Aim: There was continued interest in developing more efficient new chelating agents for metal radionuclides mostly used for positron emission tomography (PET) imaging. We focused on the development of convenient syntheses of acyclic siderophore derivatized with 

Methods & Results: 4HMS and 4HMSA were prepared through multiple steps starting with a spermine backbone to offer both chelating agents with high overall yield (72-58%). Both chelating agents exhibited strong selective coordination of $^{89}$Zr and $^{68}$Ga and offered a very fast labelling kinetic at room temperature as compared to DFO and DOTA/NOTA analogs. Achievable molar activity for $^{68}$Ga-4HMSA is almost 10 and 3 times higher compared to $^{68}$Ga-DOTA and NOTA analogs. Molar activity of $^{89}$Zr-4HMS is approximately 16 fold higher compared to $^{89}$Zr-DFO. Both radio-complexes were stable in saline, as well as against transchelation and transmetallation. $^{68}$Ga-4HMSA showed high stability in mouse plasma in vitro and in vivo over 1h and $^{89}$Zr-4HMS chelator also showed high stability in mouse plasma over 7 days. Biodistribution and imaging studies were performed in babl/c mice. The background activity in various tissues was low at 1h post-injection (p.i.) for $^{68}$Ga-4HMSA with a rapid elimination mainly through the kidneys and liver. At the same time point, the activity was largely found in kidneys for $^{89}$Zr-4HMS chelator. At 24 h p.i. most of the $^{89}$Zr-4HMS chelator was cleared from all organs and the low amount of activity in kidneys and bone is consistent with the clearance of the intact complex. Finally, the conjugation of unprotected 4HMSA to peptides of biological interest was complete within ~4 h with overall yields of 50-60%.

Conclusion: 4HMSA and 4HMS show an outstanding promise as $^{68}$Ga and $^{89}$Zr chelators. In terms of $^{89}$Zr chelation and stability, 4HMS ligand has proven to be a superior chelator compared to DFO.
0.24%; dimer 7.75 ± 0.56%; trimer 15.9 ± 0.88%). Biodistribution showed a similar profile with rapid renal excretion and moderate uptake in liver and kidneys (<10% ID/g), blood levels increased from the mono to the trimer from 1%-3%ID/g 1h p.i.

Conclusion: Our preliminary results show that the preparation of polyvalent Tz-conjugates based on a chelating scaffold is feasible. In vitro results revealed enhanced binding of di- and trimeric constructs vs. the monomer similar to the effects seen in receptor targeting ligands, biodistribution data revealed favourable biodistribution for these pretargeting constructs. Proof of tumour targeting in vivo in respective animal models is currently ongoing.

Fig. 1 (abstract OP02). Scheme: Synthetical pathway of tetraceine modified FSC-based pretargeting agents

**OP03**

**99m**Tc-radiolabeling of a poorly soluble protein, a variable heavy chain antibody domain targeting pancreatic β-cells

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP03

**Aim:** 99mTc-tricarbonyl precursor was prepared before its incubation with VH-13 in different conditions of concentration, temperature and incubation time. Due to the appearance of aggregates during radiolabelling or purification, we first studied different concentrations of several excipients to decrease their formation: 0.2, 10 and 100 mM of arginine (pH=7 or 12), 5% of tween-80 (w/w), 10 and 20% of heparin (w/w), 1 M of acetone or 4% of DMSO (v/v). In a second time, 2 variants of VH-13 were designed with mutations in the VH-VL interface of protein finally represented the best solution to reduce the aggregation and to allow a successful radiolabelling.

**Results:** The presence of arginine at 100 mM allowed to avoid the appearance of aggregates. But whatever the pH conditions, arginine was radiolabeled with Tc-99m. 10 or 20 % of heparin decreased the aggregation, quality control before purification showed a high radiochemical purity (RCP), but more than 80% of the radiolabeled product remained on the purification columns, probably because of a very light aggregation remaining. Adding DMSO was not compatible with the radiolabelling procedure with a RCP < 50%. Finally, the two mutants of VH-13 were radiolabeled and purified successfully. The RCP of radiolabeled products was higher than 95%.

**Conclusion:** Among all the tested excipients, 100 mM of arginine inhibited the aggregation of protein during radiolabeling. Nevertheless, it was also radiolabeled and greatly decreased the labeling yield of VH-13. The synthesis of the two novel mutants including a mutation in the VH-VL interface of protein finally represented the best solution to reduce the aggregation and to allow a successful radiolabelling.

**OP04**

**Synthesis of PET Radiopharmaceuticals for Cell Radiolabelling Using Anion Exchange Column and Cell Labelling**

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP04

**Aim:** In labelled radiopharmaceuticals are extensively used for WBC labelling in routine clinical practice. 64Cu (T1/2: 12.7h and 89Zr (T1/2: 7.4h) are alternative radiometals for synthesis of radiopharmaceuticals used for cell labelling that could enable long-term PET “in vivo” cell tracking with several attractive clinical applications. The aim of study was to prepare 64Cu and 89Zr tracers (oxine, tropolone) applying synthesis and concentration on an anion exchange column and radiolabel cell (WBCs, RBCs). Methods: Small volumes (1-2 mL) of tracers (oxine, tropolone) in PBS of suitable radioactive (n-octanol extraction) purity and activity (64Cu ≤ 102 MBq, 89Zr ≤ 3MBq) were prepared on the anion exchange column (SepPak QMA) using a previously described method. Cells were radiolabelled, WBCs with 64Cu using modified (incubation time 20 min) EANM recommended 111In-oxine WBCs labelling method. Labelling efficiency, cell viability, assessed by Trypan Blue exclusion assay and labelling stability (efflux of radioactivity) was determined at different time points after radiolabelling.

**Results:** Synthesis and concentration on SepPak QMA resulted in 89Zr-tropolone with > 79.1% yield, pH 7.0-7.5 and extraction into octanol >94.5%, for 89Zr-oxine solution 27.9-70.6% with pH 7.7-9 and extraction > 80%. For 64Cu-tropolone >92.5% yield was achieved, pH between 6.9-7.4, extraction into octanol >89.1%, for 64Cu-oxine 55-91.1%, pH 6.9-7.5 and extraction >90.6%. Labelling efficiency of RBCs with 89Zr-tropolone was 45-50%, with 89Zr-oxine above 64.5%. Labelling efficiency of WBCs was above 81.1% with 64Cu-tropolone independent of cell numbers, for 64Cu-oxine up to 68% highly dependent on the amount of cells available. Viability of 64Cu-tropolone radiolabelled WBCs before and immediately after the labelling was 92% and 85%, respectively 240min after labelling 88% and 81%. Labelling stability of WBCs 240min after cell radiolabelling was above 68% and remained constant up to 48h after labelling. Viability of 64Cu-oxine radiolabelled WBCs immediately after the labelling was 71% (90% unlabelled WBCs) and 70% 240min after labelling (85% unlabelled WBCs). Labelling stability of WBCs 240min after cell labelling was above 91% but decreased to 78.1% 48h after labelling. Preliminary PET studies in animal infection models with 64Cu-WBC showed expected accumulation in infected tissue.

**Conclusion:** The applied on-column synthesis and concentration method enables formation of PET tracers (oxine, tropolone) with good yields, quality and in small volumes suitable for cell radiolabelling. 89Zr and 64Cu tracers radiolabel cell with sufficient stability and viability, this way making this approach highly promising for routine clinical use.

**OP05**

**Synthesis of 18F-Ambifosfotirosan and preliminary in vitro evaluation as a novel AT1R PET radioligand in Oncology**

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP05
Aim: Angiotensin II type 1 receptor (AT1R) is a G protein-coupled receptor recognized as a promising cancer therapeutic target. AT1R expression has been reported to drive tumor development and progression for several cancers. Losartan, an AT1R inhibitor widely used for the treatment of hypertension and congestive heart failure, has been shown to inhibit cancer cell proliferation and angiogenesis. Moreover, losartan derivatives labeled with fluorine-18 (18F) and carbon-11 were reported for AT1R imaging by positron emission tomography (PET); however, they were mostly employed as AT1R PET renal tracers. The present study reports the syntheses of ammoniumethyl-trifluoroborate-losartan (19F-AmBF3-losartan), a new AT1R PET radioligand for cancer imaging.

Methods: 19F-AmBF3-losartan was prepared via a copper-catalyzed alkynyl-AmBF3 and azide-modified losartan cycloaddition at 45 °C for two hours, followed by semi-preparative HPLC purification. Then, 19F-AmBF3-losartan (25 nmol, 15.3 μg) was radiolabeled with fluoride-18 (555-925 MBq) via an 18F–19F isotope exchange reaction in aqueous phase at 80 °C for 20 minutes and purified by solid phase extraction using a C18 Light Sep-Pak cartridge. In vitro AT1R binding studies were performed at 4 °C for one hour using AT1R-positive MDA-MB-231 breast cancer cells, AT1R-expressing CHO AT1R cells, and AT1R-negative CHO cells. The in vitro studies were conducted in presence or absence of the AT1R blocker losartan potassium (100 μM). AT1R expression in cells was confirmed by quantitative real-time reverse transcription polymerase chain reaction (RT-PCR).

Results: Losartan potassium was converted into tetrazole-protected losartan, azido-modified tetrazole-protected losartan, azide-modified losartan and 19F-AmBF3-losartan with 83%, 77%, 96% and 52% yields, respectively. Mass spectrometry confirmed their identities. 19F-AmBF3-losartan was manually prepared in ~35 minutes, with 11 – 18 % radiochemical yield, > 97% radiochemical purity, and 1.8 – 2.9 GBq/μmol specific activity. The identity of 19F-AmBF3-losartan was confirmed by co-injection with the cold compound on analytical HPLC. In vitro studies showed that uptake of 19F-AmBF3-losartan increased in AT1R-expressing MDA-MB-231 and CHO AT1R cells in comparison to control AT1R-negative CHO cells. Pre-incubation with losartan potassium effectively blocked 19F-AmBF3-losartan binding to AT1R-expressing cells. Pre-incubation with losartan potassium showed specific binding to AT1R-expressing cells in vitro. These results demonstrate that 19F-AmBF3-losartan might be a promising tracer for imaging AT1R-expressing tumors.

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OP06
Time is Money and Radiation Burden - a carbon-11 ‘two-in-one-pot’ production system
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP06

Aim: The use of positron emission tomography (PET) for specific molecular examinations is increasing steadily and therefore the demand for selective and specific PET-tracers is rising accordingly. Currently, only one tracer per synthesizer can be produced (non-cassette based) within a time frame of approximately 2 h and a radiation burden of approximately 1 h. A minimum decay time of 6 half-lives (around 2 h) between two carbon-11 productions within the same hot cell is essential. Therefore, in an ideal routine (8 h day) only two syntheses of carbon-11 labeled compounds per day are possible. Consequently, the number of examinations with 11C-labeled tracers is extremely limited (number of productions; high synthesis costs and few production runs due to number of hot cells and synthesizers). To improve this situation, the aim of this study was the simultaneous production of two 11C-PET-tracers using a ‘two-in-one-pot’ reaction reducing time, cost, and radiation burden. Exemplarily, this simultaneous production was successfully performed for two commonly used brain PET-tracers, [11C]Harmine and [11C]DASB.

Methods: Production runs were performed using a commercially available GE Tracerlab FX C Pro. 1 mg of the precursors, 11CMB and Harmol, were dissolved in DMSO and 5 M NaOH was added to the solution. [11C]CH3I was subsequently bubbled through the precursor solution. After a reaction time of 2 min at 100°C, the crude dual-tracer mixture was purified by means of semi-preparative HPLC. The synthesis module was expanded with a self-constructed semi-automated formulation unit (Fig. 1) to ensure parallel SPE-purification and formulation of both tracers after HPLC (Fig. 1).

Results: Both PET-tracers were prepared simultaneously in a ‘two-in-one-pot’ reaction (n = 3) and successfully purified using one single HPLC run. Radiochemical yield was 2.0 ± 0.3 GBq (2.3 ± 0.5% not corrected for decay; based on [11C]CO2 @EOB) for [11C]DASB and 2.0 ± 0.7 GBq (2.2 ± 0.8%) for [11C]Harmine, respectively. Hence, both products were received in the same amount (ratio 1:1). The qualities of both tracers complied with the European Pharmacopoeia monographs.

Conclusion: We herewith describe the first simultaneous production of two 11C-PET-tracers in a ‘two-in-one-pot’ reaction. Both products were in full accordance with quality control parameters fulfilling the standards for parenteral human application. This simultaneous radio-photaceutical preparation lead to a significant reduction of radiation burden, reduction of amount of operator time (-50%), cost reduction (-46.2%) and, subsequently, to considerable gain in overall efficiency of the production process.

Fig. 1 (abstract OP06). Scheme of the synthesizer including the self-constructed unit for the formulation of the second tracer
Fig. 2 (abstract OP06). Exemplary RP-HPLC chromatogram for the separation of [11C]Harmine and [11C]DASB in a single run
**OP07**

18F-labelled BODIPY-steroid hormone conjugates as potential bimodal PET and fluorescence receptor imaging agents

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP07

Aim: 4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) has been used as a fluorescent probe to label a variety of different ligands. BODIPY derivatives have also been radiolabeled with radionucleides for the development of bimodal PET and fluorescent imaging agents. Previously we reported the synthesis of BODIPY-estradiol and androgen conjugates as potential fluorescent probes for receptor imaging in breast and prostate cancers. Relative binding affinities of a series of BODIPY-estradiol conjugates revealed that only the analog featuring an eight carbon spacer between the two entities showed good receptor binding affinity. We recently confirmed by in vitro fluorescence imaging that this analog localizes through a receptor-mediated process on cancer cells that over-express the estrogen receptor. As a continuation of this research, we wish to evaluate an alternative approach using 4-dimethylaminopyridine labelled analogs of BODIPY-steroid conjugates. We are currently investigating the potential to prepare 18F-labelled BODIPY-steroid conjugates as potential fluorescent probes for receptor imaging in breast cancer.

Method & Results: 18F-labelled 4-iodophenyl substituted BODIPY was first prepared by the SnCl4-assisted 18F-19F isotopic exchange method (50-70%). This radiolabeled moiety was subsequently conjugated in high yield to the C17α-position of estradiol and androgen derivatives under Sonogashira cross coupling reaction conditions using Pd-catalyst, base and CuI. Direct labelling of the conjugates using SnCl4-assisted 18F-19F isotopic exchange method resulted in the simultaneous addition of a Cl-atom to the C17α-ethynyl group and a poor molar activity.

Conclusion: Our studies confirm the potential to prepare 18F-labelled analogs of BODIPY-steroid conjugates. We are currently evaluating an alternative approach using 4-dimethylaminopyridine BODIPY as precursor to allow the efficient incorporation of 18F to potentially generating higher molar activity conjugates suitable for receptor-based bimodal imaging studies.

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**Table 1 (abstract OP08).** See text for description

| No | Precursor | Salt | Eff. % | RCC. % (n = 3) |
|----|-----------|------|--------|---------------|
| 1  | 4-Biphenyboronic acid pinacol ester | 4-DMPTs | 70±1   | 65±5          |
| 2  | 4-Biphenyboronic acid pinacol ester | 4-DMPTf | 78±1   | 96±3          |
| 3  | 3,4-Dimethoxynaphthaleneboronic acid pinacol ester | 4-DMPTF | 94±2   |               |
| 4  | 2-Methoxynaphthaleneboronic acid pinacol ester | 4-DMPTf | 85±4   |               |
| 5  | 4-Methoxynaphthaleneboronic acid pinacol ester | 4-DMPTf | 89±5   |               |

**OP08**

Copper-mediated radiofluorination of aryl pinacol boronates in the presence of pyridinium sulfonates

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP08

Aim: Nowadays copper-mediated radiofluorination of arylboronic acids pinacol esters (ArBPin) found ample application for 18F-labeling of various electron-rich arenes.1 The improvement of this methodology, in particular, 18F-recovery step, has been in focus of the recent researches.2,3 The suggested use of aqueous solutions of non-ionic bases under Sonogashira cross coupling reaction conditions using Pd-catalyst, base and CuI. Direct labelling of the conjugates using SnCl4-assisted 18F-19F isotopic exchange method resulted in the simultaneous addition of a Cl-atom to the C17α-ethynyl group and a poor molar activity.

Conclusion: Our studies confirm the potential to prepare 18F-labelled BODIPY-steroid conjugates as potential fluorescent probes for receptor imaging in breast cancer.

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**Table 1 (abstract OP08).** See text for description

| No | Precursor | Salt | EFF % | RCC. % (n = 3) |
|----|-----------|------|-------|---------------|
| 1  | 4-Biphenyboronic acid pinacol ester | 4-DMPTs | 70±1  | 65±5          |
| 2  | 4-Biphenyboronic acid pinacol ester | 4-DMPTf | 78±1  | 96±3          |
| 3  | 3,4-Dimethoxynaphthaleneboronic acid pinacol ester | 4-DMPTF | 94±2  |               |
| 4  | 2-Methoxynaphthaleneboronic acid pinacol ester | 4-DMPTf | 85±4  |               |
| 5  | 4-Methoxynaphthaleneboronic acid pinacol ester | 4-DMPTf | 89±5  |               |

**OP09**

Development of biocompatible and functionalised polymer nanoparticles for the specific vectorisation of an imaging agent

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP09

Aim: Nanomedicine, the application of nanotechnologies to the medical domain, is a fast-growing research field, especially the production of nanoparticles enabling encapsulation then controlled and targeted release of molecules of interest, such as a cytotoxic drug. Diagnostic could also benefit from the use of such nanovectors containing one or more imaging agent(s) (fluorescent dye, contrast agent, radionuclide) to image a tumour.
Methods: Benzyl polymalate and its derivatives (pegylated and bio- 
tinylated) were prepared according to the method previously de- 
scribed. (1) Different techniques have been used to formulate the nanoparticles and to incorporate an imaging agent (DID-Oil, fluores- 
cein amine and a 99mTc-based radiotracer). The resulting nanoparti- 
cles were characterised using various techniques (DLS, zetametry, 
AF4, TEM, EDS). Preliminary in vitro studies have also been done. 

Results: Prepared nanoparticles were monodisperse and stable. To en- 
capsulate fluorescent DID-Oil and lipophlic 99mTc-SSS radiotracer, nano- 
prediction method was not suitable and had to be modified. (2) The 
resulting nanoparticles were also monodisperse and stable, with a 
slightly higher diameter. Electron-dispersive spectroscopy coupled to 
TEM demonstrated the presence of sulphur in the nanoparticles, thus 
confirming the encapsulation of the radiotracer. Various peptides have 
been grafted through streptavidin and their affinity to different hepa- 
toma cell lines was tested. 

Conclusion: We have developed a family of nanoparticles based on de- 
gradable, biocompatible and functionalisable polymers, enabling the bind- 
ing of specific targeting agents (e.g. peptides) and incorporating an 
imageing agent, either for optical or scintigraphic imaging. These objects de- 
serve further investigation to gain a deeper understanding on their proper- 
ties and in vivo behaviour. Direct grafting of the most promising peptides is 
underway. Encapsulation of a therapeutic radiotracer is also planned. 

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OP10
Development of a new generation propylene cross-bridged chelator 
as versatile platform for antibody radiolabeling with Cu-64 
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP10 

Aim: Monoclonal antibodies have been widely exploited for both di- 
gragical and therapeutic purposes. For antibody radiolabeling with Cu-64, a bifunctional chelator is required which can seize the radio- 
metal quite robustly into its cavity. Thus development of cross- 
bridged chelator is in need. Unlike the other biomolecules (e.g., pep- 
tide, aptamer), antibody radiolabeling condition is tricky and needs 
sophisticated and mild labelling conditions which limits the applica- 
tion of cross-bridged chelator in immunoPET. We report a new kind of cross-bridged macromolecular chelator (PCB-TE2A-alkyne), which can be 
converted to any copper free clickable moiety or any functional 
group quite robustly into its cavity. Thus development of cross-

Methods: The propylene cross-bridged chelator was modified to differ- 
tent clickable moieties and various functional groups in a single step 
prior to radiolabeling. To confirm its applicability radiolabeled chelator 
was then conjugated to trastuzumab and biodistribution and microPET 
was performed using acetate buffer. Radiochemical purity was ana- 
lyzed by RP-HPLC and ITLC-SG. Protein binding, partition coefficient 
and stability values of 68Ga-pyoverdine in human serum and in the 
excess of competing chelator and metal were determined. In vitro 
uptake was performed using various microbial cultures. Ex vivo biodistribu- 
was studied in normal Balb/c mice. Uptake of 68Ga-pyoverdine 
by P.a. in vivo was studied in respiratory and muscle infection animal 
models using PET/CT imaging. In vivo specificity of 68Ga-pyoverdine 
for P.a. was compared with other radiopharmaceuticals. 

Results: Pyoverdin was labelled with 68Ga with high (>95%) radio- 
chemical purity. The resulting complex showed hydrophilic properties 
(log P = -3.07±0.08), low protein binding (~3% up to 120 min incuba- 
tion) and ~95% stability in human serum. In vitro uptake of 68Ga-pyo- 
verdin was highly dependent on iron load and type of microbial 
culture. In P.a. cultures high uptake under iron-deficient conditions 
was observed that could be blocked. Furthermore in all other tested micro- 
cultural conditions the uptake of 68Ga-pyoverdin was significantly lower. In normal mice 68Ga-siderophore showed rapid renal excretion and low blood values (0.09±0.01 %ID/g) even at a short time period (90 min) 

Acknowledgements
This work was supported by NRF (2016R1A2B4011546, 2013R1A4A1069507, 
2017M2C2A1014006, 2017M2A2A9A20218506, 2017R1A1B2033974, 
H17C0221, & RRF 70720161H341907667) and BK21 Plus KNU Biomedical 
Convergence Program, Korea. 

Fig. 1 (abstract OP10). See text for description
after application. PET/CT imaging in infected animals displayed specific accumulation of $^{68}$Ga-pyoverdine in infected tissues and better distribution than other, clinically used radiopharmaceuticals.

**Conclusion:** We have shown that pyoverdine can be labelled with Ga-68 with high affinity and radiochemical purity. $^{68}$Ga-pyoverdine displayed suitable in vitro characteristics and excellent pharmacokinetics. The high and specific uptake of $^{68}$Ga-pyoverdine by P.a. was confirmed both in vitro and in vivo, proving its potential for specific imaging of *Pseudomonas* infections.

**Acknowledgement**
We gratefully acknowledge the financial support of Technology Agency of the Czech Republic (Project No. TE01020028).

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**OP12**
Modifying the siderophore triacyltetrasilurin C for molecular imaging applications

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**EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP12**

**Aim:** Invasive pulmonary aspergillosis (IPA) mainly caused by *Aspergillus fumigatus* (AFU) is a major cause of mortality in immunosuppressed patients mainly due to the lack of sensitive and specific diagnostic procedures. AFU secrets the siderophore triacyltetrasilurin C (TAF) to sequester iron for acquisition and uptake via the MirB transporter. This system is essential for AFU virulence and is highly upregulated during infection. We have shown that TAFc can be radiolabeled with $^{68}$Ga thereby exhibiting excellent targeting properties in an AFU infection model [1]. Here we aimed to modify TAFc and investigate the influence of introduced substituents on preservation of AFU-targeting characteristics in *in vitro* and *in vivo* by μPET/CT imaging.

