0 mg/mL was needed for ZNP-B and ZNP-C to get similar results. SPECT/CT images showed that unmodified NPZ had a biodistribution in stomach and intestine almost constant during the first 4 hours. \textsuperscript{99m}Tc-NPZ showed a similar behaviour but faster gastric drainage and \textsuperscript{99m}Tc-NPZ-C quickly moved to intestine. All these results were confirmed by the ratios obtained from PMOD processed images.

**Conclusions:** All Zein nanoparticles could be easily radiolabelled with \textsuperscript{99m}Tc with >95% RCP. The results show a clear relationship between the biodistribution and the superficial properties which the coating material gives to the nanoparticles.
PP46
In vivo biodistribution of adult human mesenchymal stem cells I (MSCs-ah) labeled with 99mTc-HMPAO administered via intravenous and intra-articular in animal model. Preliminary results

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Introduction: The application of adult human mesenchymal stem cells from bone marrow (MSCs-ah) is being considered a promising treatment for muscularoskeletal injuries, however, their trafficking and homing are still controversial issues with further studies needed. Our work is focused on in vivo biodistribution of 99mTc-HMPAO labelled MSCs-ah after both intravenous and intra-articular injection in an animal model.

Materials and methods: 2 x 106 MSCs-ah isolated from bone marrow of healthy subjects using SEPAX system were suspended in 1 ml of PBS and labeled with up to 93 Bq/cell of 99mTc-HMPAO. Radiolabeled cell suspensions were administered to 8 adult male New Zealand rabbits (3.5-4 kg) randomized in two groups. In group A (n=4), cell suspensions were administered via intravenous (marginal ear vein) and in group B (n=4) by intra-articular injection. After dynamic acquisition of images every 30 s for 25 min, whole body static images in anterior projection at 1,6 and 24 hours were acquired, previous sedation. No immunosuppressive agents were administered.

Results: Early immune response to xenogenic MSCs-ah was not detected during the study. Neither adverse reaction nor technical complications were registered, so any and every animal was recruited for the study. In group A, high retention of the activity was observed in lung parenchyma, with kidney and bladder visualization from the start, and biliary and abdominal uptake from 1h, maintaining the pattern of distribution at 24h, with no significant activity in liver and spleen. Group B show activity in heart, spleen, liver, kidneys and bladder from the start to the late acquisition of 24h, with biliary and intestinal uptake from 6 h. No activity was observed in the lung parenchyma in this group.

Discussion/conclusion: Exogenous MSCs-ah administered via intravenous are trapped mainly in lungs, with slight posterior retrafficking to other organs. On the other hand, no lung trapping is observed after intra-articular injection, pointing out the suitability of this route of administration for the therapeutic potential of MSCs-ah in musculoskeletal pathology.

PP47
Synthesis of [18F]F-exendin-4 with high specific activity
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Introduction: A reliable method for non-invasive in vivo quantification of beta-cell mass in human pancreas is needed to understand the pathophysiology of type 1 and 2 diabetes. Based on animal studies, the [18F]F-exendin-4-peptide looks promising for imaging pancreatic beta cells but further development of the tracer synthesis is needed to obtain high specific activity (SA) and synthesis procedures amendable for GMP production. A proof-of-concept study in type 1 diabetic patients and healthy subjects showed a correlation between beta cell mass and uptake of 111In-exendin-4, which could lead to further insight into the pathophysiology of diabetes. Recent results with [18F]F-exendin-4 analogues demonstrate enhanced pharmacokinetic properties, as compared to radiometal-labelled counterparts. Here we describe a synthesis of [18F]F-exendin-4 aiming at high SA for the product

Materials and methods: We have investigated several conditions and solvents for synthesizing [18F]F-exendin-4. Alkyne tosylation and a [18F]fluoride-Kryptofix complex were allowed to react in DMSO for 5 min at 80 °C. 18F-labeled tosylate precursor ([18F]FP) was isolated by semi-preparative HPLC and concentrated with a HLB cartridge, followed by elution with THF from the cartridge. Exendin-4-azide was allowed to react with [18F]FP in presence of CuSO4/Na-ascorbate for 5 min at room temperature using vigorous mixing. [18F]F-exendin-4 was isolated by semi-preparative HPLC and concentrated with a Sep-Pak C8 cartridge. [18F]F-exendin-4 was eluted with ethanol and PBS. Radio-TLC and radio-HPLC was used for radiochemical analysis and SA determination of the [18F]FP and [18F]F-exendin-4.