**Methods:** TAFc derivatives with various substituents (different carbon chain lengths, charges, fluorescent dye) were synthesized starting from the deacetylated forms of TAFc, characterized by HPLC and MS and radiolabeled with $^{68}$Ga. Stability, protein binding and logP values were determined. *In vitro* uptake by AFU was performed in iron-depleted and iron-replete cultures. Selected compounds with highest, lowest and comparable uptake ratio to TAFc were studied regarding their biodistribution behaviors in normal BALB/c mice as well as via μPET/CT imaging in healthy and AFU-infected Lewis rats.

**Results:** 15 different TAFc derivatives with varying substitutions were synthesized in high yields and could be labeled with $^{68}$Ga at high specific activity. Lipophilicities as expressed in logP were -0.38 to -3.60 ($^{68}$Ga-TAFc -2.1). In vitro uptake studies revealed retained recognition by the MirB transporter with reduced uptake efficiency with increasing number of substitutions (mono, di, tri). Introduction of fluorescent dye (FITC) allowed imaging of uptake and processing of TAFc analogs. Three selected compounds, $^{68}$Ga(DABuFCl), $^{68}$Ga(TPFC) and $^{68}$Ga(F3SC(suc)) displayed low protein binding and were stable in PBS and serum. Biodistribution behavior and image contrast by μPET/CT of $^{68}$Ga(DABuFCl) was comparable to $^{68}$GaTAFc whereas $^{68}$Ga(TPFC) showed higher uptake in intestine. The derivative with the lowest in vitro uptake, $^{68}$Ga(F3SC(suc)) displayed no signal in μPET/CT image of infected Lewis rats.

**Conclusion:** This study shows the possibility of TAFc modification without losing its *in vitro* and *in vivo* properties to target AFU via specific recognition of the MirB transporter. Substitution of one acetyl group of TAFc by functionalities such as fluorescent dyes opens alternative strategies for theranostics of infectious diseases.

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**OP13**
**In vitro and in vivo comparison of the novel $^{89}$Zr chelator DFO-cyclo*- with DFO**

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**EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP13**

**Aim:** The current “gold standard” chelator to label antibodies with $^{89}$Zr for immunoPET is desferrioxamine (DFO). Preclinical studies have shown that the $^{89}$Zr-DFO complex is partly unstable in vivo, resulting in release of $^{89}$Zr and subsequent accumulation in mineral bone tissue. This bone uptake may prevent the detection of bone metastases, and hamper accurate estimation of the radiation dose to the bone marrow in dose planning for radioimmunotherapy. Therefore, there is a need for a more stable $^{89}$Zr chelator. Here we report DFO-cyclo*, a preorganized extended DFO derivative introducing an octa-coordination, and investigate the stability of its $^{89}$Zr complex over the unsaturated hexacoordinated $^{89}$Zr-DFO complex in *in vitro* and *in vivo*.

**Methods:** DFO-cyclo*- was prepared by coupling of a cyclic hydroxamate group to DFO, labeled with $^{89}$Zr-DFO-cyclo*- Phe-NCS or DFO-Phe-NCS and radiolabeled with $^{89}$Zr. Stability of the labeled antibody conjugates was evaluated in human plasma and in PBS with a 1000-fold molar excess of EDTA or DFO at 37°C up to 7 days. The immunoreactive fraction, IC$_{50}$ and internalization capacity of $^{89}$Zr-DFO-cyclo*-trastuzumab or $^{89}$Zr-DFO-trastuzumab were evaluated in vitro using HER2-expressing SK-OV-3 cells. The in *vivo* distribution of $^{89}$Zr-DFO-cyclo*-trastuzumab and $^{89}$Zr-DFO-trastuzumab was investigated in mice with subcutaneous SK-OV-3 xenografts by ex vivo tissue analyses and PET/CT imaging.

**Results:** Labeling efficiencies exceeded 99% and specific activities $> 150$ MBq/mg were reached for both $^{89}$Zr-DFO-cyclo*-trastuzumab and $^{89}$Zr-DFO-trastuzumab. When challenged with an excess of EDTA or DFO at 37°C for 7 days, $^{89}$Zr-DFO-cyclo*-trastuzumab showed significantly higher stability than $^{89}$Zr-DFO-trastuzumab: 99 ± 1% vs. 61 ± 1%, and 55 ± 3% vs. 44 ± 3%, respectively. Immunoreactive fractions of 65% and 61% and IC$_{50}$ values of 2.89 nM and 2.93 nM were found for $^{89}$Zr-DFO-cyclo*-trastuzumab and $^{89}$Zr-DFO-trastuzumab, respectively. Internalization after 2 h was significantly higher for $^{89}$Zr-DFO-cyclo*-trastuzumab (26.6 ± 1%) compared to $^{89}$Zr-DFO-trastuzumab (22.4 ± 1%) ($p < 0.005$). Tumor uptake and blood clearance did not differ significantly.

**Conclusion:** $^{89}$Zr-DFO-cyclo*-trastuzumab shows improved in *vivo* and *in vivo* stability compared to $^{89}$Zr-DFO-trastuzumab. In immuno-PET, less radiation exposure to bone marrow, improved bone metastasis detection and improved radioimmunotherapy dose planning may be achieved using DFO-cyclo*.

**OP14**
**Characterization by Radio-HPLC of Cell Effluaxes and Cell Extracts of 99mTc-HMPAO Human Leukocytes**

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**EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP14**

**Aim:** The labelling of autologous WBCs is a standard clinical practice for the scintigraphic detection of infectious or inflammatory disease.
The mechanism of cell labeling is based on the lipophilic complex formed upon reconstitution, which can freely cross the cell membrane and once in the cytoplasm, is transformed into a secondary complex. However, some efflux from cell has been reported, causing errors in the quantification of the component of the complex. The study of these effluxes is important due to the effect of complex formation on the viability of the cell.

Methods: Leukocytes (×10⁶) from healthy human volunteers (n = 5) were labelled following a protocol, which was split into two aliquots of 0.5 cc in plasma and saline and incubated under stirring at 37°C, for 2h and 4h. After incubation, samples were centrifuged at 2000g and the supernatant was analyzed by HPLC in a C18 (4 mm, 3.9x150 mm) column with a gradient mobile phase of 0.05M sodium acetate/tetrhydrofuran at a flow rate of 1.5 ml/min. The percentages of primary and secondary complex were determined by their retention time (tR). ⁹⁹Tc-HMPAO-human leukocytes (n = 3) were mechanically lysed and extracted in 0.5 ml of water for injection, centrifuged at 2000g and analyzed as described.

Results: Percentages of 17.8±0.7 of free ⁹⁹mTc pertechnetate (tR = 1.5 min) and 82.1±0.7 % of secondary complex (tR = 4.5 min) were found in saline at 2 and 4 h. Results in plasma were of 21.7±2.1% for ⁹⁹mTc free pertechnetate and 78.3 ± 2.1% for secondary complex. The primary complex was detected (tR = 8 minutes) at any time of incubation. Cell extracts showed percentages of 8.1±1.1 % of free ⁹⁹mTc pertechnetate and 92.1±1.2% of secondary complex. No primary complex was detected.

Conclusion: While secondary complex was detected inside and outside the cells, primary complex was absent in extracts and effluaxes at any time of study. The percentage of 99mTc pertechnetate is probably due to oxidation during the incubation period. According to our results, the activity detected in cell effluaxes is related to the exit of secondary complex, rather than the exit of primary complex after reversibility of the original complex inside the cell.

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OP15

In vivo imaging of mGluR1 neuroreceptor kinetics in mouse brain with [¹¹C]ITDM mPET

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¹¹CITDM was successfully implemented with a synthesis efficiency of 34% from EOB (end of bombardment) including formulation, molar activity (A_m) of 122.4±2.2 GBq/µmol at EOS (end of synthesis), radiochemical purity of >99% and decay corrected radiochemical yield of 3.7±1.5% (based upon [¹¹C]CO2). V_T quantification of [¹¹C]ITDM PET imaging confirmed tracer uptake primarily in cerebellum (9.1±1.2 mL/cm³) and thalamus (8.2±2.1 mL/cm³). Furthermore, YM-202074 administration showed significant blockade and displacement of [¹¹C]ITDM in all investigated brain regions, confirming target engagement of mGluR1.

Conclusion: [¹¹C]ITDM was successfully implemented in high purity and good A_m. Our initial in vivo evaluation confirmed [¹¹C]ITDM selective binding to mGluR1 in all brain regions, having repercussions for reference region selection.

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OP16

Anesthesia affects P-glycoprotein function at the Blood-Brain Barrier: A PET study with [¹⁸F]MC225 in rats

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP16

Aim: P-glycoprotein transporters (P-gp) at the Blood-Brain barrier (BBB) are efflux pumps that play an important role in protecting the brain against harmful substances. The expression and function of these proteins is of great interest in neurodegenerative diseases and drug resistance. Its function can be measured in vivo with positron emission tomography (PET) using the novel tracer 5-(1-(2-[¹⁸F]fluoro-oxy)-3,6,7,8-tetrahydro-3H-naphthalen, [¹⁸F]MC225. Preclinical PET scans are performed under anesthesia and in most longitudinal studies the animals are repeatedly anesthetized. However, the potential effect of anesthesia on P-gp function has not been explored yet using this tracer. Therefore, the aim of this study is to assess the effect of a pre-exposure to anesthesia on P-gp function in rats with [¹⁸F]MC225.

Methods: Six rats were anesthetized with isoflurane for 90 minutes. Five other rats were not subjected to anesthesia. One week later, all rats underwent a dynamic PET scan (60 min) with arterial blood sampling. [¹⁸F]MC225 with a dose of 32±6 MBq and a molar activity higher than 29000 GBq/mmol was injected into a tail vein as a bolus of 1ml/min. After tracer injection, blood samples (0.15 ml) were collected to measure radioactivity in whole blood and plasma, besides the parent compound. PET scans were performed under anesthesia and in most longitudinal studies the animals are repeatedly anesthetized. However, the potential effect of anesthesia on P-gp function has not been explored yet using this tracer. Therefore, the aim of this study is to assess the effect of a pre-exposure to anesthesia on P-gp function in rats with [¹⁸F]MC225.

Results: Significant differences in V_T of the whole brain were observed (p<0.002) between the 2 groups. The analysis of variance (ANOVA) showed a significant decrease in V_T of the whole brain in the group with prior anesthesia. This decrease was more pronounced in the cortex and cerebellum. In the group without anesthesia, V_T did not change significantly.
Tracer concentration in whole-blood and plasma, and the rate of metabolism were not significantly different between the groups. **Conclusion:** Our results suggest that anesthesia has a prolonged effect on P-gp function at the BBB, lasting at least one week. The group with additional anesthesia displayed a significant decrease of tracer uptake in brain indicating an up-regulation of P-gp.

**OP17**

An $^{18}$F-labeled derivative of baclofen for imaging GABA$_B$ receptors in mouse brain

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP17

**Aim:** GABA (4-aminobutanoic acid) is the major inhibitory neurotransmitter in the central nervous system. GABA$_B$ receptors are G-protein coupled receptor subtypes that play an essential role in various central and peripheral disorders. Molecules that modulate GABA$_B$ receptors are of great medicinal interest for the possible treatment of many disorders and conditions including autism, alcohol dependence, anti-nociception, spasticity, fragile X syndrome, Down’s syndrome, Autism’s disease and retinal ganglion cell degeneration. A PET radiotracer for quantification of GABA$_B$ receptors is not available yet. Our goal is to develop a PET radiotracer for imaging GABA$_B$ receptors that would provide a significant advance in the understanding of autism and other GABA$_B$-related CNS disorders. It could also facilitate novel GABA$_B$ drug development.

**Methods:** New GABA$_B$ agonists, fluoropyridylmethoxy analogues of baclofen, were synthesized. The in vitro potency of these new compounds was determined commercially. The most potent compound of the series, (R)-4-amino-3-(4-chloro-3-(2-fluoropyridin-4-yl) methoxy)phenyl) butanoic acid (1), was radiolabeled with $^{18}$F. The regional brain distribution of the radiolabeled $[^{18}$F]$\text{F}$ was studied in CD-1 male mice.

**Results** Substitution of the aromatic ring of $R$-baclofen with the fluoro 4-pyridyl ether moiety resulted in an increase (10+ times) of the GABA$_B$ agonistic properties. The baclofen analog $[^{18}$F]$\text{F}$ was radiolabeled via the corresponding bromo-precursor with a radiochemical yield of 12-18%, specific radioactivity in the range of 330-515 GBq/μmol and radiochemical purity greater than 97%. In the animal experiments $[^{18}$F]$\text{F}$ entered the mouse brain (1% ID/g tissue) followed by washout. The accumulation of $[^{18}$F]$\text{F}$ in the mouse brain was inhibited (35%) by pre-injection of a selective GABA$_B$ agonist, suggesting that the radiotracer binding is partially mediated by GABA$_B$ receptors.

**Conclusion:** New GABA$_B$ agonists, fluoropyridylmethoxy analogues of R-baclofen, were synthesized and radiolabeled with $^{18}$F. The mouse experiments with the most potent compound of the series, $[^{18}$F]$\text{F}$, demonstrated the feasibility of ex vivo quantification of GABA$_B$ receptors in the animal brain; however, its specific binding was insufficient for translation to human subjects. Our future research on GABA$_B$ PET imaging will target radiotracers with improved specific binding and greater blood-brain barrier permeability.

**OP18**

Measurement of blood brain barrier transport using radiolabeled antibodies

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP18

**Aim:** Antibodies targeting transporters can function as carriers for the delivery of drugs in the brain for detecting and treating diseases of the central nervous system at the molecular level. Presently there is a need to develop efficient non-invasive method to validate the transport of these antibodies across the blood brain barrier (BBB). In this study, we investigated the potential of novel radiolabeled antibodies for measuring their engagement at the BBB. We compared different modes of delivery (Intravenous (IV), intra-arterial (IA)) to estimate their brain uptake and assess their biodistribution profiles.

**Methods:** The antibodies were conjugated to NOTA chelator for $^{64}$Cu-radiolabeling. The new $[^{64}$Cu]NOTA-antibody conjugates were tested in normal rats using both IA and IV modes of administration with or without pre-injection of unlabeled material and were compared to a control $[^{64}$Cu]NOTA-antibody.

**Results** We showed that the injection in the right carotid artery of our new $[^{64}$Cu]NOTA-antibody conjugates resulted in a brief but important radioactivity exposure in the right brain hemisphere, which persisted after exsanguination. The right to left hemisphere ratios remained constant across the different concentrations of unlabeled conjugates and exceed that obtained with the control $[^{64}$Cu]NOTA-antibody.

**Conclusion:** This study demonstrates that our new $[^{64}$Cu]NOTA-antibodies allow for a transitory and specific brain uptake. These successful results are promising for the use of bi-specific radiolabeled antibody that can engage a target within the brain.

**OP19**

Synthesis and $^{18}$F-Radiolabelling of Novel Benzoimidazotriazines for Imaging of Phosphodiesterase 2A (PDE2A)

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP19

**Aim:** Cyclic nucleotide phosphodiesterases (PDEs) are a class of intracellular enzymes that inactivate the secondary messenger molecules cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Thus, PDEs regulate the signaling cascades mediated by these cyclic nucleotides and affect fundamental cellular processes, such as proliferation, differentiation, migration, survival, and apoptosis. Accordingly, they are promising therapeutic targets. Since PDE2A was found to be related to a variety of tumors, it is our aim to synthesize novel PDE2A inhibitors based on the benzoimidazotriazine (BIT) moiety that might be a prospective lead compound for the development of an F-18 labelled ligand for PDE2A imaging with PET.

**Methods:** Based on BIT key intermediates (Fig. 1a), a small series of novel fluorinated BIT derivatives was successfully prepared (overall in 7-10 steps) and the affinities towards PDE2A and other PDE subtypes were estimated. The most promising compound, BIT1, was radiolabelled by using the corresponding nitro precursor. The reaction was optimized by choosing different solvents, amounts of precursor, modes of heating (conventional or microwave), temperatures, and reaction times. Afterwards, best conditions (Fig. 1b) were transferred to an automated synthesis module (TracerLab FX2 N, GE Healthcare). The radiotracer was isolated by semi-preparative HPLC (Reprosil-Pur AQ column, 250x10mm, 46 % ACN/aqu. 20 mM NH$_4$OAc, flow 5.5 ml/min) followed by purification with a Sep-Pak C18 Plus light cartridge and formulation in isotonic saline containing 10% ethanol.

**Results** BIT1 showed a high affinity towards PDE2A (IC$_{50}$ PDE2A3 = 3.33 nM) and selectivity over other PDE subtypes. $[^{18}$F]$\text{F}$BIT1 was successfully synthesized with a radiochemical yield of 51.9 ± 1.3 % (n = 3), molar activities between 46 – 100 GBq/μmol and radiochemical purities of ≥ 99%.
Conclusion: Radiofluorination of a novel PDE2A ligand \(^{[18F]}\)BIT1 was obtained with appropriate radiochemical yield and molar activity. First biological investigations are planned to estimate the potential of \(^{[18F]}\)BIT1 as imaging agent for PDE2A.

Acknowledgement
1. Deutsche Forschungsgemeinschaft (German Research Foundation, Project Number: SCHG 1825/3-1).
2. Scholarship Program for Research and Innovation in Science and Technology Project (RIST-PRO)-Indonesia Ministry of Research, Technology and Higher Education.

OP20

\(^{64}\)Cu-labelled anti-miRNA peptide nucleic acids as probes for molecular imaging of miRNA expression

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP20

Aim: miRNA are single stranded RNAs of 18-22 nucleotides and they have been found to be promising diagnostic and prognostic markers for several pathologies, such as tumors, neurodegenerative, cardiovascular and autoimmune disease. In the present work the development and characterization of the first anti-miRNA \(^{64}\)Cu-radiolabeled probes based on peptide nucleic acids for a potential non-invasive molecular imaging in vivo of giant cell arteritis are described.

Methods: \(\text{miR-146a and miR-146b-5p were selected as targets because they have been found up-regulated in this disease. Anti-miR-PNA and scramble-PNA probes were synthesized and linked to carboxyfluorescin.}\)

Control: The experiments confirmed that anti-miR-146a PNA can selectively bind the miRNA target and its uptake is higher in miRNA overexpressing cells \(\text{in vitro.}\)

Conclusion: We conclude that there was a strong correlation between in vivo response (doubling time) and uptake of \(^{89}\)Zr-DFO-T-DM1 (incorporated into a therapeutic dose) with \(r^2 = 0.8982\). A strong indirect correlation was established between in vivo response (doubling time) and in vitro % survival, \(r^2 = 0.9208\).
mercaptoacetic acid moiety to coordinate the radiometal through the formation of a 3+1 nitrido complex.

Methods: Labelling was performed in two steps, namely: the preparation of a Tc(V)N precursor by reduction of pertechnetate (2.5-12.5mCi) with SnCl2 (0.1mg) in the presence of succinic dihydrazide (1mg) for 20 minutes followed by incubation (1 hour, 80°C) of the nitrido precursor (1mL) with the peptide (100µg), tris(2-cyanoethyl)phosphine (0.5mg) and hydroxypropyl-gamma cyclodextrin (2mg). Radiochemical Purity (RP) of the final product was assessed by RP-HPLC using CH3CN/0.1%TFA and H2O/0.1%TFA as mobile phases. Lipophilicity was determined with n-octanol and phosphate buffer (0.1M, pH=7.4). Plasmatic protein binding (PPB) was measured by size exclusion chromatography. Stability in plasma and in labelling milieu was assessed by HPLC at 2 and 4 hours after labelling, respectively. Cysteine challenge (100 fold molar excess) was also performed. Cellular uptake, internalization and membrane binding studies were performed using 1MCF-7(Atlanta HTB-22(TM)) cells.

Results Labelling of the peptide yielded a single species with a retention time of 11.4 min and a RP>90%. The complex was stable in reaction milieu and human plasma. Challenge with cysteine revealed no ligand exchange for up to 2 hours. Physiochemical evaluation showed a log P of -0.4±0.1 and a PPB of 16±2%. A cellular uptake of 3.1±0.2 (at 4 hours) was obtained. Internalization and membrane binding studies showed that 15.2% of the activity is bound to the membrane and 84.8% is internalised.

Conclusions: A 99mTc-with agonist was obtained high RP, good stability and adequate physicochemical properties. In vitro characterization showed a promising uptake in MCF-7 cells. To establish whether it is a good diagnostic agent for breast cancer, it is necessary to deepen biological studies in tumour-bearing animals.

Acknowledgements
ANII (POS_NAC_2016_1_130455), Pedeciba-Quimica.

OP23
177Lu-DOTA-MGS5: the long-awaited theranostic probe for targeting cholecystokinin-2 receptor expression in medullary thyroid carcinoma and other tumours
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP23

Aim: The theranostic use of merringatin (MG)-based radioligands targeting cholecystokinin-2 receptor (CCK2R) expression in tumours is restricted by high kidney uptake or low enzymatic stability. Recently we have reported on the possibility to stabilise MG analogues against degradation by introducing specific amino acid modifications in the C-terminal receptor binding sequence (Trp-Met-Asp-Phe-NH2). In this study we have further refined our approach leading to a highly promising new targeting probe.

Methods: The new MG analogue DOTA-MGS5 was designed based on a combination of the substitutions applied in peptide derivatives previously developed by our group (1). The in vitro and in vivo characterisation after radiolabelling with In-111 and Lu-177 included stability analyses of the radioligands incubated in rat tissue homogenates and after injection into BALB/c mice. Receptor interaction in terms of receptor affinity and cell uptake was analysed using A431 human epidermoid carcinoma cells transfected with human CCK2R (A431-CCK2R) and mock-transfected cells (A431-mock). The targeting potential was evaluated by small animal dual modality single photon emission computed tomography (NanoSPECT/CT) as well as in vivo biodistribution studies in tumour bearing BALB/c nude mice up to 4 h after injection.

Results DOTA-MGS5 and the complexes with different natural isotopes showed a high CCK2R affinity in the low nanomolar range. Interestingly, 111In-DOTA-MGS5 showed a particularly high internalisation (>40% and >80% after 1 h and 4 h respectively) into A431-CCK2R cells. The somewhat lower resistance against enzymatic degradation observed in rat tissue homogenates did not translate into a similar effect in vivo. Even though the stability in blood of 111In-DOTA-MGS5 was comparable to other radioligands previously studied (>80% intact peptide after 10 min), the tumour uptake with values of 23.5±1.3% IA/g at 4 h p.i. was incredibly high, leading to extraordinary performance in NanoSPECT/CT. Also for 177Lu-DOTA-MGS5 a similar stability in blood (>80% after 10 min) and tumour uptake (24.5±3.1% IA/g at 4 h p.i.) was found combined with favourable tumour-to-organ ratios of >3 for stomach and >6 for kidneys.

Conclusion: The combination of substitutions from earlier investigations allowed us to further improve our concept of stabilising MG analogues in the receptor-specific C-terminal sequence. 177Lu-DOTA-MGS5 with its extremely high tumour uptake seems to be the long-awaited theranostic probe for targeting CCK2 expression in tumours.

Reference
Klinger M, Rangger C, Summer D, Foster J, Sosabowski JK, von Guggenberg E, [2017], EJNMMI 44 (Suppl2), S228

Fig. 1 (abstract OP23). See text for description

OP24
In vivo evaluation of biocompatible 99mTc-bisphosphonate-coated MNPs designed as potential theranostic agents
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP24

Aim: Magnetic nanoparticles (MNPs) have the application potentiality in innovative diagnostic and therapeutic modalities because of their multifunctionality [1,2]. MNPs coated with two hydrophilic bisphosphonate ligands, i.e., methylene diphosphonate (MDP) and 1-hidroksietan diphosphonate (HEDP) were synthetized and labeled with 99mTc. The aim was to examine if the above nanosystems, can be used as theranostic nanoagents, for hyperthermia application and nuclear diagnostic imaging.

Methods: The bisphosphonate coated MNPs were synthesized using co-precipitation method [3]. The heating ability for cancer hyperthermia therapy application was quantified through the specific power absorption (SPA) measurement. Radiolabeling of both bisphosphonate-coated Fe3O4 nanoparticles with 99mTc were carried out using SnCl2 as a reducing agent. 99mTc-MNPs were
additional used for in vitro stability studies in saline and human serum and in vivo biodistribution studies in normal Wistar rats.

Results: The obtained SPA values for Fe₃O₄-MDP and Fe₃O₄-HEDP are presented in Table 1. Radiolabeling of both bisphosphonate-coated Fe₃O₄ nanoparticles with ⁹⁹mTc was performed at high yields without purification (>95%). Incubation of both radiolabeled preparations in saline and serum showed that the bisphosphonate-iron oxide bonding was very stable with only 10 and 15% of ⁹⁹mTc detaching from the iron oxide after 24 h, respectively. The highest uptake was observed at 1 h p.i. in the liver followed by the spleen (Fig. 1).

Conclusion: The obtained results of specific power absorption demonstrate potential therapeutic applications of Fe₃O₄-MDP and Fe₃O₄-HEDP. Such radiolabeled biocompatible bisphosphonate MNPs with high labeling yield (>95%), in vitro and in vivo stability, showed the great promise as theranostic nanoagent which combines magnetic hyperthermia and SPECT imaging.

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Table 1 (abstract OP24). SPA values of the aqueous dispersion of MNPs in the applied magnetic field, 23.9 kA/m

| MNPs     | SPA (W/g) |
|----------|-----------|
| Fe₃O₄-MDP | 252 kHz   |
|          | 397 kHz   |
|          | 577 kHz   |
| Fe₃O₄-HEDP | 67.3      |
|          | 143       |
|          | 183       |
|          | 55        |
|          | 78.5      |
|          | 131       |

Fig. 1 (abstract OP24). Biodistribution results of (a) ⁹⁹mTc-Fe₃O₄-MDP and (b) ⁹⁹mTc-Fe₃O₄-HEDP MNPs

OP25
In vitro therapeutic efficacy of ⁶⁷Ga-trastuzumab
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP25

Aim: Despite its desirable half-life and high-energy Auger electrons, ⁶⁷Ga therapy has been neglected due to lack of suitable chelators and targeting molecules. With the advent of ⁶⁷Ga-PET, excellent new chelators allow us to re-evaluate ⁶⁷Ga for therapy. Previously, we showed that ⁶⁷Ga causes DNA damage in cell-free systems and could kill in non-targeted cell studies. Here, we expand this work by targeting ⁶⁷Ga to breast cancer cells using trastuzumab and comparing toxicity to well-described Auger emitter, ¹¹¹In.

Methods: Trastuzumab, dialysed against 50 mM EDTA, was washed and recovered in metal-free HEPES buffer, was conjugated with tripodal tris(hydroxymethyl)aminomethane (THP) (3.23 mg trastuzumab, 0.8 mM H₃THP-Ph-SCN; 260 μL) and purified by size exclusion chromatography (SEC). DOTA-trastuzumab was also prepared similarly. DOTA-trastuzumab was labelled with ¹¹¹In chloride at 40°C, pH 5.5 for two hours whereas THP-trastuzumab was labelling with ⁶⁷Ga chloride at room temperature, pH 6.5, for 15 minutes. SEC purification was performed when labelling efficiencies were ~95%. Radiopharmaceuticals (0.19 MBq/μg) were tested for their internalisation and effects on viability (dye exclusion) and clonogenicity of HER-2-positive (HCC1954) and -negative (MDA-MB-231) cell lines. Microautoradiography of cells in 18% gelatine was also performed.

Results: Labelling efficiencies for ⁶⁷Ga-THP-trastuzumab and ¹¹¹In-DOTA-trastuzumab were 90% and 98%, respectively, giving 0.26±0.08 and 0.61±0.11 MBq/μg. At 4nM, ⁶⁷Ga-THP-trastuzumab showed significantly higher cell binding uptake (10.69±1.32%) than ¹¹¹In-DOTA-trastuzumab (6.15±1.64%; p=0.01) although the proportions internalised were equal; 62.08±1.43% and 60.78±15.45%, respectively. At 100nM however, cell binding percentages were equal: 1.15±0.98% ⁶⁷Ga-THP-trastuzumab and 0.83±0.85% ¹¹¹In-DOTA-trastuzumab. Controls ⁶⁷Ga-THP-Ac and ¹¹¹In-DOTA did not bind HCC1954 cells and binding of ⁶⁷Ga-THP-trastuzumab and ¹¹¹In-DOTA-trastuzumab in MDA-MB-231 cells was minimal (<0.2%). Microautoradiography showed that radioactivity bound to individual cells within the population varied considerably (from <10 to >90 silver grains per cell). Viability and clonogenicity decreased with increasing radiolabelled trastuzumab concentration, bound per cell. Radiopharmaceutical treatment of MDA-MB-231 cells or non-internalised activity in HCC1954 cells did not affect cell viability or clonogenicity. In HCC1954 cells, the surviving fraction after treatment at approximately 0.1 Bq/cell ⁶⁷Ga-THP-trastuzumab, reduced to 0.38±0.13, more than for ¹¹¹In-DOTA-trastuzumab (0.55 ± 0.16; p=0.03).

Conclusion: ⁶⁷Ga-THP-trastuzumab and ¹¹¹In-DOTA-trastuzumab both bind specifically HER2-positive cells and reduce their viability and clonogenicity. This shows ⁶⁷Ga holds promise as a therapeutic radionuclide as a targeted radiopharmaceutical however non-homogeneous uptake amongst cells needs further investigation.

Acknowledgments
The project was in part supported by the Academy of Medical Sciences and Malaysian Ministry of Education.

OP26
⁹⁹Y-labeled phosphate-coated magnetic nanoparticles designed for possible medical applications
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP26

Aim: Magnetic nanoparticles (MNPs) have been intensively used for a wide variety of biomedical applications. Only few reports on the in vivo biodistribution of functionalized MNPs with potential uses in magnetic hyperthermia (MHT) therapy are available [1, 2]. Radiolabeled MNPs coated with hydrophilic phosphate ligands, i.e., imidophosphate (IDP) and inositol hexaphosphate (IHP), were developed as multifunctional agents to localize both radioactivity and magnetic energy at a tumor site.

Methods: MNPs were synthesized by co-precipitation of ferric and ferrous salts in a basic solution in magnetic hyperthermia (MHT) therapy are available [1, 2]. Radiolabeled MNPs coated with hydrophilic phosphate ligands, i.e., imidophosphate (IDP) and inositol hexaphosphate (IHP), were developed as multifunctional agents to localize both radioactivity and magnetic energy at a tumor site.

Results: MNPs were labeled with 37 MBq ⁹⁹YCl₃ at room temperature for 1 h and were used in in vitro stability studies in saline and human serum and in vivo biodistribution studies in normal Wistar rats.

Methods: MNPs were synthesized by co-precipitation of ferric and ferrous salts in a basic solution [3, 4]. The heating ability of MNPs was quantified through the specific power absorption (SPA) measurements. The MNPs were labeled with 37 MBq ⁹⁹YCl₃ at room temperature for 1 h and were used in in vitro stability studies in saline and human serum and in vivo biodistribution studies in normal Wistar rats.

Results: The SPA values obtained for synthesized MNPs (46–81 W g⁻¹) in different physiological media indicated their possible application in hyperthermia treatment (Fig. 1). Both types of coated MNPs were ⁹⁹Y-labeled in a reproducible high yield (>98%) and exhibited high in vitro stability in saline and human serum. The results of
biodistribution, showed high uptake in the liver and spleen as well as in vivo stability up to 72 h. (Fig. 2).

Conclusion: $^{90}$Y-coated MNPs were radiolabeled for two purposes: to use the radiotracer to obtain an accurate biodistribution profile of the MNPs and to produce potential radiotherapeutical agents. Both $^{90}$Y-labeled phosphate-coated MNPs exhibited favorable properties that justify further investigations toward their potential use in combined radiotherapy–hyperthermia cancer treatment.

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OP27
Automation of FDG QC on Tracer-QC system
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Aim: Quality Control (QC) of PET tracers is the most labor-intensive part of clinical tracer production. Tracer-QC system is being used in the US for complete automation of QC for FDG production meeting the requirements set by United States Pharmacopeia. Here we present the technology developed to address European Pharmacopeia requirements for radiochemical and chemical purity.

Methods: A QC automation system has been developed that consists of Tracer-QC device integrated with an HPLC. The HPLC includes a quaternary pump, UV-Vis detector and a radioactivity detector. Notably, no electrochemical detector is included. An innovative coupling was developed that provides for automated HPLC injection of the radioactive sample by a pipette from the deck of Tracer-QC robot. The above described system is enabled by a consumable cartridge that incorporates all reagents needed for analysis of color, clarity, residual kryptofix, ethanol, acetoniitrile, bacterial endotoxin, radioactivity concentration, radiochemical purity (including $^{18}$F-fluoride and $^{18}$F-FDM), half-life, residual concentration of CI-DG. Pre-column derivatization of sugars with PMP reagent was utilized to quantify CI-DG content. Robotic component of Tracer-QC system allows for precise and fast mixing of the liquids.

Results: The system presented here allowed for quantification of all impurities commonly analyzed in the production of FDG in Europe, while keeping the analysis time under 45 minutes. The derivatization and separation method was optimized to separate derivatized CI-DG, Glucose, FDM and FDG with resolution exceeding Pharmacopeia requirements (Fig. 1). Limit of quantification of CI-DG was determined to be 15 ppm, Limit for detection for $^{18}$F-FDG is ~1 ppm. $^{18}$F-FDG was fully converted to $^{18}$F-FDG – PMP derivative which was detected on the radiation detector. TLC analysis confirmed that $^{18}$F-FDG – PMP is the only radioactive product of the derivatization, with no competing decomposition.

Conclusion: These developments enabled an automated QC platform that contains all hardware needed for analysis of FDG as well as other $^{18}$F-based tracers. On this platform all analyses and sample manipulations are performed automatically, providing for maximum walk-away time – the user sets up a consumable cartridge and adds one sample. Then they only need to come back to collect the complete report. It is expected that such systems will have a dramatic impact in both routine production and development of new PET tracers.

Disclaimer
Reported Research was supported by the National Cancer Institute of the National Institutes of Health under Award Number R44CA192499. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Fig. 1 (abstract OP27). HPLC analyses of the mixture of sugars commonly analyzed in FDG quality control

OP28
Simultaneous determination of the potentially toxic chemical impurities in the radiopharmaceuticals by capillary electrophoresis
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP28

Aim: The implementation of recently introduced metal-mediated “late-stage” approaches for nucleophilic n.c.a. $^{18}$F-labelling of non-activated aromatics into routine production of the radiotracers is currently on-going. These new strategies employed or copper (II) catalysts that combined with stanny- or Ni-based precursors.

Fig. 1 (abstract OP27). HPLC analyses of the mixture of sugars commonly analyzed in FDG quality control
Moreover, classical kryptofix is often replaced by tetraethylammonium hydrogen carbonate (TEAHC)\textsuperscript{2,3}. Therefore there is an urgent need in the development of simple analytical methods to control the removal of these toxic impurities from the final preparations until the allowable limits. Here we suggest the application of capillary electrophoresis (CE) for simultaneous determination of Cu, Ni, Sn and TEAHC in the analytical sample.

**Methods:** CE analysis was performed using «Capel-105M» system that equipped with UV spectrophotometric detector. The sample was loaded via electrokinetic injection (10 s, 25 kV) under following conditions: column 60 cm × 75 μm; background electrolyte: benzimidazole 20 mmol/L + acetic acid 40 mmol/L; UV 230 nm; separation voltage 25 kV, 20 °C. Stock solutions of Ni\textsuperscript{2+}, TEAHC, Cu\textsuperscript{2+} and Me\textsubscript{3}SnCl in normal saline at a concentration of 10, 1000, 100 and 200 ppm respectively were prepared. Calibration standards were than prepared by serial dilution of the stock solutions. Before analysis the samples were fourfold diluted by deionized water. Under these conditions the retention times (RT) for Ni\textsuperscript{2+}, TEAHC, Cu\textsuperscript{2+} and Me\textsubscript{3}SnCl were 4.4, 5.0, 5.6 and 7.8 min. All the peaks were well distinguished from that of sodium (3.9 min).

**Results** The conditions for simultaneous determination of toxic impurities in the buffer solution by CE technique CE analytical procedure have been developed. Our data (Fig. 1) show that the suggested method had a high sensitivity with detection limit for impurities according to ICH Guideline of Elemental Impurities Q3D.

**Conclusion:** New analytical CE method was proposed for routine quality control of the radiotracers, synthesized via metal-mediated reactions. The method is fast, simple, reliable and can be easily adapted to any CE equipment. This research was supported by RFBR grant No 16-54-12062/16.

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![Fig. 1 (abstract OP28). See text for description](Image)

**OP29**

**Evaluation of factors influencing the Ga-68 yield and Ge-68 breakthrough of a SnO\textsubscript{2} based Gallium-68 generator**

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP29

**Aim:** Breakthrough of Ge-68, and the yield of Ga-68 are important aspects of Ge-68/Ga-68 generator function. At the Western Cape Academic PET/CT Centre, SnO\textsubscript{2}-based Ga-68 generators produced by iTemba LABS are used. These Ga-68 generators are eluted with 0.6 M or 1 M HCl and Ge-68 breakthrough has been observed. The aim of this study was to evaluate the influence of the age of the generator, the number of elutions performed, and the interval between elutions on Ga-68 yield and Ge-68 breakthrough in order to optimise use of Ga-68.

**Methods:** Elution records of 7 generators, used between January 2013 and July 2017 were reviewed. The first 5 generators were eluted with 1 M HCl, and the last 2 generators with 0.6 M HCl. Ge-68 breakthrough was measured after decay of eluted Ga-68. The age of the generator and the time interval between elutions were calculated and plotted against breakthrough and yield.

**Results** The number of elutions per generator ranged between 28 and 151, and the generators were used for periods ranging from 166 to 372 days. Yield within the first month were between 119% and 133% of the nominal Ge-68 activity on the column for generators eluted with 1 M HCl, and 107% to 110% for those eluted with 0.6 M HCl. By 160 days the yields had decreased to 106% to 110% with 1 M HCl elutions and 80% to 99% with 0.6 M HCl eluant. Three generators, including those eluted with 0.6 M, showed Ge-68 breakthrough within the first 10 days of use. By 160 days age, the Ge-68 breakthrough varied between 0.02% and 1.0%, with the lower acidity eluant giving the second highest value. There were no correlation between interval between elutions and Ge-68 breakthrough and Ga-68 yield.

**Conclusion:** Due to irregular intervals between elutions, it is difficult to compare elution yield at various ages of generators. All generators however clearly functioned poorer with increased use. The increasing Ge-68 breakthrough, and especially breakthrough early in the lifespan of the 2016 and 2017 generators is worrying, as it creates long-living radioactive waste. Elution with a less acidic eluant seems to provide a slightly lower yield but also lower Ge-68 breakthrough.

**OP30**

**ITLC Method for Analysis of 68Ga Radiopharmaceuticals**

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**Aim:** Development of 68Ga radiopharmaceuticals (RPs) requires a lot of tests to be made during labeling conditions optimization, stability evaluation, etc. It is critically important not only to evaluate radiochemical purity (RCP) value, but to know exact value of every radiochemical impurity content. There’s no doubt that TLC analysis is the most convenient method for the determination of all the radiochemical species content, given that the TLC system is adequate. Previously new method for determination of RCP of 68Ga-RPs was developed \[1\]. The method is handy, fast and informative with a good correlation with pharmacopeial and other frequently used methods. The method allows to determine 68Ga-colloid, unbounded 68Ga and labeled molecule content with a single strip. The method can be routinely used with 68Ga-PSMA-617, 68Ga-DOTA/NODAGA-RGD/TOC/-TATE, etc. However the method has a drawback: sometimes adequate chromatographic separation is not available with acyclic chelators (DATA/HBED-CC/DTPA, etc.). Also molecular weight has an impact on \( R_f \) of the complexes (main peak shift can be about \(+0.3\) \( R_f \)). The aim of this study is to develop a new method allowing to overcome these drawbacks.

**Methods:** 68Ge/68Ga generator (Cyclotron Ltd, Obninsk, Russian Federation) was used. All chemicals and solvents were of high-purity or pharmaceutical grade and were purchased from Sigma-Aldrich or Panreac. Radiopharmaceutical precursors (PSMA-617, DOTA/ NODAGA-RGD/TOC/-TATE, etc.) were purchased from ABX. iTLC-SG strips of different manufacture periods were used (Pall/Varian/Thermo). PET-MiniGita (Raytest) radio-TLC scanner was used for analysis of radioactivity distribution.

**Results** The decision was made to develop a method, which allows to obtain retention factors as follows: \( R_f=0.0-0.1 \) for 68Ga-colloid, \( R_f=0.5-0.6 \) for unbounded 68Ga and for labeled molecules \( R_f=0.9-1.0 \). This separation pattern was obtained for labeled compounds listed above using mixtures with \( w:o=1:1 \) or \( 1:3 \) (w – water, saline; o – acetonitrile/ethanol/methanol). The presence of small amount of acid (TFA/HCl/HNO\textsubscript{3}, etc.) is essential. This amount should be kept very precise to provide pH 1.6±0.4. The most informative peak separation can be obtained with saline-acetonitrile mixture (1:1:1) containing 0.06-0.08% TFA. Experiment details and critical features will be presented. This system is applicable for the most of 68Ga-RPs under
study. It was found that chromatographic pattern is not the same when using iTLC-SG strips of different manufacture periods. This fact should be carefully taken into account.

**Conclusion:** New highly effective TLC method for $^{68}$Ga labeled compounds was developed. The method is applicable during pharmaceutical development and routine clinical practice.

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**OP31**
Optimization of $^{18}$F-FPSMA1007 Synthesis HPLC free on Fastlab Platform
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**Aim:** Over the past years, many different PET agents have been developed to investigating on Prostate Cancer (PC) to make the non invasive approach a reality, in order to replace the biopsy and the related complications. The PC is the more common cancer that affect the male population. Due to the high incidence of this pathology are mandatory to investigate on a fluorine-18 tracer that give the possibility to overcome the gallium-68 tracers limitations.[1] The aim of this study is to optimize an automatic synthesis for $^{18}$F-FPSMA1007 on Ge FASTLab® module, in a stable high yield with a wide range of inlet activity, and setting up a faster, efficient and EU Pharmacopoeia compliant quality control to shorten the time of product realize. [2]

**Methods:** The synthesis method is based on one step synthesis using a new precursor commercialized by ABX and is tuned on Ge FASTLab® synthesizer. All the reagents are included on a single use casette. The $^{18}$FFluorine was trapped on QMA and eluted with a mixture of TBAHCO₃/ACN or K₂22/ACN/K₂CO₃ and after drying at 105°C on synthesis reactor, the ABX precursor dissolved in DMSO was added to proceed with the nucleophilic $^{18}$F-Fluorination. The reaction mixture was heated up at 95°C for 10 min after the reaction step the mixture was cooled at 35°C to start the purification step followed by formulation. The final process takes place on 37 minutes. HPLC analysis was performed on an Agilent 1260 Infinity HPLC equipped with an Agilent 1260 UV detector and a Raytest gamma-ray detector, controlled with Gina Star software V5.9.

**Results**
Two different elution solution was used to compare the final process yields, at the same time, high activity runs were performed, in different inlet activity range, to evaluating the yield and product stability in final formulation. For stability study a range of 1-2.5 GBq/ml radioactive concentration was evaluated at room and at 40°C for up to 12h. According to final product formula specification the synthesis yield was stable on range 30-50 % at the inlet activity range (55-170 GBq) with a very high $A_s$ (800-3500 GBq/2mol) at EOS. The radiochemical purity for all the runs was > 96 % and chemical impurities were < 0.1 mg/V. The new HPLC/TLC method allow to make the quality control in terms of general indications of European Pharmacopoeia Monographs.

**Conclusion:** All the synthesis performed by using the K222 elution solution shows slightly lower yield compared to TBA, at the same time any difference, in quality control profile and stability were found, all the products collected were stable and in high chemical/ radiochemical purity. The HPLC and TLC methods setup allow to perform a complete purity evaluation on less than 15 min.

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**OP32**
Synthesis and evaluation of radioiodinated and astatinated prosthetic groups for bioorthogonal conjugation to antibodies for nuclear imaging and therapy
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP32

**Aim:** Radioiodine and astatine are increasingly studied for therapeutic or diagnostic purposes in nuclear medicine [1]. Particularly, $^{211}$At ($t_{1/2} = 7.2$ h, α-emitter) is a promising radioisotope for targeted α-particle therapy. To associate these radiohalogens to cancer targeting biomolecules (peptides, antibodies), the conventional approach consists in performing the radiohalogenation of a precursor bearing a N-Hydroxysuccinimidyl (NHS) ester function for conjugation to the biomolecule [2]. However this approach requires basic aqueous conditions leading to competitive hydrolysis of the NHS ester, resulting in sub-optimal conjugation yields (<50%). To overcome this issue, we have initiated the investigation of click chemistry based bioorthogonal conjugation approaches aiming at increasing the coupling yields and improving the radiolabelling procedure.

**Methods:** A series of bifunctional precursors bearing clickable functionalities (tetrazine, azide or alkyne) were specifically designed for radiolabelling with radioiodine and $^{211}$At. Bioorthogonal ligation kinetics of the labelled prosthetic groups were evaluated on model peptides bearing the complementary clickable functions (trans-cyclooctyne (TCO), bicyclononyne (BCN), alkyne) for identification of the best system, and transferred to antibody radiolabelling.