Results: 18F-labelling of [18F]FP proceeded in an 50% radiochemical yield (RCY). Isolation of [18F]FP by HPLC was efficient and the radiochemical purity was > 99%. The RCY of [18F]F-exendin-4 ranged between 25-60% (calculated from [18F]FP, uncorrected) and the radiochemical purity was > 96%. The SA was up to 350 GBq/μmol at EOS.

Discussion/conclusion: We now can produce [18F]F-exendin-4 with a SA of 350 GBq/μmol for synthesis batches of >500 MBq. Our further aim is to automate all synthesis procedures in order to have a GMP-level production of the tracer, suitable for clinical evaluation of [18F]F-exendin-4.

PP48
Experimental radionuclide therapy with 177Lu-labelled cyclic minigastrin and human dosimetry estimations
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Introduction: Radiolabelled cyclic minigastrin-analogues are promising candidates for possible clinical application in diagnosis and therapy of cholecystokinin-2 receptor (CCK2R) expressing tumours, such as medullary thyroid cancer (MTC) and SCLC. Within an international collaboration of the IAEA we performed biodistribution studies and an experimental radionuclide therapy in a CCK2R-specific mouse tumour model using two 177Lu-labelled cyclic minigastrin-analogues: DOTA-cyclo[γ-D-Glu-Ala-Tyr-D-Lys]-Trp-Met-Asp-Phe-NH2 (DOTA-cyclo-MG1) and DOTA-cyclo[γ-D-Glu-Ala-Tyr-D-Lys]-Trp-Nle-Asp-Phe-NH2 (DOTA-cyclo-MG2). Based on results of biodistribution studies dosimetric considerations for humans were extrapolated to evaluate the possible injected activity in first applications in patients.

Materials and methods: For biodistribution studies BALB/c nude mice were xenografted with A431 human epidermoid carcinoma cells transfected with the human CCK2R (A431-CCK2R) and transplanted with the empty vector alone (A431-mock) in both flanks. Tumours were allowed to grow for 2 weeks. Biodistribution studies with 177Lu-DOTA-cyclo-MG1 and 177Lu-DOTA-cyclo-MG2 were performed using the ICRP 30 human phantom for dosimetry calculations.

Discussion/conclusion: Preliminary biodistribution data show that the tracer (DOTA-cyclo-MG1 and DOTA-cyclo-MG2) is mainly retained in tumour tissue and has a low uptake in the liver and bone 1-24 h p.i. For the human renal equivalent (RE) calculation, an activity of 140 MBq was injected in two mice. No adverse events were observed neither clinically nor at necropsy. The ICRP 30 phantom was used for dosimetry calculations. The resulting absorbed dose was 3.6 ± 0.3 Gy/MBq at the tumour target 1-24 h p.i. and 2.5 ± 0.1 Gy/MBq at the bone target 1-24 h p.i. The absorbed dose at the liver, bone and bone marrow region area 1-24 h p.i. 31 ± 10 Gy/MBq, 0.6 ± 0.01 Gy/MBq, 0.1 ± 0.01 Gy/MBq respectively. The absorbed dose at the bone 1-24 h p.i. for 177Lu-DOTA-cyclo-MG2 was 0.5 ± 0.01 Gy/MBq.
performed at an injected dose of 1 MBq (0.02 nmol DOTA-peptide) at 30min, 4h, 24h, 48h, 72h and 168h p.i. (5 animals/group). Dose extrapolation to humans was based on linear scaling of injected activity per gram tissue between animals and humans and computed using OLINDA software. For experimental radionuclide therapy the animals were injected with both 177Lu-labelled DOTA-peptides at two different dose levels of 15 and 30 MBq corresponding to 0.6 and 1.2 nmol peptide (6 animals/group). For up to five weeks after treatment tumour volume and body weight was evaluated in comparison with a control group injected with physiological saline.