**Results**
Quantitative yields from less than 1 minute to 9 hours with the following reactivity order: [tetrazine-TCO] > [copper(I) catalyzed alkyne-azide] >> [tetrazine-BCN] > [azide-BCN], as expected from literature data.[3] The fastest system [tetrazine-TCO] was then tested on an anti-CD138 mAb, providing quantitative radiolabelling yields (>99%) without need of purification within less than a minute, confirming the high efficacy of this approach. Immunoreactivity against CD138 was preserved (80 ± 2%).

**Conclusion:** The results obtained highlight the higher efficiency of click chemistry in comparison with the conventional approach. They open perspectives for quantitative radiolabelling with reduced conjugation time that may facilitate the transfer of $^{211}$At labelled antibodies to clinical applications. Assessment of the in vivo behaviour of such radiolabelled biomolecules is now in progress to fully validate this promising approach.
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OP33
Relative biological effectiveness (RBE) of 177lutetium-NOTA-pantinumab F(ab\’)2 fragments for radioimmunotherapy of pancreatic cancer cell lines
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP33

Aim: The 5-year survival for PnCa patients is only 6% due to late-stage diagnosis and inadequate treatment options. There is an urgent need for new treatment strategies. Human epidural growth factor receptor (EGFR) is overexpressed on up to 90% of PnCa tumours. Pantinumab is an antibody against EGFR that can be modified with a NOTA chelator for radiolabelling with 177Lu to treat PnCa cells as a radioimmunotherapy agent (177Lu-RIT). This study aims to determine the RBE of 177Lu-RIT compared to γ-radiation and examine EGFR positivity as a predictor of PnCa response to 177Lu-RIT.

Methods: Flow cytometry: EGFR density was measured on AsPC-1, PANC-1, MiaPACA-2, and Capan-1 cell lines. Cells were incubated with panitumumab for 60 min, then with Alexa Fluor 647 anti-human IgG immunoconjugates for 30 min at 4°C, then run on a flow cytometer. Clonogenic survival assays: Cells were treated with γ-radiation (0-8Gy) or 177Lu-RIT (0-3MBq/72nM) then seeded into 6-well plates and allowed to grow at 37°C, 5% CO2, for 14 days. Then colonies were fixed/stained and counted. Subcellular fractionation: Cells were treated with 177Lu-RIT (1MBq/72nM) for 1, 3, 4, and 24 hours. Radioactivity associated with cell surface, cytoplasm, and nucleus was separated then measured in a γ-counter.

Results: EGFR density was ++++, +++, +, and +, for AsPC-1, PANC-1, MiaPACA-2, and Capan-1 cells, respectively. Clonogenic survival after γ-radiation was reduced to 10% (D10) for AsPC-1, PANC-1, MiaPACA-2, and Capan-1 cells at 3.2 Gy, 5.0 Gy, 4.0 Gy, and 2.5 Gy. 177Lu-RIT caused D10 at 3.1MBq, 1.1MBq, 6.0MBq, and 1MBq for AsPC-1, PANC-1, MiaPACA-2, and Capan-1 cells, respectively. Subcellular fractionation studies showed that PANC-1 had the highest nuclear localization of 177Lu-RIT and results from these studies allowed microdosimetry to be performed to convert MBq to Gy. The D10 after 177Lu-RIT was 16.9 Gy, 5.7 Gy, 23.3 Gy, and 8.2 Gy for AsPC-1, PANC-1, MiaPACA-2, and Capan-1, respectively, resulting in RBE of 0.2, 1.0, 0.2, and 0.3.

Conclusion: RBE of 177Lu-RIT is less than γ-radiation and varies significantly between PnCa cell lines with no correlation to EGFR density. EGFR positivity is not an adequate predictor of response to RIT with 177Lu-RIT.

OP34
Biological Assessment of a Radiolabelled LXXLL-Peptide for Breast Cancer Theranostics
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):OP34

Aim: Breast cancer (BC) remains the most common invasive cancer diagnosed among women and the second most frequent cause of cancer-related death in women worldwide. The majority of BC cases are hormone-responsive and approximately 75% express the estrogen receptor (ER), a well-established biomarker for prognosis and guiding treatment of patients. ER has also been used as a target for BC imaging. In spite of the improved BC survival rate due to endocrine therapies that modulate ER action, resistance to treatment is a major clinical concern. Thus, new molecular imaging agents and more effective therapies are needed to improve BC management. Theranostics is particularly suitable for that purpose as the ER status profiling acquired by imaging can be used to target treatment. Peptides containing the LXXLL sequence have demonstrated high ER affinity and could be of potential value to develop targeted radiotherapeutics. Thus, our goal was to assess the potential value of a radiolabelled LXXLL peptide as theranostic agent for BC. For that purpose, we have selected from the literature a peptide with recognized ER affinity and have radiolabelled it with two different radionuclides, 111In and 129I.

Methods: A LXXLL-peptide was synthesized and conjugated to the bifunctional chelator DOTA by microwave-assisted solid phase synthesis. The peptide conjugate was radiolabelled with 111In. 129I-labeling was performed by direct radiiodination in the histidyl residue using the oxidative method of chloramine-T. ER binding affinities were evaluated by a fluorescent polarization assay with the corresponding inactive In(III) complex or iodinated peptide. Cellular uptake was assessed in MCF-7 (ER+) and MDA-MB-231 (ER-) human breast cancer cells. Biodistribution was assessed in tumor-bearing Balb/c mice injected with MCF-7 cells and microSPECT imaging studies are underway.

Results: The LXXLL peptide was successfully radiolabelled with both radionuclides with high radiochemical yield and purity at high specific activity. Chemical identity was ascertained by comparing its HPLC profile with that of the inactive congeners. Inactive LXXLL-peptide derivatives retained ER binding affinity. Both radiolabelled peptides have showed rapid and high uptake in MCF-7 cells (ER+). Biodistribution studies in tumor-bearing mice indicated high in vivo stability, fast blood clearance and high uptake in ER rich organs and MCF-7 xenografts.

Conclusion: The favourable biological performance of radiolabelled LXXLL peptides in cellular and tumor-bearing mice models suggests their potential as theranostic agents.
PP01
Optimization of Biological Quality Control of Radiochemical Precursors used for Radiopharmaceutical Formulations – A step towards Good Radiopharmacy Practice
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Aim: The present study was designed to develop and validate methods for biological quality control (bacterial endotoxin and sterility testing) for various radiochemical precursors (RCPs) viz. 68GaCl3, 177LuCl3, H15F, Na99mTcO4, Na188ReO4 and CH3COO90Y. These RCPs are considered to be active pharmaceutical ingredient (API) for various radiopharmaceutical formulations. The validation of biological quality control procedures for RCPs is a mandatory requirement for small scale radiopharmaceutical preparations (SSRP) in order to maintain good radiopharmacy practice (GRPP).

Methods: 68GaCl3, 177LuCl3, H15F, Na99mTcO4, Na188ReO4 and CH3COO90Y were used as precursors. The endotoxin limit (EL) for RCPs was fixed at 5EU/mL, based on literature describes interactions of this material with [18F]Fluoride material for microfluidic devices for radiopharmaceutical synthesis. Literature describes interactions of this material with [18F]Fluoride material for microfluidic devices for radiopharmaceutical synthesis.

Results: In BET assay, 177LuCl3 and CH3COO90Y exhibited inhibition due to extreme pH conditions. Inhibition could be resolved by neutralizing with endotoxin negative (<0.25EU/mL) 0.25N Tris-base at first dilution. 68GaCl3 exhibited enhancement due to presence of excess Na+ and inhibition because of extreme pH. The enhancement in BET test was resolved by using endotoxin negative PBS as diluting agent instead of LRW and inhibition was taken care of by neutralization with 0.25N Tris-base. BET assay of Na99mTcO4, Na188ReO4 and H15F did not demonstrate any inhibition. In sterility testing, luxuriant growth was observed for positive control culture in the presence of 1-2 mL (50-100 mCi) of Na99mTcO4, Na188ReO4 and H15F following incubation for 48 hours. Growth of all these microorganisms was inhibited (<100 CFU) in 0.5-1.0 mL 68GaCl3, 177LuCl3 and CH3COO90Y. However, inhibition was reversed on neutralization with sterile Tris-buffer (0.2/0.4/2N). This was also confirmed by counting CFU by spread plate method. No growth was seen at bacterial concentration <100 CFU (serial dilution- spread plate method). No growth was seen for biological quality control (bacterial endotoxin and sterility testing) for various radiochemical precursors (RCPs) viz. 68GaCl3, 177LuCl3, H15F, Na99mTcO4, Na188ReO4 and CH3COO90Y. These RCPs are considered to be active pharmaceutical ingredient (API) for various radiopharmaceutical formulations. The validation of biological quality control procedures for RCPs is a mandatory requirement for small scale radiopharmaceutical preparations (SSRP) in order to maintain good radiopharmacy practice (GRPP).

Post Data: 236 n/cm 2/s respectively during the irradiation. The HPGe spectrum showed a broad peak at 1.34 MeV corresponding to 68Ga along with other peaks. The quantity of 64Cu produced was estimated to be 29 μCi at the EOB.

Conclusion: The indigenous developed solid target assembly was found to be compatible with GE-PET trace 800 cyclotron, having only liquid and gaseous target cavities. The target was successfully tested for irradiation at 2mA current for 30 m time duration with natural Ni target. This indigenous target can be used for production of higher amount of 64Cu while using enriched Ni with high current.

PP03
Microfluidic reactor in a PDMS chip for [18F]-radio pharmaceuticals
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Aim: Polydimethylsiloxane (PDMS) is a cheap and easily available material for microfluidic devices for radiopharmaceutical synthesis. Literature describes interactions of this material with [18F]Fluoride material for microfluidic devices for radiopharmaceutical synthesis. Literature describes interactions of this material with [18F]Fluoride material for microfluidic devices for radiopharmaceutical synthesis.

Objectives: To investigate the interaction of the [18F]Fluoride material with the PDMS material by synthesizing [18F]Fluoride using a microfluidic chip fabricated from PDMS.

Methods: Ten 30 μl reactor chambers of PDMS microchips for PET radiopharmaceuticals were tested to evaluate possible adsorption phenomena. No cover films were used, so the [18F]Fluoride interacted directly with the PDMS. Activities from 8 MBq to 2500 MBq of [18F]Fluoride were assayed. First, [18F]Fluoride was preconcentrated in a QMA resin and eluted with K2CO3 6 mg/mL. The radioactive solutions (K2CO3 aqueous solution/acetonitrile 1:1) were pipetted manually into the reactors, heated until complete evaporation (100-140°C) with vacuum (700 mbar, Sykam S1021 pump) to mimic azetroptic distillation and nucleophilic substitution. An average temperature of 130°C was maintained during 10 minutes, measured by a coupled termocouple. [18F]Fluoride was then eluted with water for injection and the reactor chambers were dried with compressed air. Radioactivity was measured after filling, evaporation and water elution in a Cameca IBC Dose Calibrator.

Results: In our experience, [18F]Fluoride was practically not adsorbed to PDMS when this material is under heating and vacuum conditions commonly used in [18F]Fluoride preconcentration and
nucleophilic substitution. On the contrary, [18F]Fluoride was retained and almost completely eluted with water for injection, so we can suggest this cheap and easily available material for lab-on-chip Radiopharmacy.

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Table 1 (abstract PP03). See text for description

| PDMS Reactor | Loading [18F]- Activity (MBq) | Activity After evaporation (MBq) | Elution Water for injection (MBq) | Residual activity at reactor after elution (MBq) |
|--------------|-------------------------------|---------------------------------|----------------------------------|-----------------------------------------------|
| 1            | 8.62                          | 7.88                            | 6.96                             | 0.10                                          |
| 2            | 12.58                         | 11.08                           | 10.20                            | 0.20                                          |
| 3            | 28.12                         | 26.27                           | 22.60                            | 1.03                                          |
| 4            | 51.80                         | 48.84                           | 40.70                            | 1.70                                          |
| 5            | 66.60                         | 59.29                           | 50.90                            | 1.80                                          |
| 6            | 240.05                        | 217.6                           | 206.88                           | 0.74                                          |
| 7            | 802.10                        | 726.90                          | 672.66                           | 18.07                                         |
| 8            | 925.30                        | 804.22                          | 710.45                           | 7.05                                          |
| 9            | 1257.01                       | 1122.7                          | 955.34                           | 7.03                                          |
| 10           | 2544.80                       | 2372.1                          | 2152.03                          | 7.40                                          |

PP04
Development and comparison of dual generator elution methods on a MultiSyn Synthesizer (iPHASE technologies Pty Ltd) - application to the synthesis of 68Ga-peptides
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):1

Aim: Increasing patient demand for 68Ga-peptides in our facility has created the need for higher number of doses per batch produced. We have investigated several options for dual generator elution for the synthesis of 68Ga-DOTATATE on an iPHASE MultiSyn Synthesizer, using two ITG 68Ge/68Ga generators.

Methods: The efficiency of three double elution methods of ITG generators using HCl 0.05M were compared:

(i) Fractionated elution: The elution profile of one ITG generator was first determined. The most concentrated fractions from two generators were then collected and measured. (ii) “In series” elution: Two generators were connected in series and the elution profile determined. The optimum fraction and rate of elution were established.

(ii) Pre-concentration (1), the individual eluates from both generators were then collected and measured. (ii) was first determined. The most concentrated fractions from two elutions were established.

(iii) TLC of the crude reaction mixture was performed before purification. After synthesis, activity of all the synthesis components were measured and decay corrected to EOS.

Results: The % recovery for the three methods was determined as follows: double elution recovered activity/sum of activity recovered from 4 mL single elution of each generator (1-3 days prior) x 100. (i) Fractionated elution: 87% of 68Ga was recovered in 4.5 min from the collection of the 2-5 mL fractions from both generators. (ii) “In series” elution: the 2-8mL fraction was collected in 6.5 min, yielding 80% of recovered activity. (iii) The pre-concentration/elution method took 5.5 min and recovered 91% of the activity. 68Ga-DOTATATE synthesis: Using method (i) the precursor (25μg) in 1.5 mL 0.25M Na-acetate buffer was reacted with the 6 mL eluate (pH reaction 3.5-4) for 7 min at 90-95°C. The RCP of 68Ga-DOTATATE before purification was 98.4% (TLC). 68Ga-DOTATATE was obtained in 83% yield (n.d.c.), 13% activity was found in the waste and was mostly accounted for the discarded fractions. Using method (iii), the precursor in 1 mL buffer + 3 mL Trace Select water was reacted with 0.8 mL eluate (pH reaction 3.5-4). Before purification, the RCP of 68Ga-DOTATATE was 95.6% (TLC). 68Ga-DOTATATE was obtained in 84.5% yield (n.d.c.), 4.2% activity remained on the Strata X and 6% in the waste.

Conclusion: 68Ga-DOTATATE was successfully synthesized in excellent yields on a MultiSyn Synthesizer starting from the fractionated and pre-purification double elution methods of two ITG generators.

PP05
Radiolabelling of DTPA-silk fibroin nanoparticles with 111In for nanoparticle biodistribution studies
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):1

Aim: In the last decades the Silk Fibroin Nanoparticles (SFNs) have received considerable attention for drug delivery due their high binding capacity for various drugs and controlled drug release properties. Up to date, multiple formulations of SFNs with different encapsulation methods and drugs have been described but it is still unknown their biodistribution in vivo after intravenous administration or tissue injection. Our aim in this work is to label the SFNs with a radioisotope to allow the visualization of the SFNs distribution in vivo. Post-labelling approach is proposed, nanoparticles are first functionalized with DTPA and then incubated with 111InCl3.

Methods: SFNs were prepared by a nanoprecipitation in MeOH [1]. The DTPA-functionalized silk fibroin nanoparticles (DTPA-SFNs) were prepared by amide coupling reaction between the amine groups of SFNs and one of the carboxylic groups of DTPA by using the EDC/NHS activating system [2].

Suspensions of DTPA-SFNs (Zaverage = 191.1 ± 0.7 nm, Pdl 0.115 and (ζ = -32.1 ± 0.6 mV; pH 7.5) at concentration of 7 mg/mL were labelled with activities ranging from 22 to 79 MBq of 111InCl3 (Mallinckrodt Radiochemicals Spain, S.L.U) at room temperature up to 3 hours under stirring. After incubation, suspensions were washed twice with deionized water. Labelling yield was calculated as radioactivity in SFNs divided by total radioactivity (SFNs and supernatants). Results: Radio labelling yield was 63.69±2.15 % (n = 8). Activity measured in supernatants after washing was under 3% (n = 8). 111In labelled nanoparticles were stable in suspension along the incubation experiment.

Conclusion: The coupling of DTPA followed by incubation with 111In chloride could be a promising method for SFNs radiolabelling. Further studies are needed in order to optimize the radiolabelling procedure, including stability tests, cytotoxicity in cell cultures and biocompatibility in vivo.
PP06

First approaches to radiolabelling of silk fibroin nanoparticles with $^{99m}$Tc

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Supp 1):PP06

Aim: Nanoparticles have recently gained great interest in biomedical applications. Among them, silk fibroin nanoparticles (SFNs) present excellent properties as vectors for drug delivery and several biomedical applications have been recently reviewed [1]. In vivo excellent properties as vectors for drug delivery and several biomedical applications. Among them, silk fibroin nanoparticles (SFNs) present

Methods: SFNs were prepared by a previously described nanoprecipitation method [3]. Aqueous suspensions of SFNs (Zav=168±1±2.5 nm, Pdl = 0.095, = -21.2±0.7 mV; pH 7.5) at two concentrations (5 and 12.5 mg/ml) were radiolabeled with activities ranging from 250 to 1050 MBq of $^{99m}$Tc-DTPA (Technescan DTPA, Mallinckrodt Radiopharmaceuticals Spain, S.L.U.) and $^{99m}$Tc-HMPAO (Exametazima, Radiopharmacy Laboratory Ltd), either at room temperature or 4 °C, up to 3 hours under constant stirring and recovered by centrifugation and washed twice with deionized water in order to remove weakly bounded radioisotopes. Labelling yield was calculated as radioactivity in SFNs divided by total radioactivity (SFNs and supernatant). Radioisotope-labelled silk fibroin nanoparticles were further characterized in diameter (Z-Average) and Z-potential (ζ) by DLS.

Results: Results of labeling yield in the assayed conditions are summarized in the following figure.

Conclusion: According to our results, labelling with $^{99m}$Tc-HMPAO reaches higher yields (32.1 ±1.4 %) compared to $^{99m}$Tc-DTPA (6.4±0.4 %). Higher concentration of nanoparticles shows higher isotope retention. Temperature of incubation does not significantly affect labelling yields. On one hand, DLS measurements showed that $^{99m}$Tc-DTPA-SFNs are significantly bigger in diameter but are less negatively charged than the unloaded SNFs. On the other hand $^{99m}$Tc-DTPA-SFNs are significantly bigger in diameter but are less negatively charged than the unloaded SNFs. These are very preliminary outcomes and further studies are needed to optimize the radiolabelling procedure.

PP07

Investigation of radiopharmaceutic potential of a new radiolabelled graft polymer for using in the therapy and in the molecular imaging on albino wistar rats

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Supp 1):PP07

Aim: The main reason to use the nanoparticles is to make active substrate not to decay at the injection site, to target impact zones, to provide continuous active substrate oscillation and to improve the bioeffect of administration of drug. To assist intravenously applied anticancer drugs in overcoming barriers (such as Chemical, Biological, Physical, and Cellular Barriers) and to improve the balance between their efficiency and their toxicity, a large number of drug delivery systems have been developed over the years, ranging in nature from ‘simple’ liposomes, polymers [1] and micelles, to bacterially derived ‘Minicells’ and temporally targeted ‘Nanocells’ [2].

Methods: This study deals with grafter lysine aminoacid (Lys), metacryl derivate of a hydrophobic side chain aminoacid containing poly(2-hydroxyethylmethacrylate) based magnetic graft-Lys-poly(HEMA) nanoparticles, and the attempts to radiolabel this compound with a appropriate radionucleide (such as $^{99m}$Tc) and evaluate the usefulness of the resultant radiopharmaceutical for the diagnosis and management of malignant tumors using nuclear methods. Hydrophobic nature of the nanoparticle, possibly increases the uptake of nanoparticle in different organs and tissues.

Results: Magnetic graft-Lys-poly(HEMA) was labeled with $^{99m}$Tc and radiopharmaceutical potential was investigated using animal models in this study. Quality control procedures were carried out using thin layer radiochromatography (TLRC). The labeling yield of radiolabeled polymer, $^{99m}$Tc-m-graft-Lys-poly(HEMA), was found to be about 100%. Then, stability and lipophility studies were done for this radiolabeled polymer. The n-octanol/water partition coefficient (lipophility) of $^{99m}$Tc-m-graft-Lys-poly(HEMA) was determined. The lipophilicity was found to be 0.17. The results of the serum stability experiments demonstrated that approximately 100% of $^{99m}$Tc-m-graft-Lys-poly(HEMA) existed as an intact complex in the human serum within 240 min. Biological activity of $^{99m}$Tc-m-graft-Lys-pol(HEMA) was determined on female Albino Wistar rats by scintigraphy and biodistribution studies. The biodistribution study showed high uptake in the stomach, the pancreas, brain, ovarian, intestines and the breast.

Conclusion: In conclusion, $^{99m}$Tc-m-graft-Lys-poly(HEMA), which has diagnostic and therapeutic application potentials in nuclear medicine, was first radiolabeled using the SnCl2 method and investigated to evaluate its biodistribution. Radiolabeled m-graft-Lys-poly(HEMA) proved a useful tool for assessing the in vivo behavior of the drug in rats.
PP08
Production of Lutetium-177 DOTATATE/PSMA-617 on a MultiSyn radio-synthesizer module for use in Molecular Radiotherapy

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP08

Aim: To provide 177Lu-labelled PSMA and DOTATATE for clinical use at Sir Charles Gairdner and the PET Centre, Turku, Finland (grant no. 266891 and 307924).

Methods: The preliminary development phase presented here was investigated with 10 GBq per synthesis. Methods: The syntheses were carried out on a MultiSyn automated synthesizer located into a dedicated shielded hotcell. 177Lu-DOTATE was prepared by incubating 177LuCl3 (~10 GBq in 500μL of 0.04M HCl) with 120 μg of DOTATE in 500μL of 0.4 M sodium acetate/50μL of 20% ascorbic acid solution at 85 °C for 30 min. After cooling, 0.5 mL of DTPA (1 mg DTPA in 0.9%NaCl) was added. The final product was diluted and transferred through a sterile filter (Cathivex) into the final collection vial containing 25 mg of sodium ascorbate. Radiochemical purity as well as radiochemical stability of the product was determined by radio-TLC and radio-HPLC methods.

Results: 177Lu-DOTATE was prepared in yield 86.7% ± 0.2% (n = 3) with a radiochemical purity greater than 99%. The final product passed all recommended quality control specifications (pH, sterility, pyrogen test & bubble test). The synthesis of 177Lu-DOTATE was completed in ~ 45 min. A similar approach for the 177Lu-PSMA is in progress.