Results and conclusion: Biodistribution studies in tumour-bearing mice revealed an uptake of >3.5% ID/g for both 177Lu-labelled DOTA-peptides in the A431-CK2R-tumour 30min p.i. This uptake remained stable for 4h p.i. and declined to >2% ID/g at 24h p.i.. A much lower uptake was observed in the A431-mock tumour. The tumour-to-kidney ratio resulted to be in the order of 3. In the experimental radionuclide therapy a clear therapeutic effect could be observed. In comparison to the control group the mean tumour volume doubling time of A431-CK2R-tumours was increased by a factor of 1.8 and 2.6 in the 15 and 30 MBq group, respectively. For the A431-mock-tumours lower factors of 1.2 and 1.7 were observed in both treatment groups proving a receptor-specific effect. On the basis of dosimetric extrapolation to humans first therapeutic applications in patients should start with 4 repeated administrations of 3-5 GBq to limit the cumulative kidney dose to <27Gy. Based on patient individual dosimetry accompanying the first therapy the injected activity may be adapted accordingly.

PP49
Synthesis of radiochemicals for cell radiolabelling using anion exchange column
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Introduction: 111In labelled radiochemicals are extensively used for cell labelling in routine clinical practice. Being members of coordinating radioisotopes group 68Ga and 89Zr are suitable candidates for synthesis of radiochemicals used for cell labelling. Due to its half life (78,4h) 89Zr labelled radiopharmaceuticals could be used for cell labelling and potentially suitable for cell labelling in routine clinical practice. Being members of group 68Ga and 89Zr are suitable candidates for synthesis of radiopharmaceuticals used for cell labelling. With this automation and their use in human could be done under clinical trial.

Materials and methods: Preclinical IGG100 was used as 68Ge/68Ga generator. Purification of the generator eluate have been explored in the literature, although recent improvement on some generator brands (i.e. low 68Ge breakthrough and low metallic impurities content), makes this pre-purification unnecessary. Development of a labelling process, GMP-compatible and reproducible, using a commercial synthesis module for every peptide labelling is a real challenge for the nuclear medicine. The method presented herein uses a cassette-based approach and a MiniAIO (mAIO, Trasis®) module and has been tested with the IGG100 68Ge/68Ga generator.

Materials and methods: Preclinical IGG100 was used at 68Ga generator. Precursors of radiolabelling were bought from ABX. Automated 68Ga-labelling was performed without pre-purification in mAIO module. Reaction parameters such as sodium acetate concentration, precursor quantity, temperature and time were optimized for each peptide. Labelling efficiency was determined on Waters HPLC system.

Results: DOTANOC, DOTATOC, HBED-PSMA-11 and NODAGA-RGD were obtained in 68% min that is ideal for labelling small peptides as radiochemicals thanks to the use of a chelating agent with several clinical applications. Numerous gallium-68 labelled peptides (e.g. [68Ga]DOTA-TOC/-NOC, [68Ga]HBED-PSMA-11, [68Ga]NODAGA-RGD) have shown their interest (1,2). Developing an easy, rapid and performing labelling method is important. Different methods for the pre-purification of the generator eluate have been explored in the literature, although recent improvement on some generator brands (i.e. low 68Ge breakthrough and low metallic impurities content), makes this pre-purification unnecessary. Development of a labelling process, GMP-compatible and reproducible, using a commercial synthesis module for every peptide labelling is a real challenge for the nuclear medicine. The method presented herein uses a cassette-based approach and a MiniAIO (mAIO, Trasis®) module and has been tested with the IGG100 68Ge/68Ga generator. Precursors of radiolabelling were bought from ABX. Automated 68Ga-labelling was performed without pre-purification in mAIO module. Reaction parameters such as sodium acetate concentration, precursor quantity, temperature and time were optimized for each peptide. Labelling efficiency was determined on Waters HPLC system.

Results: DOTANOC, DOTATOC, HBED-PSMA-11 and NODAGA-RGD were tested for 68Ga-labelling without pre-purification. Optimal and reproducible conditions were determined for each peptide. 68Ga-peptides were synthesised with excellent incorporation yields, (90-99%) and high synthesis yields > 60% in less than 15 min. 68Ga-peptides were synthesised with excellent incorporation yields, (90-99%) and high synthesis yields > 60% in less than 15 min. 68Ga-peptides were synthesised with excellent incorporation yields, (90-99%) and high synthesis yields > 60% in less than 15 min. 68Ga-peptides were synthesised with excellent incorporation yields, (90-99%) and high synthesis yields > 60% in less than 15 min.
Introduction: The development of dry kits containing the active ingredient and suitable excipients permits more efficient use of 68Ge/68Ga radionuclide generators, despite the limitations in the variety of generator eluent's composition and pH. Our aim was to develop a dry kit formulation for 68Ga radiolabeling of the PSMA inhibitor, PSMA-11 (Glu-CO-Lys(Anh)-HBED-CC). Methods: A series of experiments on "wet" labelling in the presence of various amounts of sodium acetate and ascorbic acid were performed taking into consideration the challenge to find the composition of buffering agents ready to maintain pH value between 4 and 5 after addition of various volumes of 68Ga eluate (volumes of 1-4mL 0.1M HCl). For quality control of the 68Ga-PSMA-11 the RP-HRPLC (Kinetex C18 150mm: A: 0.1%TFA/H2O, B: 0.1%TFA/CAN) was used with gradient conditions (according to M. Eder et al. Pharmaceuticals 2014, 7, 779-796) as well as in isocratic elution (optimally set to 17% B) which visualized the radiolabelled and non-labelled PSMA-11 in chromatogram. The radiochemical purity also was tested by TLC (ITLC SG: 1. 0.9%NaCl/Methanol 80/20 v/v; 2. 0.9%NaCl/Methanol/25%H3PO4, 80/20/5 v/v/v; 3. 1M NH4OAc/Methanol 50/50 v/v).

Results and discussion: Ascorbic acid (25mg/mL) added to the "wet" labelling mixture increased the radiolabelling yield (98.8%) compared to the labelling performed in acetate buffer only (95.0%). It also increased the buffering capacity of the formulation. These results were further used to formulate the dry kit composition using 30g of PSMA-11, 60mg of sodium acetate and 12.5mg of ascorbic acid. The yield of 68Ga labelling of PSMA-11 from the freeze-dried kit was > 95%. The biodistribution of such prepared 68Ga-PSMA-11 was investigated in healthy Balb/c mice (n=5) after intravenous injection of 0.1mL (38 MBq/mL) (at 15, 30, 60 and 120min). 68Ga-PSMA-11 was rapidly cleared from the blood and excreted by kidney route with >70% urine elimination at 1h p.i. These results confirmed high stability of the 68Ga-PSMA-11 complex in vivo, with low contribution of free gallium-68, which circulates longer in blood stream. It is worth noting that the addition of ascorbic acid changes the biodistribution of free gallium and favors its blood clearance. This is probably due to the formation of week Ga complex with ascorbic acid which blocks Ga access to ferritin, hence resulting in lower liver and spleen accumulation.

PP52 Development of an experimental method using Cs-131 to evaluate radiobiological effects of internalized Auger-electron emitters

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Introduction: The low energy electrons emitted in the Auger cascade of certain isotopes are expected to have a disproportionately high radiobiological effect. This is seen as the effect of the multiplicity and predominance of very low energy electrons in dose delivery. Despite many years of research in Auger emitters, only few direct measurements of the radiobiological effect of such Auger cascades have been published. One problem in making such fundamental dose-effect experiments is the method to bring Auger-emitting isotopes inside the cell and close to the nucleus in a predictable and quantifiable manner. Only when the location, activity, and time-activity profile are known it is possible to compare the observed damage with damage induced by the same dose and dose rate given by external irradiation. We have used the biological uptake of the Auger-emitting potassium analogue Cs-131 (9.7 d) into mammalian cells in culture as a test system for measuring the dose-effect of Auger electrons. Materials and methods: Cs-131 was repeatedly extracted from Ba-131 (11.5 d) made by high flux neutron irradiation at ILL (6 to 10 days at (1.1-1.3) 1015 n.cm-2s-1) of either natural Ba (17 mg as carbonate) or 49% enriched Ba-130 (0.15 mg as nitrate). Such targets were dissolved in hydrochloric acid, and re-precipitated with ammonium carbonate. The supernatant was spiked with sodium hydroxide, then dried and fired, leaving cesium-131 as a chloride salt with NaCl carrier. The re-precipitated targets were stored for 1-2 weeks for buildup of Cs-131, and then the process can be repeated. We have milked up to 4 batches of useful Cs-131 activity from a single activated barium batch from ILL. The harvested, 131Cs/NaCl salt was dissolved in water to reach isotonicity, and was offered to V-79 and HeLa cells in normal growth media. The cells invariably up-concentrated the cesium activity, reaching a plateau after 8-10 hours.