Conclusion: High activities of 177Lu-DOTATATE were successfully prepared on a MultiSyn radiosynthesizer, passing all QC requirements. The automated synthesis was performed in a dedicated hotcell for proper radiation protection. This optimised method will be used to provide 177Lu-labelled PSMA and DOTATATE for clinical use at Sir Charles Gairdner Hospital in 2018.

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PP09
Production of norepinephrine transporter tracer, [18F]NS12137, via copper-mediated nucleophilic 18F-fluorination

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP09

Aim: [18F]NS12137 (3-[(6-[18F]fluoro-2-pyridyl)oxy]2azabicyclo[3.2.1]octane) is a highly selective norepinephrine transporter (NET) tracer [1]. Recently [18F]NS12137 has been produced via electrophilic pathway [1]. Herein we introduce the synthesis of protected [18F]NS12137 by a copper-mediated 18F-labelling starting from a stannylated NS12137 precursor (Fig. 1).

Methods: Radiolabelling was performed with a partly automated synthesis device, by applying a previously reported 18F-labelling procedure [2]. Dry [18F]KF/MeCN complex was formed by azeotropic drying of the cyclotron produced [18F]fluoride at 120 °C. Cu(OTf)2(py)4 was added to the reaction vessel in anhydrous MeCN at room temperature and the reaction mixture was stirred for 10 minutes. The stannylated precursor was added to the reaction vessel in dimethylacetaamide (DMA) or MeCN. When using DMA as a solvent, MeCN was first evaporated at 120 °C. The reaction vessel was then heated at 120 °C and samples for analytical radio-HPLC were collected during 30 minutes every 5 minutes. When using MeCN as a solvent, the reaction vessel was heated at 80 °C and samples for analytical radio-HPLC were collected during 75 minutes every 15 minutes. Deprotection has been performed by changing the solvent (DMA to THF), evaporating THF to dryness and then treating with 48 % HBr.

Results: Protected [18F]NS12137 was successfully prepared from the stannylated precursor via copper-mediated nucleophilic [18F]fluorination. The radiochemical yield (RCY, non-decay corrected, based on radio-HPLC analysis of the crude reaction mixture) was up to 94% when using DMA as a reaction solvent after 5 minutes reaction. When MeCN was used, the RCY was up to 0.5% after 30 minutes reaction.

Conclusion: Protected [18F]NS12137 was produced with a high RCY by the copper-mediated synthesis. The RCY obtained is higher than with electrophilic 18F-fluorination. Future work will include deprotection and isolation of the product.

Acknowledgements
We are grateful to Dan Peters from DanPET AB (Sweden) for providing the stannylated NS12137 precursor. This work was supported by the Academy of Finland (grant no. 266891 and 307924).

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Fig. 1 (abstract PP09). Synthesis of protected [18F]NS12137

PP10
Production, applications and status of zirconium-89 immunoPET Agents; an IAEA new Coordinated Research Project

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP10

Zirconium-89 has attracted huge interest and is used in tracing and quantification of slow biological processes and labelling of long half-life biomolecules such as monoclonal antibodies for pharmacokinetic studies and clinical trials. A new Coordinated Research Project (CRP), planned to be initiated by IAEA in late 2018 will investigate the target, irradiation data, separation and coordination chemistry of zirconium-89 by all participants from developed and developing countries. A detailed work plan on conjugation and 89Zr...
Radiolabelling of biomolecules shall be addressed. The participation of 15 Member States for 4 years is expected in this project.

**Keywords**: IAEA, CRP, 89Zr, Production, Radiolabelling, Monoclonal antibody

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**PP11**

**Assay of Bacterial Endotoxins in Radiopharmaceuticals by Microplate Reader**

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**EJNMMI Radiopharmacy and Chemistry** 2018, 3(Suppl 1):PP11

**Aim**: Radiopharmaceutical preparations for parenteral use must comply with the test for bacterial endotoxins according to the European Pharmacopoeia [1], whereby the endotoxin test can be carried out after release for the in-vivo application. For this purpose dedicated endotoxin testing devices are commercially available. Some nuclear medicine sites additionally perform bioanalytical tests using an optical microplate reader. We aimed to approve and validate a method for endotoxin testing of radiopharmaceuticals with analysis and evaluation by a microplate reader.

**Methods**: For endotoxin testing the quantitative kinetic chromogenic LAL assay Kinetic-QCL, (Lonza Group Ltd, Basel, Switzerland) with a 96 well pyrogen-free microplate was used. The dynamic measurements were performed with a FLUOstar OPTIMA multi-detection reader (BMG Labtech, Ortenberg, Germany) [2]. The specific analysis was programmed with the operating software MARS. The test measures the reaction time (y) to reach the predetermined absorbance (threshold of 0.2 OD). 100 μl of standards (0.005, 0.05, 0.5, 5.0 EU/ml) and unknowns were measured at 405 nm in duplicate for a total of 100 min. The endotoxin concentrations (x) were calculated by linear regression fit with the formula log(y)=a*log(x)+b. As assay control, spiked samples (0.5 EU/ml) of the unknowns were measured in duplicate. To validate interfering factors, 6 samples of unknowns ([68Ga]-peptides in PBS matrix, 5 % EtOH) were diluted 1:10; 1:20; 1:50 and 1:100 and measured without/with a spike in duplicate.

**Results**: In 3 validation runs accuracy, reparability, precision, and linearity were studied. All samples in the studies were valid with a spike recovery between 75% and 100%. The correlation of the calibration fit was >0.997 and LLOQ was found to be 0.005 EU/ml. The solvent matrix of [68Ga]-peptides showed no interferences at a dilution of 1:10. Considering this dilution, the range of the endotoxins test stretched from 0.05 to 50 EU/ml. According to Ph. Eur. the limit for bacterial endotoxins in [68Ga]-peptides solution for injection is defined with 175 EU per dose. Calculating 10 ml per dose the limit of 17.5 EU/ml is well covered.

**Conclusion**: We have demonstrated that an optical microplate reader is well suitable to test bacterial endotoxins in retain samples of [68Ga]-peptides solutions. Up to 20 samples can be tested as bundle onto a 96 well microplate within 3 hours. After validation this analysis method was integrated successfully into the parametric release of our routinely prepared [68Ga]-peptides.

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**PP12**

Ga-68 labeled quinazoline monomers and dimers bearing the HBED-CC chelator as PET tracers for EGFR-TK imaging

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**EJNMMI Radiopharmacy and Chemistry** 2018, 3(Suppl 1):PP12

Ga-68 labeled quinazoline monomers and dimers bearing the HBED-CC chelator as PET tracers for EGFR-TK imaging

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**EJNMMI Radiopharmacy and Chemistry** 2018, 3(Suppl 1):PP12

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Aim: Epidermal growth factor (EGFR) receptor is over-expressed in several solid tumors. EGFR tyrosine kinase inhibitors (EGFR-TKIs) bind to the intracellular TK domain and compete with adenosine triphosphate (ATP), preventing thus tumor cell proliferation, angiogenesis, and protection from apoptosis. In the present study, we aimed towards the synthesis of potential 68Ga labeled monomers and dimers of the EGFR-TKI pharmacophore (3-bromo-phenyl)quinazoline-4,6-diamine pharmacophore) in order to evaluate their functionalized propionic acid moieties, resulting in monomers 3 and 4 or dimers 5 and 6, respectively. Ligands 3-6 were tested in vitro for TK inhibition, radiolabeled with 68Ga and tested in vivo for their biodistribution profile in mice bearing A431 (human epidermoid carcinoma cell line) tumors.

Methods: The cytotoxicity of 1-6 was determined by the MTT assay against A431 cells by treating them with varying concentrations of 1-6 for 72 h (10 nM-1000 μM). HEBD-CC analogues 3-6 (1.0 mM in HEPES buffer 2.4 mM, 90-100 μl) were radiolabeled with 68Ga eluate (40 μl, 40-50 MBq), 98°C, 10 min, and evaluated for radiolabeling efficiency via RP-HPLC. Tracers (68Ga) (i = 3-6), were investigated in vivo for their tumor accumulation and biodistribution in A431 tumor-bearing SCID mice at 5, 60 and 120 min post injection.

Results: In vitro MTT assays for 3-6 showed IC50 values in the μM range. Monomer 3 (43.60 ± 7.49 μM) was significantly better than 4 (62.84 ± 6.34 μM) and 6 (68.80 ± 3.71 μM) and identical with dimer 5. Radiolabeling of 3-6 resulted in a single radioactive product (68Ga) (i = 3-6), while radiochemical yield ranged between 98-100%. Biodistribution experiments for (68Ga) (i = 3-6) showed tumor (A431) uptake which ranged between 0.90-1.34 %ID/g at 5 min. Monomer 3 and dimer 6 showed a fast tumor clearance (0.49-0.34 %ID/g at 60 min p.i.), while for monomer 4 and dimer 5 tumor uptake remained rather constant after 60 min p.i. (0.73-0.70 %ID/g). All compounds showed extensive uptake in the intestines at 60 and 120 min p.i., ranging between 23.23-43.18 %ID/g. Among the ligands studied monomer 4 presented less off-target uptake in combination with the higher tumor accumulation.

Conclusion: Four novel quinazoline monomers and dimers based on the HEBD-CC chelator (3-6) were synthesized, radiolabeled with 68Ga and evaluated as biomarkers for EGFR-TK imaging. Among the tracers studied (68Ga) (i = 3-6), monomer 4 presented the best pharmacokinetic characteristics regarding tumor uptake and off-target accumulation. Tracer 4 is being considered for further evaluation as PET tracer for EGFR-TKI imaging.

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PP13
Prostate-specific membrane antigen (PSMA) and gastrin-releasing peptide receptor (GRPR) PET-imaging for prostate and breast cancer: tumor models and interactions with clinical relevance
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP13

Aim: PSMA and GRPR are both highly expressed on prostate cancer (PCa), and thus considered attractive targets for imaging and therapy. GRPR has been also been suggested for imaging breast cancer (BC), while on the contrary for PSMA a limited number of positive cases has been reported. We compared two ligands linked to the chelator N,N′-bis[2-hydroxy-5-(carboxyethyl)benzyl]ethylenediamine-N,N′-diacetic acid (HEBD-CC), H2N-(Ahx)-Lys-NH-CO-GLu (PSMA-11) and a GRPR antagonist, H2N-4-amino-1-carboxymethyl piperidine-[IR-(R)-Phe], Sta13B[6N-14] (68Ga)2. Both tracers were tested in vitro in PC-3, LNCaP (both PCa), T47D, MDA-MB-231 (both BC) and in the PSMA-transduced and PSMA-overexpressing cell lines PC-31, T47D and MDA-MB-231. In addition, a GRPR probe for fluorescence-based flow cytometry (FACS), 3, was synthesized by the replacement of HEBD-CC with Alkyne-BDP-FP (Jena Bioscience).

Methods: PSMA overexpression in PC-3, T47D and MDA-MB-231 cell lines was accomplished by means of lentiviral transduction and was subsequently confirmed by western blot in the respective cell lines. 1 and 2 were synthesized and radiolabeled with 68Ga according to published methods. Total cell bound 68Ga1 and 68Ga2 over time (0-90 min) was determined according to previously published methods. FACS analysis with 3 was also performed on the following cell lines T47D (PSMA), T47D (vector only), T47D (wild type).

Results: Maximum cell binding of 68Ga1 and 68Ga2 (PSMA- and GRPR-specific respectively) was achieved after 45 min of incubation. Cell binding of 68Ga1 and 68Ga2 was increased after PSMA transduction in all cell lines tested after 1h incubation. More specifically, (results are expressed as time% above control, % given radioactivity) 68Ga1 (PSMA) was higher for MDA-MB-231 (100x, ~80 %), T47D (4x, ~2.5 %), PC-3 (10x, ~13 %) than controls (Mock or blocked with addition of 10,000-fold excess pharmacophore), while for LNCaP it was ~66 %. Interestingly, alongside PSMA overexpression caused by the transduction the binding of the GRPR ligand was also increased, thus 68Ga2 was higher for MDA-MB-231 (2x, ~3 %), T47D (2x, ~9 %), PC-3 (2x, ~30 %) than controls (Mock or blocked with addition of 1000-fold excess pharmacophore). These observations have been also confirmed by FACS using 3 in T47D and T47D-

Conclusion: The transduction of PSMA positively affected the amount of cell bound ligand for both 68Ga1 (PSMA) and 68Ga2 (GRPR) in all PCa and BC cell lines studied. These results indicate a possible connection between the two receptors (PSMA/GRPR); further studies are underway to prove this hypothesis. The findings of this study might be of high impact for the development of novel radio-pharmaceutical treatment strategies addressing tumor heterogeneity.

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PP14
Radiolabeling of single domain antibodies with Tc-99m: evaluation of the best parameters for complexation allowing to preserve 3D conformation
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP14

Aim: Camelid single-domain antibodies (sdAb), also called VHHLs, constitute a promising “new” class of imaging agent for nuclear medicine. When using the tricarbonyl method for radiolabelling VHHL with Tc-99m, the optimal conditions must be specifically determined 1) by determining the parameters such as temperature and heating times to obtain the maximum of complexation with Tc-99m, and 2) by evaluating the effects of temperature on their secondary structure.

Methods: Five VHHL (VHH-r1, VHH-r2, VHH-r3, VHH-r4 and VHH-r5) were radiolabeled with technetium-99m using tricarbonyl method at their C-terminal hexahistidine-tag (His-Tag). For each compound, various parameters such as specific activity, time and temperature of in complexes, when using the tricarbonyl method for radiolabelling VHH with Tc-99m, the optimal conditions must be specifically determined:

1) by determining the parameters such as temperature and heating times to obtain the maximum of complexation with Tc-99m, and 2) by evaluating the effects of temperature on their secondary structure.

Results: When using the tricarbonyl method for radiolabelling VHH with Tc-99m, the optimal conditions must be specifically determined:
Results The conditions enabling to acquire the highest complexity with Tc-99m were found to depend on the evaluated VHH. For instance, they were of 45 min at 75°C for VHH-r1, VHH-r4 and VHH-r5, of 60 min at 60°C for VHH-r2, and of 90 min at only 40°C for VHH-r3. Similarly, conformational changes observed by CD were found to vary between the evaluated VHHs, with maximal thermal stabilities ranging from 40°C to 75°C. As a result, for 3 VHHs out of 5, the incubation temperature had to be reduced to achieve the maximum of complexity in order to preserve their 3D conformation (50°C for 90min for VHH-r2 and 60°C for 60min for VHH-r4 and VHH-r5). Once successfully radiolabeled and purified (PRC>95°C), we showed that all 99mTc-VHH-r's had preserved affinity for their target, except for 99mTc-VHH-r2.

Conclusion: This study underlines the fact that despite their structural similarities, each VHH responds differently to the conditions of radiolabeling with Tc-99m. Therefore, to radiolabel a VHH using 99mTc-tricarbonyl method it is very important to define optimal conditions of incubation in agreement with the maximum of complexity without affecting its 3D conformation.

PP15
A comparison of four different dose calibrators using various isotopes and sample geometries
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP15

Aim: Dose calibrators are medical devices used in nuclear medicine to determine the amount of radioactivity to be administered to patients. According to Dutch Nuclear Medicine Guidelines, dose calibrators should be validated for all radionuclides and geometries with a maximum deviation of 5% with respect to the true radioactivity. This validation requires the use of well calibrated radioactive standards or measurements with calibrated equipment. Because this is expensive and time consuming, validation is often limited to a comparison to other dose calibrators. It is questionable if this limitation is justified, especially when looking at the effect of the (type of) isotope, the dose calibrator type/manufacturer and the geometry of the radioactive sample.

Methods: In this study, we used and compared four different dose calibrators (ISOMED 2000, ISOMED 2010, Capintec CRC-25R, Veemstra V8-200). In these, we measured activity from four commonly used isotopes (18F, 68Ga, 99mTc and 111In) using different volumes in both syringes and vials. Additionally, we analyzed the effect of using different geometries (3 different brands of syringes and 4 different brands of vials) on the measurements in more detail on one system. All measurements were compared to a fully calibrated gamma spectroscopy semi-conductor system (GR1018, Canberra) to determine the deviation from the true radioactivity.

Results For 18F and 99mTc, the deviations were mostly within the 5% limit on all devices and for all geometries. For 68Ga and 111In however, deviations of more than 30% from the true radioactivity and 50% between different dose calibrators were observed. Within one type of dose calibrator, the deviations are small for all isotopes: the standard deviation for repeated measurements is below 3%, while the effect of varying syringes and vials is about 5-10%.

Conclusion: Even though all dose calibrators showed acceptable deviations for 18F and 99mTc measurements, the deviations for 68Ga and 111In are not conform legal regulations and require on-site modifications of the dose calibrator settings. Although the effect of sample geometry was generally limited (compared to the differences between isotopes), it frequently still exceeded the 5% limit as posed in the guidelines. We therefore advise to validate dose calibrators (at least one per type) for every isotope and for every geometry, using a well-calibrated standard. As this may affect the ’medical device’ status of the system, discussion with the manufacturer is required.

PP16
A first synthesis of 11C-labelled analog of 4’-O-methylhonokiol as a potential PET radiotracer for inflammation
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP16

Aim: Neolignan 4’-O-methylhonokiol (MH) isolated from Magnolia grandiflora is known to have several biological activities, including anti-cancer, anti-inflammatory and anti-neurodegenerative effects [1]. MH has recently been shown to inhibit cyclooxygenase (COX) activity with a higher selectivity for COX-2 (IC50=0.062 μM) over COX-1 (IC50=2.4 μM) [2]. The development of COX-2 inhibitor radiolabeled analogs is of special interest for PET visualization of inflammation and tumors. Therefore we suggested a MH analog labeled with carbon-11 (4’-[11C]methoxy-5-propyl-1,1-biphenyl-2-ol or [11C]MPbP) as a potential PET radiotracer. Unlabeled MPbP has demonstrated a high anti-inflammatory activity comparable to that of specific COX-2 inhibitor celecoxib in the preliminary tests on mice with a carrageenan-induced inflammation. The present study describes the radiolabeling procedure of [11C]MPbP.

Methods: The synthesis of [11C]MPbP was performed by two-step procedure starting from precursor with Boc-protecting group and operated with home-made fully automated module. The radionuclide carbon-11 (T1/2 = 20.4 min) in the form of [11C]CO2 was produced by a nuclear reaction 14N(p, α)11C in a PETtrace cyclotron (GE Healthcare). [11C]CH3I was produced using ‘wet’ method (LiAlH4/HI) and transferred under nitrogen flow (~10 ml/min) into reaction vessel with the precursor solution (2 mg in 1.2 ml of acetone and 0.7 M NaOH in water-ethanol solution (1:1, v/v) as a base). [11C]-methylatation reaction was performed at room temperature in 5 min. The hydrolysis step was accomplished at 60°C with 12 M HCl in 5 min. The efficiency of O-11C-methylation as well as yield of [11C]MPbP were determined in crude reaction mixture using radio-TLC on Silicagel 60 F254 UV-plates with hexane/ethyl acetate (4/1) as a mobile phase and radio-HPLC (Waters X-Bridge column 4.6x150 mm, MeCN/H2O (60/40) as eluent with flow-1.3 ml/min). The radiochemical identity of [11C]MPbP was evaluated by comparison with the reference compound.

Results For the first time, [11C]MPbP, a novel potential COX-2 inhibitor was produced. The 11C-methylation yield was about 60% and [11C]MPbP was resulted in 45% yield based on [11C]CH3I.

Work is now in progress to further optimize synthesis and purification of [11C]MPbP and to investigate its biochemical characteristics (plasma stability, lipophilicity, BBB permeability, binding affinity to COX-2). This study was funded by RFBR according to the research project 17-04-02119 A.

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**PP17**

**Synthesis of WX-360-derived, uPAR-binding PET Tracers and Development of a Platform to Evaluate Their in Vitro Binding Affinities**

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP17

**Aim:** Binding of the urokinase-type plasminogen activator (uPA) to the membrane anchored receptor uPAR is defined by its amino terminal fragment (ATF) consisting of a kringle and a growth factor-like domain (GFD). The latter is buried deep in the binding cavity and harbours the majority of the high-affinity interactions. A cyclic peptide (WX-360: cyclo21-29[D-Cys21]-uPA21-30[21C;H29C]) was derived from this original structure demonstrating a capability to inhibit tumour growth and spread [1,2]. As the uPA system is frequently associated with the process of invasion/metastasis and a more aggressive phenotype in a great variety of cancers, there is a great interest in PET imaging using uPAR-directed tracers to visualise the tumour’s potential for metastasis formation. Hence, this study uses the cyclic peptide WX-360 as a starting point for the development of new PET tracers.

**Methods:** Ligands were synthesised on solid phase. A competitive bioassay based on immobilised rhuPAR was developed to assess their binding affinities. Briefly, increasing concentrations of ligand (0-1000 µM) were incubated in the presence of a 0.1 nM solution of a monoclonal mouse IgG1 antibody (clone #62022, R&D Systems) directed against the uPA-binding pocket of uPAR. Binding of the hors eradish peroxidase conjugated secondary goat anti-mouse IgG1 antibody and subsequent conversion of a chromogenic substrate generated the signal which was read at 450 nm. A competitive cell-based binding assay to re-evaluate the initial results is currently being developed.

**Results** Saturation and competitive binding studies employing the natural ligand uPA revealed specific binding of the primary mAb (Kd = 1.0 ± 0.1 nM). Assay specific IC50 values of the peptide-based, literature-known structures DOTA-AE105 and WX-360 were in the low micromolar range while no binding was detected for DOTA-AE105M (non-binding mutant) [3,4]. Studies on the optimisation of the binding motif were promising for methylation of the central lysine residue and exchange of the disulphide bridge for a peptide bond. Conjugation of a DOTA chelator for radiometal complexation was accomplished by insertion of various linkers at the C-term inus of WX-360, the resulting compounds displaying similar inhibitory potentials as compared to the original structure. Placing the chelator at the N-term inus, however, slightly reduced the binding affinity.

**Conclusion:** With initial positive results, this study presents the potential of deriving uPAR-binding PET tracers from the peptide WX-360. The platform for compound characterisation is currently expanded to re-evaluation of IC50 values in a cell-based assay as well as determination of logP values and serum stabilities.

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**PP18**

**Fully-automated production of 2-[18F]fluoroflumazenil without using gradient HPLC purification**

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP18

**Aim:** 2-[18F]fluoroflumazenil (FFMZ) has proven useful for benzodiazepine receptor imaging. Our goal was to implement the fully-automated synthesis of [18F]FFMZ in a Tracerlab FXFN module.