Results: Using enriched Ba-130, higher activity is obtained, but even with natural barium activities up to 80 MBq have been available repeatedly for the uptake experiments. Under optimal conditions, the cells in culture can reach intracellular activities over 1 Bq/cell during 4 hours incubation. The alkali-metal nature of cesium gives us reasons to assume a homogeneous intracellular distribution, allowing a first-principles calculation of the absorbed dose. With this method and unique isotope, we can compare the effect of equivalent absorbed doses of external gamma and internal, predominantly Auger delivered dose.
tracers show that the uptake and retention on different tumor cells depends on tumor type, receptors expression and dimerization. Very good uptake-retention profile of 68Ga-DOTA-E-(cRGDKR)2 makes it our option for further investigations as therapeutic agent. Research contracts CRP16500 IAEA and MEN UEFISCDI, PN II 228/2014 are acknowledged for financial support.

**PP54**

Synthesizer- and Kit-based preparation of prostate cancer imaging agent 68Ga-RM2

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Introduction: Prostate cancer (PCA) is the most common cancer in men and the second most common cause in cancer-related deaths. 68Ga-RM2 is a nona-peptide with optimized binding sequence for Gastrin Releasing Peptide receptor (GRPr) which is overexpressed in PCA. In clinical studies 68Ga-RM2 has demonstrated its ability for detection of primary prostate cancer with high specificity due to its low uptake in normal prostate and in benign hyperplasia.2 Recent studies showed further potential of 68Ga-RM2 for detection of recurrent PCA and for imaging of estrogen receptor positive breast cancer.3,4. To provide 68Ga-RM2 for further clinical research, robust methods for preparation of this promising tracer are needed. Herein we describe the results of automated synthesizer based 68Ga-radiolabelings of RM2 as well as of simplified “shake-and-bake” kit-preparations.

**Materials and methods:** The automated 68Ga-radiolabeling was optimized on a PharmTracer module (Eckert&Ziegler). The influence of several scavengers was investigated, as well as a processes with and without subsequent purification via C18 cartridge. Optimized conditions were used on further synthesizers (Scintomics, ITG). In a second approach, a simplified kit preparation was established. The 68Ga-generator eluate was added directly into a vial containing RM2 precursor, buffer and scavenger. The vial was heated and optionally buffered was used for pH-adjustment.

**Results:** Automated procedures on various synthesizers provided up to 850 MBq of 68Ga-RM2 in >70% uncorrected yield in 20 min including purification and formulation. The radiochemical purity was >97% (HPLC and TLC) if acetic acid was used as scavenger. The simple and easy to use kit preparation approach provided 68Ga-RM2 in 90% uncorrected yield and purity of >95%.

**Discussion/conclusion:** 68Ga-RM2 can be reliably obtained on automated synthesizers as well as by a simple “shake-and-bake” kit preparation strategy. Both methods provided 68Ga-RM2 in high yields and high radiochemical purity.

**PP55**

Synthesis of pancreatic beta cell-specific [18F]fluoro-exendin-4 via strain-promoted aza-dibenzylo cyclooctyne/azide cycloaddition

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Introduction: Diabetes has dictated many lives, and its lifetime treatment has accumulated into one of the most costly medical conditions, and yet, its pathophysiology is still undisclosed. However, efforts to develop sensitive noninvasive methods to quantify beta cell mass, which underlies the development of diabetes, have been challenged by the low abundance (1-2 %) and high dispersion of beta cells in the pancreas [1]. Recent preclinical studies using 18F-labelled exendin-4 showed specific targeting of pancreatic islets and insulinomas with favourable clearance kinetics that would potentiate clinical utility [2]. The focus of the present study is the radiosynthesis of a novel 18F-labelled exendin-4 analogue with a clear clinical prospective for imaging pancreatic beta cell mass.

**Materials and methods:** The synthesis of [18F]fluoro-exendin-4 is depicted in Scheme 1. The dried [18F]fluoride-Kryptofix complex was reacted with tosylated prosthetic compound 1 in dimethylsulfoxide at 80 °C. After chromatographic isolation, the 18F-labelled azide 2 was allowed to react with cyclooctyne-derivatised exendin-4 in ethanol/water at 60 °C for 30 min. Isolation and radiochemical analysis of [18F]fluoro-exendin-4 was performed using high performance liquid chromatography (HPLC) with radiodetector.

**Results:** Preliminary results showed efficient reaction between prosthetic reagent [18F]2 and exendin-4 precursor with a radiochemical yield of 30 %. The absence of major radioactive by-products assured neat separation using HPLC. The average isolated yield of [18F]exendin-4 after HPLC purification was 180 MBq starting from 3.5 GBq aqueous [18F]fluoride.

**Discussion:** Currently, the scope of this reaction is being explored and its labelling conditions optimized. Preclinical studies with [18F]fluoro-exendin-4 will include in vivo stability, in vivo distribution kinetics, and dosimetry calculations in healthy and diabetic animal models.

**PP56**

Automated systems for radiopharmacy

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Introduction: Automated modules for target processing and production of radiopharmaceuticals become to be a more and more required in both R&D and routine practice. However, it turns out that available systems for processing of targets for radiometal production and for labeling performed via microfluidic technology are still rather limited. Our aim was to develop a multipurpose automated systems for processing solid state targets and for operating microfluidic PMMA-based chip.

**Materials and methods:** Stingray – Automated microfluidic system. The device is a versatile automated system that allows for control of variety of processes performed on a microfluidic chip. This platform makes possible operations like precise mixing, separation, extraction or small-scale nanoparticle formation. It consists of 3 independent inlets for 3 different mobile phases with an in-built degasser, 4 peak solvent valves, 2 independent nanopiston pumps, 2 selectors (6/7), 2 manual inject valves with 100 μl loop volume (adjustable), 2/10 valve, 3 check valves and universal automated manifold for inserting microfluidic chip. This platform offers enough variability of reaction parameters and allows for a repeated return of a sample to the microfluidic chip. It may enhance the yield dramatically. CRAB – versatile platform for separation, formulation and simple labelling processes. The main parts of the system are two reactors, two selectors, peristaltic pump, 3/2 way valves, and the column. Prime reactor R1 allows for transport solid target material from shielding container to process position and for handling liquid target content. It is leak-proof for 5 bars. There is an in-built solid phase extraction (SPE) column for separation processes driven by peristaltic pump and solvents. Four positions are available for uploading the solvents into the reactor R1 or on the SPE column. Splitting the separated radionuclide from the target matrix, including enriched material, is enabled thanks to the smart software checking the column output activity and controlling the splitting valves position. Final activity concentration can be precisely set via the case software without losses on the walls of reactor R2. There are 3 positions for uploading the solvents to the reactor R2 for formulation or for simple labelling steps like chelation.

**Results and conclusion:** Both automated platforms are versatile, reliable tools that offer user-friendly settings for wide range of processes. Stingray is operable up to 200 bar back pressure allowing thus the flow rate of 20 ml/min. Crab was tested on separation of 61Cu and 64Cu from Ni targets in 99% separation yield of copper radionuclides and almost quantitative recovery of target matrix.
PP57

Simple, suitable for everyday routine use quality control method to assess radionuclidic purity of cyclotron-produced 99mTc

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Introduction: Formulations of cyclotron-produced 99mTc contain trace amounts of other Tc isotopes depending on the isotopic composition of starting 100mTc. Presence of other nuclides may affect image quality and patient dosimetry. Therefore, quantification of radioisotopic impurities is very important. Currently, radioisotopic purity of cyclotron-produced 99mTc is measured using gamma-ray spectrometry and is time-consuming. We sought to identify another approach better suitable for quality control during routine productions.

Materials and methods: 100Mo targets (99.815% enrichment) were irradiated in a cyclotron at 24 MeV for 2 h. The targets were processed and radioisotopic purity of resulted 99mTc was evaluated using three different procedures. Method 1, "Dilution": An aliquot of diluted 99mTc solution was measured using gamma-ray spectrometry. Percentage of each radioactive contaminant was calculated dividing activity of each isotope by total activity in the sample. Method 2, "Shielding": A vial containing formulated 99mTc was measured in a calibrated ionization chamber. Then, it was inserted into a 6 mm thick lead canister, used in nuclear medicine for 99mMo breakthrough determination, and measured using gamma-ray spectrometry. The activity of each radioactive contaminant was calculated and divided by total activity in the vial as measured in ionization chamber. Method 3, "High-energy breakthrough": A vial containing formulated 99mTc was measured in a calibrated ionization chamber as is and inside the lead canister. The ratio of two measurements gave relative proportion of high-energy gamma-rays.