**Methods:** Synthesis was performed via nucleophilic substitution using the chemical precursor Tosyloxyethylflumazenil (TFMZ), adapting the method reported by Yoon et al. [1] but avoiding the use of gradient HPLC. Briefly, [18F]Fluoride ion was trapped in a QMA cartridge and eluted with 1.5 mL solution of K22.2.2 (5.3 mg/mL)/ K2CO3 (1.4 mg/mL) in MeCN/H2O (95/5). After drying of the azetrophic mixture, 5 mg of TFMZ in 1.7 mL MeCN/0.3 mL DMSO were added to the reaction vessel and heated at 110°C for 12 min under intermittent purging with helium. The reaction mixture was diluted with 2 mL (x2) of water and passed through an activated alumina cartridge to the HPLC-injection vial. Solution was then loaded onto the HPLC-loop for purification in a semi-preparative C18 reverse phase column (250/10 mm, Nucleosil 100-7 C18, Macherey-Nagel). Purification was performed under isocratic elution using two mobile phases at a flow rate of 3 mL/min: first with 100% water for 10 min then switching to 40% MeCN in water. The collected product-fraction was diluted with water (1:5) and loaded onto a preconditioned C18 cartridge. After rinsing with 3 mL of water, [18F]FFMZ was eluted with 1 mL of 70% EtOH, diluted with 10 mL of physiological saline, and sterilised by filtration (0.22 µm Millex-FG).

**Results:** [18F]FFMZ was eluted at 20 min after injection as shown in Fig. 1. Synthesis was satisfactorily accomplished yielding the final product in radioactive yields of 20-25% decay corrected at the end of bombardment after a synthesis time of 60 min (n = 5), with a radiochemical purity >99% as determined by analytical HPLC.

**Conclusion:** A convenient and reliable synthesis of [18F]FFMZ was successfully adapted in an automated module by using two successive isocratic elutions instead of gradient HPLC. Final product showed no radioactive or non-radioactive impurities obtaining [18F]-FFMZ in enough quantity and quality for clinical applications.

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**Fig. 1 (abstract PP18).** Full HPLC chromatogram of the purification of [18F]FFMZ.
PP19

Radioiodination of Small Molecules and Short Peptides; the Effect of Oxidant Reagents Choice on the Radiochemical Yields
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP19

Aim: The radioiodinated products are used as diagnostic or therapeutic agents. The diversity of the labelling methodologies used to introduce the radioactive iodine into the target molecule are wide enough to make the selection of a specific route is of great interest. Several oxidant agents were used in labelling biomolecules. Three famous ones are Chloramine-T, lodogen and lodo-beads (the solid state of the Ch-T). However, the mentioned agents are producing varies radiochemical yields and more importantly stabilities of the produced labelling product always questioned. The current report is detailing the findings out of a study that was aimed in investigating several reaction factors on the above labelling agents when used with small molecules and short peptides.

Methods: The direct electrophilic method via Chloramine-T or similar oxidant was used. Following the end of the reaction the purification step was done using Sep-Pak C-18 Classic cartaghes that should be activated with 3 ml water prior the purification. Then the Sep-Pak was then washed with 1 ml water, then collect the product (labeled peptide) in the second wash which done by eluting with 0.5 ml MeCN and 1.5 ml mix of the HPLC eluent from a 68Ge/68Ga generator is used as the generator is attached to a biological molecule (peptide) dissolved in 50 μlH 2O.

Results: Chloramine-T gives constant RCY most of the time under all conditions except at basic PH (8.5) in which heat is required. lodogen gives better RCY when heated and higher PH 7.4 and 8.5 were used. The effect of the heat was clear at PH 6.5 as the RCY was nine times higher at 60°C. Also lodo-beads gives higher RCY than the other two labeling agents except at PH 8.5 and 60°C, it was about 25 % (±5) less.

Conclusion: We can conclude that RCY and purity are very dependent on the right choice of the labeling agent and reaction conditions. However lodo-beads give higher RCY than the other two labeling agents which make it the targeted intermediate oxidant reagents.

Acknowledgements
This work was supported by King Abdullah City for Science & Technology (AT-29-15), RAC # 2080 047.

PP20

The Use of Nano-Sized Particles in Labelling New Class of Radiopharmaceuticals
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP20

Aim: Radiosynthesis an imaging radiotracer for a carefully selected biological process and choosing the right imaging modality become the top aims for all biomedical researchers. Positron Emission Tomography (PET) is the first on the list used for such investigations as it fulfills all informations required for imaging of biological process. On the other hand human cancer cells over express many peptide receptors which can be used as molecular targets. Therefore, radiolabeled receptor-binding peptides have emerged as an important class of radiopharmaceuticals for tumor diagnosis and therapy. As small peptides for receptor imaging and targeted radiotherapy have advantages over proteins, antibodies and antibody fragments as they are small and show rapid diffusion in target tissue and they have low molecular weight which will result in rapid clearance from blood and non-target tissues and that in turn will result in high tumor-to-background ratios.

Methods: In a routine process a 0.1mg of nanoparticles that been attached to a biological molecule (peptide) dissolved in 50μlH 2O. 68GaCl3 eluent from a 68Ge/68Ga generator is used as the generator is fractionally eluted beginning with 5.0 ml of sterile 0.1N HCl. For fractional elution, the first 1.6 ml of eluate always discarded, the next 2.0 ml is collected for use and the final 1.4 ml also discarded. 200 μl of the fractionated Ga-68 eluate (approximately 1 μCi/37 MBq) is added to nanoparticle solution then 100μl of sodium acetate buffer (1.25M) or ammonium acetate buffer (0.25M) is added and allowed to react at 65-90°C for 30 minutes, the pH of the reaction mixture should be kept at 5.5. Following incubation, the labeled nanoparticle is then tested for radiochemical yield and purity using Radio-TLC and HPLC.

Results: The labelled nanoparticles attached to the biological molecule is monitored by both TLC and HPLC. Radio-TLC example of labelling reaction mixture: 68Ga-anoparticle remains at the origin with retention factor of 0.00, the purity approximately 85%, and the free 68Ga migrating to the solvent front with retention factor of 0.96.

Conclusion: New efficient methodology to label nanoparticles with Ga-68 was developed in order to promote the development of PET-nanoradiopharmaceuticals that in the future will overcome the limitation of the regular PET-radiopharmaceuticals in use nowadays.
PP21
Biological Investigations of a laminin Class Peptide that has a potential as a Diagnostic and Therapeutic Properties
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP21

Aim: Melanoma is a tumor of constantly accumulative occurrence for which novel methods of imaging and targeted therapy are generally pursued. Developing a newly laminin class peptide that been labeled with position emitter Ga-68 or I-124 and with SPECT emitter I-131 will be discuss. In order to evaluate the potential relevance of positional emitter Ga-68 or I-124 and with SPECT emitter I-131 pursuing. Developing a newly laminin class peptide that been labeled with position emitter Ga-68 or I-124 and with SPECT emitter I-131 was labelled with 131I to be tested as therapeutic approach. Methods: High performance liquid chromatography (HPLC) system and Thin Layer Chromatography (TLC) were used for quality control purposes. The radiochemical purity were > 99.00% (n = 5). The stability was persistent over 6 h and amounted to > 98.55% ±0.35% (n = 15) for the 68Ga-peptide. In vitro receptor binding was performed on SK-MEL 28 melanoma cell line. Also the same peptide was investigated in SK-MEL28 melanoma cell line. The uptake of 68Ga/124I-peptide was measured in 10 cm strip and 70% (v/v) methanol, the Rf for iodate was 0.5-0.7 and for iodide was 0.7-0.9; with 85% (v/v) methanol, the Rf were 0.3-0.5 for iodate and 0.7-1.0 for iodide, respectively, taking an hour and a half for the chromatographic run. The resolution obtained with 85% (v/v) methanol was better when compared to methanol 70% (v/v).

Conclusion: By the use of the staining test for iodate and iodide proposed by the American and Argentine pharmacopoeia methods present iodate-131 as the main radiochemical impurity but differ in the mobile phase composition and in the color development method for iodide and/or iodate 1-2. The systems advocate the use of 30 and 20 cm long stationary phases which require several hours for separation of the species, and method 70% and 75% (v/v), respectively. In a 10 cm strip and 70% (v/v) methanol, the Rf for iodate was 0.5-0.7 and for iodide was 0.7-0.9; with 85% (v/v) methanol, the Rf were 0.3-0.5 for iodide and 0.7-1.0 for iodide, respectively, taking an hour and a half for the chromatographic run. The resolution obtained with 85% (v/v) methanol was better when compared to methanol 70% (v/v).

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PP22
Improvement of iodide and iodate identification method in the radiochemical analysis of Iodine-131 radiopharmaceutical
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP22

Aim: Sodium Iodide (131 I) is a radiopharmaceutical available as oral capsule or solution, and is largely used in nuclear medicine for thyroid scintigraphy imaging and in radioiodine therapy. Radiochemical purity (% RqP) is defined as the percent of total radioactivity in the desired chemical form. The main methods to determine % RqP is paper chromatography (PC), thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC). Some compendial methods also establish procedures for retardation factor (Rf) determination 1-2. The objective of this work was to evaluate the mobile phase effect on the Rf of iodide (I) and iodate (IO3-) in the % RqP analysis of Sodium Iodide (131 I).

Methods: Whatman 3MM PC (1.5 x 12.5 cm), methanol and glacial acetic acid were from Merck Millipore. Methanol and purified water in the proportion of 50%, 70%, 75% and 85% (%/v/v) were the mobile phases. Diluent solution containing 0.1 mg/mL KI, 0.2 mg/mL KI, 1.0 g/mL Na2CO3, and standard solutions of 0.2 g/mL KI and 0.4 g/mL KIO3 were prepared. In the origin position of a 10 cm paper strip, 5 μL of the diluent solution were applied. After chromatographic separation in each mobile phase, the suitable standard solution was dripped on the iodide and iodate expected positions followed by drops of glacial acetic acid to reveal the Rf of the species with formation of a brown spot 3.

Results: As determined by HPLC, the 68Ga-short peptide efficiency was >70% and radiochemical purities always > 99% in short reaction time. These synthetic approaches hold substantial promise as a rapid and efficient method amenable for automation for the labelled of peptides with high radiochemical yield and short synthesis time. For radioliodination direct electrophilic method were used with High RCY.

Conclusion: The uptake of 68Ga-peptide was high by pheomelanotic melanoma cells. In vivo characterization in normal mice revealed rapid blood clearance of 68Ga-peptide with excretion into the kidney and hepatobiliary pathways. In vivo imaging using animal PET/CT is confirming the later findings yielded a high tumor-to-background ratio at 1 h and at 2 h. In vitro tests have shown that significant amount of the 68Ga-peptide associated with melanoma cell fractions. Due to its easy handling and quite high uptake by melanoma cells, we expect that this peptide could be successfully used in routine application for melanoma imaging or eventual radiotheraphy suggesting great potential for noninvasive clinical evaluation of suspected metastatic melanoma.

Acknowledgements
This work was supported by King Abdualaziz City for Science & Technology (AT-29-15), RAC # 2080 047.

PP23
Delivery of DTPA through liposomes as a good strategy for enhancing plutonium decorporation
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP23

Aim: Internal contamination with plutonium (Pu), either in the context of accidental occupational exposure or as a result of possible terrorist use, continues nowadays to be a potential hazard. The practical way to reduce Pu body burden and the associated radionuclide risks is decorporation by chelation therapy by injection of marketed Na2-Ca-DTPA solution. This study aimed at assessing the efficacy of 110nm-sized liposomes encapsulating the DTPA in plutonium-exposed rats. The comparative effects of liposomal and free DTPA at similar doses were examined in terms of limitation of alpha activity burden in rats receiving various treatment regimens.

Methods: Unilamellar liposomes (DSPC/Cholesterol/DSPE 69:30:1) were used to encapsulate DTPA (25mM). For decorporation experiments, rats were first contaminated by intravenous administration of the soluble citrate form of 238Pu (4 to 10.3 kBq), before injection of DTPA, marketed free form or encapsulated in liposomes (2.25 at 6.74 μmol/kg). Treatment schedules started at one hour (prompt treatment) or at seven days (delayed treatment) after contamination and were given as a single injection or repeated injections. A prophylactic single treatment was also tested given at three days before contamination. Organs of interest (liver, bone and spleen) and excreta were collected at different times for measurement of Pu alpha activity.

Results: Liposomal DTPA given at 1h post-contamination reduced significantly Pu retention by 2.5%, 1.6- and 3-fold in liver, bone and spleen respectively, compared with free DTPA. For delayed administration, liposome-entrapped DTPA decreased hepatic, skeletal and
splenic Pu levels by, respectively 68, 39 and 74% of the levels in untreated control rats (only 19, 12 and 24% with a similar dose of free DTPA). In addition, repeated injections of liposomal DTPA improved the removal of Pu compared to single injection and the efficacy of the prophylactic treatment was also observed.

Conclusion: The advantage of liposomal DTPA was undoubtedly directly and indirectly due to the better cell penetration of DTPA when loaded within liposomes, mainly in the tissues of the mononuclear phagocytic system. The decorporation induced by liposomal DTPA may result firstly from intracellular chelation of Pu deposited in soft tissues, predominantly in the liver. Afterwards, the slow release of free DTPA molecules from these tissues may enable a sustained action of DTPA, probably mainly by extracellular chelation of Pu available on bone surfaces. To conclude, Pu decorporation can be significantly improved by liposomal encapsulation of DTPA regardless of the treatment regimen applied.

PP24
Validation of a clean room for the production of radiopharmaceuticals at Turku PET Centre
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP24

Aim: According the EU GMP Guide the radiopharmaceuticals should be manufactured in controlled areas and the premises maintained so that the level of particles and microbiological contamination are low. At Turku PET Centre (TPC) the production of radiopharmaceuticals is performed in EU GMP class C clean room. Thus validation of the clean room shall ensure that the area fulfills all EU GMP requirements for this classification.

Methods: In order to grade our production premises, we have compiled a validation plan which was accepted by the quality assurance person of TPC. The validation process according the accepted validation plan was performed by an outside contractor according to valid standards (methods, calibrations of the measurement equipment and documentation). The tests include measurements of HEPA filter integrity, air volume flow, room differential pressure, airflow visualization, air change rate, airborne particle levels and viable microbial particles. The microbiological testing and measurement of airborne particles were performed both at rest and in operation. For microbiological testing, active and passive air samples are performed annually: HEPA filter integrity test, air volume flow, room differential pressure, air change rate, airborne particle levels and homogeneous perfusates, gravity technique was found to deliver a variable content perfusate making difficult the control of cumulative perfused activity, and perilous the planning administration of partial activities following dose adjustments. The infusion of the entire content requires at least 81mL of saline, significantly higher than the SPC requirements.

Results: The contractor compiled a validation report including all measurement records. All the requirements were fulfilled, the report was checked and accepted by the responsible person at TPC.

Conclusion: To maintain the classification, following measurements are performed annually: HEPA filter integrity test, air volume flow, room differential pressure, air change rate and airborne particle levels. In addition, the room differential pressures are monitored automatically all the time giving alarm when out of specification. Microbiological sampling and measurement of airborne particles are performed regularly according to a standard operation procedure. Microbiological sampling is done at least once a month. Airborne particles are measured at least once a week during end product sterile filtration. While doing aseptic work, microbiological sampling and the measurement of particles are always performed.

PP25
Use of Gravity Perfusion Method in PRRT: Experimental Evaluation and Optimization
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP25

Aim: 177Lu-DOTATATE (Lutathera®) indicated in endocrine tumors PRRT should be administered according to its SPC by a slow intravenous infusion (7.4Gbq within 30min-or-50mL/h) with either the "2-pump" or the "gravity" methods. This latter method consists in using a saline solution placed in height and connected to the Lutathera® vial by an infusion line, which flows the vial causing volume expansion and overpressure, pushing the diluted product into a second infusion line that goes to the patient. The first trials we made with this technique showed that it was as imprecise as difficult to control. The aim of this work was to optimize experimentally this technique and compare to the other ones.

Methods: A saline bag (250mL) was placed at 2m height, connected to an infusion line (set at 16drops/min -or-50mL/h) ending with a 22G/30mm-needle. The needle was inserted into the Lutathera vial placed at 1.5m height. A second long 19G/88mm-needle deeply inserted into the vial septum until touching its bottom -was connected to the venous perfusion line going to the patient. For radiation safety reasons, 177Lu-DOTATATE was substituted by a cold saline solution stained by Red Ponceau and placed a Lutathera empty vial sealed with a new rubber stopper and an aluminium ring. The evaluation of photometric absorption replaced the radioactive measurements. Once the assembly of this system set up, the infusion was started, 150 mL the colored solution was gradually collected at the line outlet in 5mL fractions which content was assessed by UV-visible spectrophotometry at 550nm. Measurements permitted to evaluate 3 parameters: volumic activity of the perfusate (VAp), (in %, AT) and residual activity in the bottle (in %, AR). Results are expressed as mean±SD (n = 3).

Results: Collected perfusate showed a volumic activity which decreased gradually in exponential way [VAp=6.0e-0.062. Perfused-Volume]/R2=0.99]. Similar evolution was found in the residual activity of the vial, which disappeared according to the same mode. Symmetrically, the total perfused activity gradually increased in an exponential mode. After 30 minutes of infusion (infusion time requested in the SPC), a significant amount of the product (21±±1.1%) remained in the vial. We calculated that to administer 99% the total delivered activity, it was necessary to infuse a a total volume between 81mL and 86 mL. After 100mL of infusion, only 0.45±0.26% remained in the vial.

Conclusion: While the pump infusion techniques produce uniform and homogeneous perfusates, gravity technique was found to deliver a variable content perfusate making difficult the control of cumulative perfused activity, and perilous the planning administration of partial activities following dose adjustments. The infusion of the entire content requires at least 81mL of saline, significantly higher than the SPC requirements.

PP26
Injection of 177Lu-DOTATATE with pharmaceutical Vehicles and Peptides
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP26

Aim: 177Lu-DOTATATE is indicated in neuroendocrine tumor PRRT. Its therapeutic efficiency and safety are tightly related the maintenance during all the treatment cycle of the chelate stucture integrity until its internalisation in tumour cells. Any alteration on its structure may lead to the complex destruction or unmettlealation. These reactions may be suspected during co-infusion of other pharmaceuticals impacting pH, such as alkaline vehicles, or in patient freshly pre-treated with cold somatostatin analogues which may be competitive to the bound peptide. The aim of this work is to study chemical interactions of 177Lu-DOTATATE in co-infusion conditions with various pharmaceutical vehicles and non-radioactive somatostatin analogues.

Methods: 177Lu-DOTATATE (Lutathera®) was diluted to 1/5th in saline, 5% glucose, Bicarbonate 8.4% solution, Ringer-Lactate,
octracetate solution, octracetate LAR or incubated at 37°C in plasma or plasma enriched with 10% de octreotide. Radiochemical purity (RPC) was determined by ITLC-SG in 0.1mol/L sodium citrate pH=3. The labelled peptide had retention factor (RF) of 0.1 (0.2 in plasma) and the free radionuclide migrates at RF=1.0. RPC was also determined using C18 Sep-Pack-Plus cartridge (Waters, USA). The free radionuclide was eluted with 5mL of 0.1mol/L acetate buffer pH=3 and the labelled peptide with 5mL of methanol, and hydroxylated Lu-177 remained inside the cartridge. Mixtures were then analyzed immediately, 30min and 24h after dilution. Results were expressed as mean±SD (n = 3-4) and RPC was considered as compliant when it was ≥95%. Binary comparisons to the undiluted product were made by a Mann-and-Whitney U test and stability over time checked by a 1-way ANOVA (p < 5%).

Results: 177Lu-DOTATATE diluted in saline or glucose or with cold peptides showed compliant RPC levels during the 24hours study duration. In Bicarbonate and Ringer Lactate, significant amounts of unbound Lutetium were found during Sep-Pack extraction, respectively 10.5±3.3% and 8.9±4.2%. In plasma mixtures, significant amounts of either hydroxylated Lutetium and unbound Lutetium found in incubation: respectively 4.9%±1.3% and 4.2%±0.6% in plasma and re- sulted only within 24h. Physiological 89Zr-Oxalate Solution for PET Diagnosis: Purification, Formulation, Biological Evaluation. V.B. Bubenschikov, M.V. Zhukova, A.S. Krasnopyorova, A.Ya. Maruk, A.A. Larenkov

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP27

Physiological 89Zr-Oxalate Solution for PET Diagnosis: Purification, Formulation, Biological Evaluation.

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP27

Aim: The positron-emitting 89Zr (T 1/2=78.42h) is widely studied for PET. Monoclonal antibodies labeling is the main focus of these studies. But should we disregard the diagnostic potential of simple 89Zr compounds?! The possibility of effective diagnosis of inflammation, skeletal disorders and even tumors using 89Zr-oxalate and other complexes was previously demonstrated [1,2]. The aim of this study is to develop handy procedure for production of 89Zr-oxalate in physiologically acceptable form and evaluate its potential for PET diagnosis.

Methods: All chemicals and solvents were of high-purity or pharmaceutical grade and were purchased from Sigma-Aldrich or Panreac. Quality control was carried out with TLC chromatography (ITLC-SG – 50 mM DTPA solution and other eluents; PET-MiniGita radio-TLC scanner). Dowex 1, Chelex-100 (Sigma-Aldridge), ZR (Triskem), Chromafix HCO3 (Macherey-Nagel) resin were used. 89ZrCl4 in 5 M HCl was purchased from Cyclotron Ltd, Obninsk, Russian Federation. In vivo experiments were carried out in BALB/C mouse models with different pathologies. Wizard 2400 gamma-counter and G4 microPET (Sofie) were used for biological evaluation.

Results: It was found that 89Zr resin allows to obtain 89Zr-oxalate solution with highest yield and lowest metal impurities content. Chromafix HCO3 and Chelex-100 resins allow to obtain high purity as well. However, 89Zr-oxalate solution purified with 89Zr resin does not meet all the requirements for the PET radiopharmaceutical under development. Its virtues and shortcomings will be presented in detail. Using Chelex-100 resin allowed to develop the procedure for production of pH-neutral isotonic 89Zr-oxalate solution with high purity and specific activity. Radiochemical yield of the preparation and purification procedure is ≥90%. Radiochemical radiochemical purity remains ≥95% for at least 2 weeks. 89Zr complexes with other carboxylic acids were studied as well. Biological data indicate that 89Zr complexes with carboxylic acids are promising visualizing agents for infection and inflammation.
Aim: Radiolabeled peptides have become very important in nuclear medicine and oncology in recent years mainly because they represent the molecular basis for in vivo imaging and radiopharmaceutical therapy with high specificity and affinity for overexpressed receptors in tumors. The objective was to evaluate the radiolabeling efficiency of the $^{131}$I-peptides, as well as their interaction with overexpressed receptors on tumor cells related to glioblastoma.

Methods: The EEEEYFELV peptide and its analogue DEDEYFELV both with affinity for EGFR receptor and the fragment GRGDYV with high affinity for the integrin receptor were synthesized accordingly to the Fmoc protocol and purified by preparative HPLC. All the peptides were radiolabeled with the radioisotope $^{131}$I-1. The radioiodination was evaluated and optimized using the methodology of Chloramine-T. Radiochemical yield analyses of $^{131}$I-EEEYFELV and $^{131}$I-DEDEYFELV peptides were performed by Whatmann 3MM using MeOH 95% and by thin layer chromatography on silica gel TLC-SG (Al) in ACN 95% for $^{131}$I-GRGDYV peptide. The stability studies in vitro were realized at 2, 24, 48 and 72 hours in room temperature and refrigerate and in human serum at 37°C up to 24 hours. Partition coefficient and binding to plasma proteins were determinate for all radiopeptides. The interaction of radiolabeled peptides with tumorigenic cells was assessed by using culture cells (C6) and brain homogenate of the glioblastoma animal models ($n = 6$).

Results: The peptides EEEEYFELV, DEDEYFELV and GRGDYV were efficiently synthesized, radiolabeled and showed radiochemical yield of 90.32% ± 0.30, 94.67% ± 0.27 and 97.95% ± 0.05 ($n = 7$), respectively. The stability studies showed that all the peptides were stable within 72 hours when stored in the refrigerator and up to 24 hours in human serum showing an average of radiochemical purity of 91.65% ± 1.33 ($n = 3$) for all peptides. All $^{131}$I-peptides have hydrophilic features and showed a binding percentage to plasma proteins of 59.47% ± 0.74, 57.68% ± 1.29 and 34.92% ± 2.53 ($n = 3$). Furthermore, the peptides presented an interaction affinity with the tumorigenic cells of 4.48% ± 0.22; 4.40% ± 0.30 and 1.73% ± 0.38, respectively and a internalization of approximately 45% for all fragments.

Conclusion: The peptides were efficiently synthesized and the tested radiolabeling strategies showed successful results. Moreover, all the peptides demonstrated affinity for the tumor cells evaluated. These results obtained in this study are consistent to adapt in the clinical application.

Funding: FAPESP, CAPES and FCMSCSP (FAP).

Aim: Ubiquicidine (UBI) 29-41 is currently being investigated as a potential infection imaging agent. All published methods for radiolabelling of this tracer to date describe manual processes. The current manual method for labelling UBI 29-41 with Ga-68 has several disadvantages, including unnecessary radiation exposure to operators, and difficulty to meet GMP requirements. The aim of this study was to develop an automated synthesis method for the labelling of Ga-68 UBI.

Methods: Ga-68 for radiolabelling was freshly eluted from an iThemba Labs Ge-68/Ga-68 generator, using fractional elution with 0.6M HCl. NOTA-UBI was provided by BL Biochem (Shanghai, China). The approach to developing an automated method was to first duplicate the manual method developed by Ebenhan et al using the generator, eluant and consumables available at our PET Centre. Next, the manual method was adapted to suit a Scintomics protocol, e.g. adapting volumes for the synthesis unit. The radiolabelling yield and radiochemical purity were determined after each labelling experiment.

Results: Initial manual labelling attempts indicated that the volume (± 0.55 ml) of sodium acetate required to adjust the pH of the radiolabelling mixture was too small for use in an automated synthesis process. It was decided to use a 1.5 M HEPES buffer to adjust the eluate to an acceptable pH. Eluate volumes ranging from 1.0 to 2.0 ml were used to which 1.2 to 1.6 ml of HEPES buffer was added and the pH measured in order to find the most suitable combination of eluate volume and buffer volume to render a mixture with a pH between 3.5 and 4.0. Results indicated that 1.4 ml to 2.0 ml eluate and 1.2 ml HEPES buffer was suitable for use in the development of an automated synthesis method. Four successful automated labellings were performed using the HEPES buffer with an average decay-corrected radio-yield of 88.97% and a radiochemical purity of 91.65% for $^{131}$I-GRGDYV peptide. The stability studies in vitro were realized at 2, 24, 48 and 72 hours in room temperature and refrigerate and in human serum at 37°C up to 24 hours. Partition coefficient and binding to plasma proteins were determined for all radiopeptides. The interaction of radiolabeled peptides with tumorigenic cells was assessed by using culture cells (C6) and brain homogenate of the glioblastoma animal models ($n = 6$).

Results: The peptides EEEEYFELV, DEDEYFELV and GRGDYV were efficiently synthesized, radiolabeled and showed radiochemical yield of 90.32% ± 0.30, 94.67% ± 0.27 and 97.95% ± 0.05 ($n = 7$), respectively. The stability studies showed that all the peptides were stable within 72 hours when stored in the refrigerator and up to 24 hours in human serum showing an average of radiochemical purity of 91.65% ± 1.33 ($n = 3$) for all peptides. All $^{131}$I-peptides have hydrophilic features and showed a binding percentage to plasma proteins of 59.47% ± 0.74, 57.68% ± 1.29 and 34.92% ± 2.53 ($n = 3$). Furthermore, the peptides presented an interaction affinity with the tumorigenic cells of 4.48% ± 0.22; 4.40% ± 0.30 and 1.73% ± 0.38, respectively and a internalization of approximately 45% for all fragments.

Conclusion: The peptides were efficiently synthesized and the tested radiolabeling strategies showed successful results. Moreover, all the peptides demonstrated affinity for the tumor cells evaluated. These results obtained in this study are consistent to adapt in the clinical application.

Funding: FAPESP, CAPES and FCMSCSP (FAP).

Aim: The current treatment methods such as chemotherapy have limited effects on cancer or other cells because of using conventional cytotoxic drugs. For this reason, using multifunctional nanoparticles in drug delivery systems become more important.[1,2] Some drug molecules are encapsulated nanoparticles as drug delivery systems.[3] Ampicillin is a one of effective antibiotics against Gram-positive and Gram-negative bacteria. The present study aims to label ampicillin loaded graphene oxide nanoflake (AMP-GO) with $^{99m}$Tc and evaluate of its in vitro binding to S. aureus and E. coli.

Methods: Graphene oxide nanoflake were prepared according to Hummers’ method.[4] Ampicillin was loaded onto graphene oxide nanoflake prepared. AMP-GO was characterized by Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM) techniques, and the amount of loaded ampicillin onto GO was determined by UV-Vis absorption spectroscopy. AMP and AMP-GO were labeled with $^{99m}$Tc using stannous chloride reducing agent. The stability of $^{99m}$Tc-AMP and $^{99m}$Tc-AMP-GO were determined in phosphate buffered saline (pH=7.2). The in vitro bacterial binding studies were performed by using both radiolabelled AMP and AMP-GO to S. aureus and E. coli.

Results: Labeling efficiency of $^{99m}$Tc-AMP was 92.6±5.94 % while labeling efficiency of $^{99m}$Tc-AMP-GO was found to be 97.6±2.06%. The stability results of radiolabeled compounds in phosphate buffered saline are summarized in Fig. 1. In vitro binding results of $^{99m}$Tc-AMP/$^{99m}$Tc- AMP-GO to S. aureus are seen in Fig. 2. In vitro binding results of $^{99m}$Tc-AMP/$^{99m}$Tc- AMP-GO to E. coli are seen in Fig. 3.

Conclusion: AMP and AMP-GO were labeled with $^{99m}$Tc with high yield. According to in vitro binding results, the binding efficiency of $^{99m}$Tc-AMP-GO was higher to S. aureus and E. coli than $^{99m}$Tc-AMP. $^{99m}$Tc-AMP-GO could be promising candidate as agent infection nuclear imaging. Furthermore, in vivo studies of $^{99m}$Tc-AMP-GO with infected rats are planned to be done.

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PP32  
**Room-temperature radiolabeling can be achieved by Al$^{18}$F chelation**  
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*EJNMMI Radiopharmacy and Chemistry* 2018, 3(Suppl 1):PP32  

**Aim:** $^{18}$F is the most commonly used radionuclide for PET imaging. Its half-life of approximately 110 min is suited for same-day imaging of many compounds that clear quickly from the body to allow visualization of uptake in the intended target. Recently, $^{18}$F-labeling method using Al$^{3+}$ complex in aqueous solution was devised and offered a straightforward $^{18}$F-labeling procedure. The radiolabeling of various chelates with Al$^{18}$F at high temperature ($\sim$100-110°C) is well documented in literature. But high temperature radiolabeling is unsuitable for heat sensitive biomolecules. So facile method for radiolabeling at lower temperature is highly needed. We examine the efficiency of Al$^{18}$F chelation by a variety of available macrocyclic chelators at low temperature (Fig. 1).

**Methods:** $^{18}$F$^{-}$ aqueous solution was first treated using a Sep-Pak cartridge equilibrated with 0.4 M KHCO$_3$. The sample was then loaded, washed with water and eluted with 200-$\mu$L fractions. The radiolabeling was performed by adding 10 $\mu$L of the 2 mM Al$^{3+}$ stock solution, 10 $\mu$L of the chelator stock solution, and ~500 $\mu$Ci Na$^{18}$F to a vial containing 100 $\mu$L 0.1 M ammonium acetate buffer (pH 5.5) solution and 100 $\mu$L ethanol at different temperature.

**Results:** It was found that, chelators with TACN backbones bearing two acetate pendant arms and one benzyl-NCS moiety (NODA-MP-NCS) resulted quantitative labeling in a temperature as low as 30 °C within 2 min.

**Conclusion:** These results represent an important step toward rapid and efficient radiofluorination of biomolecules at room temperature.

**Acknowledgements**  
This work was supported by NRF (2016R1A2B4011546, 2013R1A4A1069507, 2017M2C2A1014006, 2017M2A2A6A02018506, H17C0221) and BK21 Plus KNU Biomedical Convergence Program, Korea.

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trapping [11C]MeI in a solution of peptides was determined in FBS, and the in vivo stability test was a standard Fmoc method and an extra tyrosine residue was added.

**Methods:** [11C]MeLi was prepared via lithium-halogen exchange by trapping [11C]MeLi in a solution of n-BuLi. The prepared [11C]MeLi was further used in a palladium catalyzed cross-coupling reaction with aryl bromides.

**Results** Using this procedure, three classes of tracers with applications in amyloid plaques imaging, breast cancer imaging and VACHT imaging had been successfully labelled. Radiolabeled products were obtained in good yields with high radiochemical purity (>99%).

**Conclusion:** A new labeling methodology was developed and successfully applied to the synthesis of clinically interesting radiotracers. This procedure fulfilled essential requirements for the introduction of carbon-11, providing the target molecule in high yields and high radiochemical purity within 30 to 40 minutes from the end of bombardment (EOB).

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**PP34**

**Effect of structural forms on the stability of linear and cyclic apoptosis-targeting peptides**

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP34

**Aim:** Apoptosis, a genetically determined process of programmed cell death, has a crucial role in various processes including normal cell turnover, tissue homeostasis, and development. Moreover, apoptosis occurs in many medical disorders and hence the development of non-invasive evaluation method is highly demanded. We have reported high potential of the radiolabeled apoptosis-targeting peptide (ApoPep-1) for apoptosis detection, however in vivo stability of radiolabeled peptide was not acceptable to monitor apoptosis for a long time [1]. In this study, we prepared cyclic ApoPep-1 peptides (Fig. 1) to compare the stability with original linear ApoPep-1. A targeting ability of cyclic ApoPep-1 peptide for apoptosis was also investigated in acute myocardial infarct model.

**Methods:** Linear and cyclic ApoPep-1 peptides were synthesized by a standard Fmoc method and an extra tyrosine residue was added for labeling with radio-iodine. The in vitro stability of the radiolabeled peptides was determined in FBS, and the in vivo stability test was performed by blood analysis. To evaluate its targeting efficacy of radiolabeled cyclic ApoPep-1, autoradiography was done in isoprenaline-induced acute myocardial infarct model.

**Results:** All linear and cyclic peptide was synthesized and radiolabeled with I-131 in good yield. The radiolabeled peptides showed significant difference in vitro stability at 24 h (linear ApoPep-1: 72%, cyclic ApoPep-1: ~95%). In vivo stability of radiolabeled linear and cyclic peptides at 1 h showed drastic difference between linear and cyclic peptides (linear ApoPep-1: 60%, cyclic ApoPep-1: 87%). Apoptosis induced in heart by myocardial infarction was clearly detected by the radiolabeled cyclic ApoPep-1.

**Conclusion:** Effect of structural forms on the stability of linear and cyclic ApoPep-1 was demonstrated.

**Acknowledgement**
This work was supported by NRF (2016R1A2B4011546, 2013R1A4A1069507, 2017M2C2A1014006, 2017M2A2A6A02018506, 2017R1D1A1B03033974, H17C0221, & KRF 7072016H1D3A1907667) and BK21 Plus KNU Biomedical Convergence Program, Korea.

**Reference**
1. Wonjung Kwak, et al (2015), Apoptosis, 20, 110-21.

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**PP35**

**Synthesis and evaluation of a [18F]fluorinated quaternary α-amino acid-based arginase inhibitor**

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP35

**Aim:** Arginase catalyzes the hydrolysis of arginine to ornithine and urea, being the final enzyme of the urea cycle, which is a ubiquitous pathway to excrete toxic ammonia from organisms. Lately, it also has emerged as a key regulator of nitric oxide by competing with NO synthase for the same substrate. Therefore, arginase overexpression has been associated with a series of pathogenic processes that can go from cardiovascular, immune-mediated or inflammatory conditions to tumor cell metabolism.11 Several research groups have been involved in the development of small-molecule arginase inhibitors reaching to data holding great promise. However, only recently the drug development industry is attaining clinical trials.[2] Since the association to PET labeling techniques has great relevance not only for the evaluation and characterization of some of these molecules, but also to increase the library of radiotracers available, our goal with this work was to synthesize and evaluate the [18F]fluorinated equivalent of a late-generation arginase inhibitor.[3]

**Methods:** An arylboronic acid pinacol ester-derived precursor was synthesized in view of a Cu-mediated nucleophilic [18F]fluorination. Radiochemical yield for the conversion of the intermediate species was assessed by TLC-SG and/or radioHPLC. Deprotection of the amino acid moiety was achieved by hydrolysis and the final radiochemical purity was determined in FBS, and the in vivo stability test was performed by blood analysis. To evaluate its targeting efficacy of radiolabeled cyclic ApoPep-1, autoradiography was done in isoprenaline-induced acute myocardial infarct model.

**Results:** All linear and cyclic peptide was synthesized and radiolabeled with I-131 in good yield. The radiolabeled peptides showed significant difference in vitro stability at 24 h (linear ApoPep-1: 72%, cyclic ApoPep-1: ~95%). In vivo stability of radiolabeled linear and cyclic peptides at 1 h showed drastic difference between linear and cyclic peptides (linear ApoPep-1: 60%, cyclic ApoPep-1: 87%). Apoptosis induced in heart by myocardial infarction was clearly detected by the radiolabeled cyclic ApoPep-1.

**Conclusion:** Effect of structural forms on the stability of linear and cyclic ApoPep-1 was demonstrated.

**Acknowledgement**
This work was supported by NRF (2016R1A2B4011546, 2013R1A4A1069507, 2017M2C2A1014006, 2017M2A2A6A02018506, 2017R1D1A1B03033974, H17C0221, & KRF 7072016H1D3A1907667) and BK21 Plus KNU Biomedical Convergence Program, Korea.

**Reference**
1. Wonjung Kwak, et al (2015), Apoptosis, 20, 110-21.
Results: In summary, radiochemical yield of the conversion of the arylboronic ester-derived precursor reached ~80% when using 60 to 15 μmol (lower amounts brought significant losses in yield). Final [18F]fluorinated compound was obtained with radiochemical purity ≥95% in an overall yield of 11% (d.c., non-automated synthesis). The radiotracer showed stability in solution up to 4 h and an experimental log D of -0.67±0.05.

Conclusion: The [18F]fluorinated arginine inhibitor was efficiently labeled with fluorine-18 in good yield. Preliminary in vitro studies using R22v1 cell lines, which express Arg2, revealed a cellular uptake of the radiotracer susceptible of being blocked after treatment with arginine inhibitors. Further studies are currently being performed in different cell lines which either overexpress Arg1 or Arg2 to evaluate the potential of the developed radiotracer towards arginine mapping.

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PP37 Physico-chemical assessment of labeled freeze dried kits of trastuzumab-immunoconjugates significant for breast cancer therapy

Aim: Monoclonal antibodies appears as an important therapeutic agents for cancer treatment and have shown high complexity in the manner of action and their biological properties. Due to the significant potency in various malignancies and easy detection of radioactivity with outside scintigraphy, radioimmunoconjugates have become a part of many clinical trials. The antibody manipulation and exposure to stress conditions during the processes of conjugation, lyophilization and labeling, can cause disruption of the native structure of the protein. The most appropriate technique for protein integrity and purity examinations is reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Attenuated total reflectance-infrared (ATR-IR) and Raman spectroscopy as powerful, non-destructive and easy-to-use techniques provide valuable molecular structure information and are convenient for verification of changes in the secondary structure. The successful experience in formulation of ready-to-use kits of rituximab-immunoconjugates, inspire us to use another attractive monoclonal antibody for therapy of HER2 positive breast cancer. The same protocols, with minor adjustments, were implemented for structural characterization of labeled and non-labeled lyophilized trastuzumab-immunoconjugates formulations.

Methods: The purified trastuzumab from Herceptin® was conjugated with bifunctional chelators (BFCAs), p-SCN-Bn-DTPA, p-SCN-Bn-DOTA, p-SCN-Bn-14BAM-DTPA in ratio of 1:20 and lyophilized to solid state. The freeze dried conjugates and cold labeled samples with LuCl3 and YCl3, were used for further examinations and physico-chemical characterization by applying of SDS-PAGE, ATR-IR and Raman spectroscopy.

Results Under reducing conditions migration of the trastuzumab provided separation of two bands of fragments with molecular weight of 25 kDa for light chain and 50 kDa for heavy chain, proven with molecular weight marker. The same intensity of the fragments of lyophilized and labeled conjugates with the fragments of pure trastuzumab was indicated that there is no degradation of the antibody. ATR-IR and Raman spectra also have indicated that all samples have retained native structure expressed in terms of assignment of the amide bands (amide I, II and III bands), characteristic for

Fig. 1 (abstract PP35). See text for description
IgG1 structures principally composed of β-sheets. Characteristic amide I band at ~1670 cm⁻¹ and amide III band (1230-1300 cm⁻¹) were detected in Raman spectra. IR spectra also contain the amide I (1700-1600 cm⁻¹), amide II (1480-1575 cm⁻¹) and amide III bands (1230-1300 cm⁻¹) specific for secondary structure of the proteins.

**Conclusion:** The promising results from electrophoresis and vibrational spectroscopy are good basis for further radiolabeling of immunoconjugates with ¹⁷⁷Lu and ⁹⁰Y for treatment and imaging of HER2 positive lesions.

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**PP38**
Development of activity-based PET probes for selective detection of active caspase-3
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**EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP38**

**Aim:** Apoptosis represents the most abundant type of regulated cell death, and plays a key role in disease treatment. Many therapies targeted at promoting tumor apoptosis are currently in use for the treatment of cancer patients. Therefore, assessment of response to anticancer therapies can be achieved by readout of apoptosis in tumors. Non-invasive molecular imaging of therapy-induced apoptosis can be achieved by using positron emission tomography (PET) radiotracers that specifically target hallmarks of the apoptosis process such as the activation of caspase-3. We therefore aimed to develop [¹⁸F]labeled activity-based probes (ABPs) to selectively monitor caspase-3 activity by PET imaging.

**Methods:** Our concept is based on turning selective and potent peptide-based inhibitors into ABPs. For this Hyx-(Ahx)₂-DW₃-KE was developed bearing an optimized peptide sequence (DW₃) for the selective detection and inhibition of caspase-3 in cells, and an electrophilic center (warhead, KE) for covalent binding to the enzyme active site. Radiolabeling of Hyx-(Ahx)₂-DW₃-KE with [¹⁸F] was performed using the copper-catalyzed azide-alkyne cycloaddition reaction. The binding efficiency of the [¹⁸F]-labeled ABP, [¹⁸F]MICA-304, to human caspases will be evaluated using SDS-PAGE analysis. Radiotracier metabolic stability will be evaluated in vitro. The in vitro affinity and selectivity of the non-radioactive ABP will be profiled against a panel of human caspases.

**Results** “Click radiolabeling” was used to obtain [¹⁸F]MICA-304 from [¹⁸F]fluoroethylazide in a radiochemical yield ranging from 55 to 81% (based on HPLC analysis of the crude product, Table 1).

**Conclusion:** Optimal radiochemical reaction conditions were defined to be 10 minutes at room temperature. In vitro and preliminary in vivo data will be reported. [¹⁸F]MICA-304 is a candidate ABP for selective detection of caspase-3 activity, and will be modified for in vivo PET imaging in mouse models of apoptosis.

**Table 1 (abstract PP38). Radiochemical yield of [¹⁸F]MICA-304**

| Reaction time | Amount of precursor | Radiochemical yield (%) |
|--------------|--------------------|-------------------------|
| 10 min       | 4 mg               | 81%                     |
| 20 min       | 8 mg               | 76%                     |

**PP39**
Chemical analysis of cyclotron-based [⁶⁸Ga]GaCl₃ by ICP-MS
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP39

**Aim:** Current limitations with existing [⁶⁸Ga]GaCl₃ generators have led to a recent interest in the direct cyclotron production of [⁶⁸Ga]Ga by the [⁶⁸Zn(p,n)⁶⁸Ga] reaction. To this end, we recently presented a new automated two-column scheme to isolate [⁶⁸Ga]GaCl₃ following proton irradiation of enriched [⁶⁸Zn]Nair, M. et al [2017], Eur J Nucl Med Mol Imaging 44: S119–S596. Optimization of this process was focused not only on maximizing [⁶⁸Ga]Ga recovery, but also on meeting specifications (e.g. <10 μg/GBq iron and zinc) of the European Pharmacopoeia (EUP) generator-based [⁶⁸Ga]GaCl₃ monograph. A detailed assessment of other chemical impurities was, however, considered warranted and forms the basis for this study.

**Methods:** A selection of sample retains (Zn Lot A: Feb-May, 2017; Zn Lot B: Aug-Oct, 2017) from experiments performed on a GE PETtrace [⁶⁸Ga] liquid target/FASTlab system were submitted for ICP-MS analysis (ALS Scandinavia). Samples were either irradiated (but not chemically processed), or, samples were chemically isolated as ~2.8 mL (ALS Scandinavia). Samples were either irradiated (but not chemically processed), or, samples were chemically isolated as ~2.8 mL (ALS Scandinavia). Samples were either irradiated (but not chemically processed), or, samples were chemically isolated as ~2.8 mL (ALS Scandinavia).

**Results:** Click reaction mixture: 2.2 eq. CuSO₄; 8.2 eq. sodium ascorbate; 1.1 eq. bathophenanthroline disulfonate. *Using half of the amount of the click reaction mixture.

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**EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP39**
Conclusion: While the levels of Zn and Fe have been optimized to meet EUP specifications, a detailed assessment of additional chemical impurities has revealed that the starting contribution (i.e. post irradiation, but pre-chemical processing) for impurities of potential concern is generally quite low – e.g. at most ~1-2 μg/mL, with post-purification reducing such impurities to the single-to-low-ten of ng/mL range. This analysis supports the current applicability of cyclotron-based production of 68Ga and anticipated robustness as novel ligands and chelators are developed and introduced.