Results: There was a tendency to slightly overestimate the radioisotopic purity of cyclotron-produced 99mTc with "dilution" method (99.98±0.007%) compared to "shielding" method (99.97±0.009%). Since both methods require measurements by gamma-ray spectrometry, they are time-consuming, involve elaborate calculations, and demand specific expertise. "High-energy breakthrough" measurements showed good correlation with the results of gamma-spectrometry.

Conclusions: With "shielding" method, lead canister attenuated low-energy gamma-emission (including from 99mTc) and allowed detecting impurities present in trace amounts with better sensitivity. While "high-energy breakthrough" measured in ionization chamber does not give true radioactivity values for each contaminant, it offers sufficient information about relative amount of high-energy radioactive impurities in the product. This could serve as a surrogate test during routine productions when cross-calibrated with gamma-spectrometry data for given isotopic composition of 100Mo targets.

PP59

Chemical analysis of the rituximab radioimmunoconjugates in lyophilized formulations intended for oncological applications

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Introduction: For a protein based drug including antibody based radiopharmaceuticals, a structural characterization is mandatory before a clinical trial. Vibrational spectroscopic techniques, as Fourier transform infrared (FTIR) spectroscopy and Raman spectroscopy, are one of the biophysical methods for structural characterization of proteins because of their sensitivity to the composition and architecture of molecules. Here we used vibrational spectroscopy to characterize the physico-chemical stability and structural changes of three immunono conjugates of rituximab, intended for labeling with radioisotope of choice (131I, 99mTc and/or 68Ga), relevant for the stability of therapeutic/diagnostic antibodies during preparation, storage and/or transport.

Materials and methods: Rituximab, conjugated with three different bifunctional chelating agents (BFCAs), p-SCN-Bn-DOTA, p-SCN-Bn-DTPA and 184M-DTPA in a form of lyophilized preparations non-radioactive labeled with above mentioned radioisotope analogues, was subjected to characterization and determination of secondary structure and quality parameters (purity, integrity, fragmentation and aggregation of the antibody) by FT-IR and Raman spectroscopy.

Results: Based on the frequencies assigned for amide I, II and III bands, the studied formulations contain highest percentage of β-sheet conformation (antiparallel and parallel) in the structure, followed by α-helices. Significant changes upon processes of conjugation and lyophilization were not observed in comparison with spectra of native antibody.Vibrational spectroscopic data allow detection of alterations in investigated protein models as well as rapid assessment of conformational changes resulting from ligand binding.
aggregation or macromolecular interactions. Appearance of strong absorption bands below 1620 cm\(^{-1}\) can be correlated with aggregation-usually associated with the formation of new strong β-sheets. According to the obtained spectra, it is important that we observed retaining of native structure of the antibody and no obvious aggregation (the lowest band frequency detected was 1620 cm\(^{-1}\) with weak intensity) in all samples of lyophilized conjugates.

**Conclusion:** We investigated the application of vibrational spectroscopy in assessment of conformational changes during stress conditions, as lyophilization and non-radioactive labeling are, using different rituximab-conjugates. The results are a good foundation for further radio-labeling studies of the lyophilized formulations for possible therapeutic application.

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**PP61**

**The need and benefits of established radiopharmacy in developing African countries**

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**Introduction:** Our work is to present the current status, and in the same time the need and benefit of establishment of Radio-pharmacy practice in Eastern Africa using the perspective of Kenya and Ethiopia. The exact information on the status and size of Radiopharmacy units, regionally, is still not clearly documented, as well human resources, education, suitable training and local demand for the Radiopharmacy and Nuclear Medicine services. The Radiopharmacy Practice requires well-defined and controlled conditions to avoid the risk contamination with microbes, pyrogens and particulate matter as well as cross contamination with other radiopharmaceuticals. Corresponding to the expected improvement, the principles of Good Practices in all levels should be planned, introduced by the planned priority and strictly observed in the production, preparation, testing and the packaging of the final product ready for use. Because non-communicable diseases (NCDs) are a challenge of epidemic proportion and that they will be the commonest cause of mortality in Africa by 2030, early detection and treatment can significantly improve patient outcomes. Radiopharmaceuticals should have become of invaluable benefit because they offer the most sensitive tools in the detection, diagnosis and targeted therapy of NCDs and also infectious diseases. In light of the foregoing, therefore, radiopharmacy has a huge role to play in responding to the unfolding new disease trends in sub-Saharan Africa. The preparation of radiopharmaceuticals for human use requires that it is carried out in well-defined and controlled conditions to avoid the risk contamination with microbes, pyrogens and particulate matter as well as cross contamination with other radiopharmaceuticals. Every procedure undertaken should be done according to the clearly defined protocol and under the right conditions so as to build quality into the product. Radiopharmacy professionals should have adequate training in all aspects of sterile production, quality control, GMP, GLP, radiation safety and radiochemistry to ensure that they are competent to handle radioactive materials and that they can take responsibility for their level of practice.

**Conclusions:** We are expecting that the good education and continuing training of all professionals working in Radiopharmacy will be the key point how to create the network of all professionals and state authorities for establishing and develop Good Radiopharmacy Practice, qualified personnel and appropriate regulation according to the local and international parameters will be step forward to have advanced health care system and confidence of the patients.
Synthesis and preclinical validations of a novel 18F-labelled RGD peptide prepared by ligation of a 2-cyanobenzothiazole with 1,2-aminothiol to image angiogenesis.

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Introduction: The RGD-recognising αVβ3, αVβ5 and α5β1 integrins are known to be involved in carcinogenesis and are overexpressed in many types of tumours compared to healthy tissues; thereby they have been selected as promising therapeutic targets. Positron emission tomography (PET) is providing a unique non-invasive screening assay to discriminate which patient is more prone to benefit from antiangiogenic therapies, and extensive research has been carried out to develop a clinical radiopharmaceutical that bind specifically to integrin receptors. However, despite promising clinical results, one still needs to identify an integrin radioligand that could be easily deployed. We herein report the synthesis of a 18F-labelled RGD peptide, namely [18F]FPyPEGCBT-c(RGDfK), by condensation of a 2-cyanobenzothiazole prosthetic group to a cysteine-modified cyclic RGD peptide and the characterisation of its preclinical biologic properties.

Materials and methods: A novel bifunctional synthon ([18F]FPy-PEGCBT) has been developed to accommodate: i) an efficient [18F]-incorporation and ii) a mild conjugation to biomolecules. Then, [18F]FPyPEGCBT has been conjugated to a cyclic RGD peptide known for its high binding affinity and selectivity for integrin αVβ3. The in vitro binding characteristics of FPyPEGCBT-c(RGDfK) were analysed by standard binding assay in U-87 MG and SKOV-3 cancer models and its selectivity towards integrins by siRNA depletions. Its preclinical potential was studied in mice bearing subcutaneous tumours by ex vivo biodistribution studies and in vivo microPET/CT imaging.

Results: [18F]FPyPEGCBT was prepared from its corresponding 2-trimethylammonium triflate precursor and purified by solid-phase extraction. Ligation of [18F]FPyPEGCBT to the cysteine-modified cyclic (RGDFK) peptide was carried out in DMF at 43 oC. The 18F-peptide was obtained, after HPLC purification and reformulation, in 124–132 min from the end of bombardment with a final decay-corrected yield of 7% ± 1. In vitro, FPyPEGCBT-c(RGDfK) bound efficiently to RGD-recognising integrins as compared to a control c(RGDfV) peptide (IC50 = 30.8 × 10⁻⁷ M vs. 6.0 × 10⁻⁷ M). In vivo, this new tracer demonstrated specific tumour uptake with %ID/g of 2.9 and 2.4 in U-87 MG and SKOV-3 tumours 1 h after injection. Tumour-to-muscle ratios of 4 after 1 h of uptake allowed good visualisation of the tumours. However, unfavourable background accumulation and high hepatobiliary excretion were noticed.

Discussion/conclusion: [18F]FPyPEGCBT-c(RGDfK) specifically detects tumours expressing RGD-recognising integrin receptors in preclinical studies. Further optimisation of this radioligand may yield candidates with improved imaging properties and would warrant the further use of this efficient labelling technique for alternative targeting vectors.