Table 1 (abstract PP39). See text for description

| Zn (administration) | Zn (administration) |
|---------------------|---------------------|
| Isolated | Isolated |
| (n=3) | (n=9) |
| 48583 ± 365 | 51138 ± 1066 |
| 118 ± 11 | 147 ± 152 |
| 37 ± 20 | 22 ± 24 |
| 3 ± 3 | 2 ± 25 |
| 21 ± 35 | 18 ± 34 |
| 9 ± 12 | 33 ± 192 |
| 46 ± 3 | 1940 ± 87 |
| 0.17 ± 0.12 | 0.24 ± 0.12 |
| 0.98 ± 0.13 | 1.03 ± 0.12 |
| 1 ± 0.5 | 1 ± 0.55 |
| 0.14 ± 0.11 | 0.14 ± 0.11 |
| 21 ± 29 | 21 ± 29 |
| 0.22 ± 0.12 | 0.22 ± 0.12 |

PP40 Automation of Click Chemistry for the synthesis of 18F-labelled PSMA-tracers using the FlowSafe

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP40

Aim: Automated PET-tracer syntheses are essential to enable productions in a quick and robust way for (pre)clinical studies [1]. In this study, azide-alkyne Huisgens click couplings of 18F-labelled azide building blocks (azide-BB) with acetylene-ligands are used for the development of 18F-labelled PET-tracers for diagnosis of prostate cancer by targeting prostate specific membrane antigen (PSMA). Due to purification issues of the azide-BB, the radiochemical yields (RCY) of these click reactions (CR) were low. Automated microfluidic systems, such as the FlowSafe, could improve these syntheses. The advantages of 18F-labeling in flow-mode comprises the more dynamic mixing and heating of the azide-BB precursor and 18F-fluoride, leading to higher yields and less side-products due to shorter reaction times and higher surface-to-volume ratios [2]. Therefore, we elaborated an automated synthesis route for 18F-labelled PSMA-tracers (18F-PSMA-Tracer) in the FlowSafe, using a 2-steps procedure in which an azide-BB is 18F-fluorinated in a microreactor, purified by Solid Phase Extraction (SPE) and subsequently underwent an in-batch CR.

Methods: Azeotropically dried [18F]fluoride and the precursor for azide-BB are redissolved in acetonitrile, transferred through a 100μL glass microreactor with a flowrate of 80μL/min at 110oC, giving an effective reaction time of 75s. A back-pressure regulator adjusted the pressure inside the microreactor to 5.0bar and increased the boiling point of acetonitrile. The intermediate is dissolved in an excess of water and transferred to an Oasis HLB Plus-cartridge. It is eluted with ethanol and collected in a vial containing click reagents and PSMA-targeting acetylene-ligand. CR is performed in a batch-reactor in at least 50% water, heated to 80oC for 20min and subsequently purified by HPLC.

Results: Manually 18F-fluorination of the azide-BB gave a RCY of 21%. Performing the 18F-fluorination of the azide-BB in the FlowSafe reduced radiochemical impurities from 2.7% “by hand” to 0.3%. With the purer intermediate, the SPE purification-method was improved, providing the azide-BB in >99% radiochemical purity and a RCY of 39%. With this purified azide-BB, a CR was performed yielding a 18F-PSMA-tracer with an isolated RCY of 5%. The same azide-BB was successfully coupled in-batch by CR in the FlowSafe.

Conclusions: Automation of 18F-PSMA-tracer synthesis was successful. Less side-products were formed during the 18F-fluorination of the azide-BB in the microreactor, which simplified the purification and led to an improved RCY. With the purified 18F-labelled azide-BB, a novel 18F-PSMA-tracer was successfully coupled in-batch by CR in the FlowSafe.

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PP41 [18F]-FEPPA, a 2nd generation of TSPO radioligand: optimized radiosynthesis and quality control

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP41

Aim: [18F]-FEPPA, a radiotracer specific for TSPO (18 kDa translocator protein), is used in PET imaging (Positron Emission Tomography) as a biomarker of cerebral inflammation. Literature [1] describes the use of methanol and formic acid in the mobile phase during semi-preparative HPLC purification. Aims of this work were to implement a radiosynthesis of [18F]-FEPPA using HPLC purification more suitable for in vivo use and to optimize the analytical HPLC control method.

Methods: [18F]-FEPPA is synthesized by nucleophilic substitution (90° C, 10 min) from a tosylateprecursor (N(2)-(4-phenoxypyridin-3-yl)acetamido)(methyl)phenoxyethyl-4-methylenesulfonate followed by semi-preparative HPLC purification (mobile phase: ethanol/water for injection 70/30 v/v + 0.1% phosphoric acid, FEPPA retention time = 13 min) on an AllInOne™ radiosynthesis automate. Chemical, radiochemical purity (RCP) and specific activity (SA) are determined by analytical HPLC (Kinetex® C18 column (50 x 2.1 mm, 2.6 μm), injection volume: 5 μL, mobile phase: H2O/CH3CN 75/25 v/v at 0.6 mL/min).

Results [18F]-FEPPA radiosynthesis were carried out in 49 min with a non-decay corrected yield enhanced from 19 to 29%. The RCP obtained was greater than 99%. The mean SA was 198 GBq/μmol. The new analytical HPLC method allowed a quality control in 7 min. Solvent consumption and injection volume have been reduced by a factor 12 and 4 respectively. This new control method induced a 3-fold decrease of the received dose by the operator in terms of ionizing radiation.

Conclusion: [18F]-FEPPA radiosynthesis was implemented with high yields. The final product was obtained in a new formulation more suitable for in vivo studies. Operator exposure was improved with the new faster control method.
PP42
Bioorthogonal chemistry applied to bispecific antibody manufacturing
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Aim: Bispecific antibody (BsAb) can be obtained both chemically and bio-engineered for imaging and therapeutic applications in nuclear medicine1-2, if all the described tools are satisfactory for imaging, the in vivo instability of engineered BsAb or the poor yield of BsAb obtained by chemically limit their use in radioimmunotherapy (RIT). We propose in this study a new way to generate BsAb using click chemistry tools.

Methods: The 9E7.4 IgG2a (anti-CD138) antibody was chosen for proof of concept. 9E7.4 was first modified with 100-500 equivalents of N-ethylmaleimide (NEM) to saturate the accessible thiol groups on the antibody surface. The modified antibody was then purified by gel filtration chromatography. The modified (9E7.4-NEM) and unmodified antibodies (9E7.4) were cleaved with optimized pepsin quantities under acidic conditions to give (Fab')2 fragments. The (Fab')2 obtained were then reduced with 2-mercaptoethylamine to generate the Fab fragments. These fragments were separated into two fractions and functionalized with dibenzocyclooctyne (DBCO) for the first fraction and with azide (N3) for the second one. Both functionalized Fab' were then mixed 1 hour or 1 month after production to obtain BsAb in order to validate their stability and their reactivity over time. 9E7.4 IgG, 9E7.4-NEM IgG, 9E7.4 (Fab')2, 9E7.4-NEM (Fab')2 and BsAb were monitored by UPLC and electrophoresis for chemical purity, and after 131I radiolabeling for antigen recognition.

Results Immunoreactivity was verified after each step described above. For BsAb, the antibody tested had the best final yield (20%) and the lowest aggregates percentage corresponding to a pre-saturation with 100 eq NEM and addition of 20 eq of DBCO and 20 eq of N3 for bioorthogonal chemistry. The results are summarized in the following Table 1.

Conclusion: We demonstrated that click chemistry can be used to generate BsAb and that cysteine pre-saturation by NEM limits the formation of aggregates during the antibody functionalization. The yields obtained are comparable to those described in the literature with phenylethanesulphimide30. The immunoreactivity decrease is probably due to the large spacing between the Fab' fragments provided by the PEG chains. This problem can be solved by the use of new click modifiers presenting a minimal distance between the fragment and the click function.

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Table 1 (abstract PP42). See text for description

| Antibody  | IgG| IgG-NEM | IgG-NEM | IgG-NEM | IgG-NEM | (Fab')2-NEM | (Fab')2-NEM | BsAb-NEM | BsAb-NEM |
|-----------|----|---------|---------|---------|---------|--------------|--------------|----------|----------|
| 9E7.4     | 100| 100     | 500     | 500     | 100 eq  | 100 eq NEM/ NEM | 100 eq NEM/ NEM | 2h | 2h |
|           | eq | eq      | eq      | eq      | eq      | eq 2h        | eq 2h        | 2h        | 2h        |

Immunoreactivity (%) 73 70 71 52 45 74 73 45

PP43
A fast and robust quantification of residual solvents in radiopharmaceuticals by UHPLC
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP43

Aim: Radiopharmaceutical preparations for human purpose may contain residual solvents. Headspace Gas Chromatography (HS-GC) remains the standard method for the analysis of volatile solvents, according to the European Pharmacopoeia. Dimethyl sulfoxide (DMSO), dimethyl formamide (DMF) and dimethyl acetamide (DMA) are less volatile solvents that are widely used in carbon-11 and fluorine-18 labeling. However, in that case, HS-GC quantification takes too much time for the release of short half-lives radiopharmaceuticals. Therefore, we developed a fast and robust Ultra High Pressure Liquid Chromatography (UHPLC) method for the simultaneous quantification of those 3 solvents.

Methods: Analyses were carried out on an UHPLC (Waters) using a BEH C18 column (Waters). Validation was performed within the requirements ofICH validation guidelines (Q2A, Q2B and Q3C) and based on Good Laboratory Practices (GLP). It consisted in the evaluation of linearity, limit of quantification (LOQ), limit of detection (LOD), selectivity, specificity, carry-over, accuracy, precision and matrix effect.

Results The raw data were collected on six different days with a minimum of five days of exploitable data for each solvent. Separation of the three solvents can be achieved within 4 minutes with high resolution. Solvents in samples can be quantified with good inter and intraday accuracy and precision. LOQ is far below the permitted daily exposure of each solvent. Selectivity and specificity were satisfactory. No matrix effect was observed.

Conclusion: This fast UHPLC method is routinely used for the quality control of radiopharmaceuticals produced in our facilities. Moreover, stability of stock solutions and frozen quality controls is achieved for at least 10 months. The use of a 98% aqueous mobile phase reduces the risk of coelution of radiolabeled compounds which makes this method suitable with most of them.

PP44
Comparison between two octreotide derivatives for somatostatin receptor scintigraphy
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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP44

Aim: Somatostatin receptor scintigraphy reveals various types of neuroendocrine tumors. The radiotracer historically used is Indium-111 pentetreotide (111In-DTPA-Phe-Octreotide) = Octreoscan®. It is an analog of somatostatin’s specific membrane receptors labeled with Indium111. A new derivative of octreotide coupled to Technetium 99m (99mTc-HYNIC-[D-Phe,Tyr-octreotide) = Tektrotyd® is marketed. In this study, we propose to compare between these two products according to our experience.

Methods: This comparative study focused on several criteria: method of preparation and administration of the product, dosimetry, patient examination preparation, image acquisition and quality. For this, we relied on the summary of products characteristics and on the examinations made at the department.

Results Both products require extemporaneous labeling; Tektrotyd® nevertheless has some preparation steps in addition to Octreoscan®. The preparations quality control is carried out for both of them by the same method (Thin Layer Chromatography) which is convenient for the hospital routine. The dosage varies from one product to another. Therefore, for an adult, the effective dose received for the maximum dose of 220 MBq of 111In-pentreotide is 12 mSv while 740 MBq of 99mTc-Octreotide gives an effective dose of 3.7 mSv. Image
acquisition is done the day of the injection for Tektrotyd® (after 4 hours) unlike OctreoScan®, where the acquisition is done after 24 hours. Patient exam preparation is done the same way for both products. Our physicians noticed that scintigraphy performed with Tektrotyd® showed a better image quality making it easier to interpret.

Conclusion: Technetium 99m is a gamma emitter (141kev) with short half-life (6 hours), while Indium 111 is a 94% gamma emitter (247kev) with a longer half-life (2.8 days). From a dosimetric point of view, Indium 111 is much more irradiant. Thus Tektrotyd® is more convenient to use either for patients or for the staff. Some studies demonstrated that both products are equally effective despite all these differences. In our department, doctors reported a better image quality with Tektrotyd®. The fact that Tektrotyd® is presented in the form of cold kit with Technetium 99m labeling, with less radiation and better images’ quality than Indium 111, makes us choose it for scintigraphy of neuroendocrine tumors in our department.

**PP45**

**Comparison of automated and manual labeling methods for somatostatin analogues with the example of DOTANOC and Somakit TOC®**

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP45

**Aim:** 68Ga-edotetritide, PET-agent for somatostatin receptor imaging in GEP-NET, has recently obtained a marketing authorization as a radiopharmaceutical kit (Somakit TOC®). We wanted to compare the performance, ease of realization, radiation protection parameters of automated labeling of DOTANOC as compared to the labeling of DOTATOC (using Somakit TOC) with Ga-68.

**Methods:** Automated radiolabeling of DOTANOC was performed on the ModularLab PharmTracer® module (Eckert & Ziegler) with C4-Ga68-PP cassette. Process is fully automated and includes generator elution, cation exchange post-processing, heating, purification on C18 cartridge and dilution with sterilizing filtration. Manual preparation of Somakit was performed according recommendations and with equipment supplied by AAA. The generator is eluted with a syringe pump directly into the Somakit vial. Buffer is then added by hand before transferring the vial to a heating block with a forceps. Radiochemical purity (RCP) was evaluated with reverse HPLC for DOTANOC and TLC for Somakit. Overall yields, including elution yield and synthesis yield, were calculated corrected for decay in order to compare the labeling performance. Hand radiation exposure was evaluated using APLV-ED3 detector with two probes fixed on the first phalanx of each middle finger.

**Results**

Overall yields were respectively 57.9 +/- 11.3 % (n = 157) and 64.8 +/- 5.6 % (n = 35) respectively for DOTANOC and Somakit. RCP was always > 98 % for DOTANOC and 96.9 +/- 6.9 % for Somakit include 5 (15 %) non-compliant RCP. To note that 30 to 35 min are required between generator elution and batch release for both processes, but preparative step lasts at least 30 min for DOTANOC versus less than 10 min for Somakit. The radiation exposure measurements (extrapolated for a generator at calibration) gave 70 and 132 μSv for the middle finger (left and right hand) and 179 and 152 μSv respectively for DOTANOC and Somakit.

**Conclusion:** Somakit radiopharmaceutical kit not only allowed to turn the preparation of 68Ga-DOTATOC into a straightforward routine process, but it also led to an increase in the overall synthesis yield compared to DOTANOC. However, the lack of purification step in Somakit radiolabeling process prevents its injection to patient in case of incomplete complexation of Ga-68. Also, process including purification provides PRC consistently higher for DOTANOC vs. Somakit. Shifting from an automated to a manual process in routine also raises the question of radiation safety. This last encourage automation of Somakit labeling which should also improve the reproducibility and allow moving towards full GMP. This work is in progress.

**PP46**

**Optimization of automated 68Ga-PSMA-11 preparation by switch from tubing to GMP synthesizer**

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP46

**Aim:** The value of 68Ga-PSMA-directed PET imaging as a diagnostic procedure for recurrent prostate cancer is assessed and more particularly in early biochemical relapse. Synthesis of 68Ga-PSMA-11 is relatively straightforward due to HBED-CC chelation allowing significant synthetic yield even at room temperature. Automation of 68Ga-PSMA-11 synthesis can nevertheless be optimized to increase overall radiochemical yield i.e. increase of the available activity to inject more patients by synthesis during the shelf life of gallium-68 generator.

**Methods:** PSMA-11 was purchased from IASON and gallium-68 chloride was obtained by elution of GalliaPharm® generator. Current preparation process has been developed on the ModularLab Standard® (Eckert&Ziegler AG, Berlin) tubing synthesizer in a procedure similar to that described by Ceci et al. Briefly, the process includes an acetone-free cation exchange post-processing purification (Mueller et al.), 95°C heating for 5 min in acetate buffer (2 M, pH 4.5) and purification on disposable Sep-Pak C18 Plus® cartridge (Waters SASS France) by elution with 2 mL ethanol/water (1:1). The resulting product is sterilized by filtration and diluted in physiological saline. Then, the process was transferred on the ModularLab PharmTracer® (Eckert&Ziegler AG) cassette-based synthesizer with the C4-Ga68-PSMA cassette and the same steps and reagents as above. Radiochemical purity was evaluated by reversed-phase HPLC.

**Results**

Up to now, 62 syntheses were performed on the tubing synthesizer. Mean observed radiochemical yields (RCY) was 86.1 +/- 4.6 % and mean radiochemical purity (RCP) was 99.0 +/- 0.9 %. Among 10 % of the initial activity is retained on the C18 cartridge. With this process, synthesis is completed in 14 min. Validation of the GMP-cassette synthesis is ongoing. First results were 96.8 % RCY and 99.2 % RCP in 17 min process.

**Conclusion:** Optimization of the process was motivated by safety improvement by using GMP cassettes. Transfer from tubing to cassette module lead also to an increase in RCY. This is explained by the opportunity of using a Sep-Pak C18 Light® cartridge with a lower residual 68Ga-PSMA-11 retention. In spite of the intrinsic longer time process, the final product activity is increased and should allow to inject more patient per batch.

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**PP47**

**Effective treatment of microscopic cancers with Tb-161**

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP47

**Aim:** Prostate cancer is one of the most common cancers among men. Prostate specific membrane antigen (PSMA) has proven to be a useful diagnostic and therapeutic agent in radiotherapy. PSMA-617 is currently one of the most interesting molecules that binds specifically to the PSMA-receptor. 177Lu and 161Tb are beta emitting radionuclides with similar radiochemical properties. In addition, Tb-161 emits
a high number of low energetic conversion- and auger-electrons. Therefore, Tb-161 could be a promising radionuclide for treatment of small tumors and metastases.

**Methods:** PSMA-617 was purchased from ABX, Germany, and used without further modifications. The PSMA-617 was radiolabeled with \(^{177}\)Lu (ITG, Germany) and \(^{161}\)Tb (IFE, Norway). Cell toxicity studies with \(^{177}\)Lu-PSMA-617 and \(^{161}\)Tb-PSMA-617 were conducted on the cell line LVCap (ATCC, USA).

**Results** In this study, PSMA-617 was labeled with Lu-177 and Tb-161 using different reaction conditions to optimize radiochemical yield and purity. The formed complexes \(^{177}\)Lu-PSMA-617 and \(^{161}\)Tb-PSMA-617 were compared with regard to specific activity, stability and shelf-life.

**Conclusion:** \(^{177}\)Lu-PSMA-617 and \(^{161}\)Tb-PSMA-617 complexes have been obtained in high radiochemical yield and purity. In the next step, we will use an AR-positive hormone responsive prostate cancer cell line to investigate the therapeutic efficacy of \(^{177}\)Lu-PSMA-617 and \(^{161}\)Tb-PSMA-617 in vitro.

**PP48 Development of targeted radiopharmaceuticals with terbium-161 for use in radioimmunotherapy**

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP48

**Aim:** Breast cancer is the most common cancer in women and causes over 500 000 deaths worldwide every year. Overexpression of HER2 receptor tyrosine kinase occurs in 30 % of metastatic breast cancers and correlates with poor prognosis. Trastuzumab (Herceptin®, Roche, United Kingdom) is a recombinant humanized anti-HER2 monoclonal antibody used for treatment of HER2-positive breast cancer. Studies have shown that anti-HER2 radioimmunotherapy (RIT) with \(^{177}\)Lu-labelled trastuzumab is effective in treatment of breast cancer in vitro. \(^{161}\)Tb exhibits similar radiochemical properties to \(^{177}\)Lu, but also yields a significant number of short-range Auger/conversion electrons (≤50 keV). In this study, we want to label and compare the therapeutic efficacy of \(^{177}\)Lu-DOTA-trastuzumab and \(^{161}\)Tb-DOTA-trastuzumab on the HER2-overexpressing cell line SKBR3.

**Methods:** Trastuzumab was conjugated with p-SCN-Bz-DOTA (Maccyclics, USA) to give the DOTA-trastuzumab conjugate, which was radiolabelled with \(^{177}\)Lu (ITG, Germany) and \(^{161}\)Tb (IFE, Norway) under identical reaction conditions. Immunoreactivity studies with \(^{177}\)Lu-DOTA-trastuzumab and \(^{161}\)Tb-DOTA-trastuzumab on the SKBR3 cell line (ATCC, USA) are ongoing.

**Results** The average number of p-SCN-Bz-DOTA molecules per antibody was determined radiochemically to 5.4 ± 1.1. Radiochemical yield and purity >98 % was achieved for both \(^{177}\)Lu and \(^{161}\)Tb. Quality control parameters like pH, appearance, radiochemical purity and in vitro stability were studied. The radiolabeled preparations were stable up to 120 h within the tested range of specific activities.

**Conclusion** This study indicates that the used conjugation and radiolabeling procedures give preparations of high stability and high radiochemical purity. Further evaluation of the efficacy of \(^{161}\)Tb-DOTA-trastuzumab and \(^{177}\)Lu-DOTA-trastuzumab in vivo should be pursued.

**PP49 National regulations in Radiopharmacy: Is the present situation generally acceptable?**

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PP49

**Aim:** The aim of this overview was to evaluate the regulations in Republic of Macedonia dealing with radiopharmaceuticals with emphasis on Marketing authorisation, keeping in mind that the way how radiopharmaceuticals is developed and introduced to the users are completely different from what was typical for conventional pharmaceutical products.

**Specific issues:** The quality of the radiopharmaceuticals is defined in the applicable pharmacopeia monographs of individual radiopharmaceuticals. Regarding the processes of registration of radiopharmaceuticals and production of radiopharmaceuticals according to Good Manufacturing Practises, similar uniformity is not present in all countries. The Good Radioisopharmaceutical Practice Code was defined to comprise the application of the GMP guidelines for Pharmaceuticals with the relevant guidelines for radiation protection during production, distribution and hospital handling of radiopharmaceuticals to ensure the safety and efficacy of the product administered to the patient. Over the years the need for regulation of the radiopharmaceuticals became necessary. The discussions lead to the realisation that radiopharmaceuticals could not be considered as something extraordinary, and that the same quality standards would have to apply for these products as for conventional Pharmaceuticals. The first formal systems for obtaining market authorisation for radiopharmaceuticals were established in the USA, United Kingdom and Denmark. It was obvious that the requirements 653 used for non-radioactive drugs could not be applied directly to the radiopharmaceuticals. Most countries have established national registration procedures to obtain official market authorisation for a radiopharmaceutical. In Republic of Macedonia radiopharmaceuticals are include under the general legislation for medicinal products. Republic of Macedonia is a small country and use of radiopharmaceuticals is small. Therefore the interest in registering these products is small.

**Status of radiopharmaceutical regulations in Republic of Macedonia**

1) Law on Medicines and Medical Devices (Official gazette No. 106/07,88/2010, 36/11, 53/11, 136/11, 11/12, 147/13, 27/14, 43/14, 88/15) in which radiopharmaceuticals are regulated

2) Rulebook on the contents of the application, the documentation and detailed requirements in respect of the premises, equipment and staff for granting authorisation for production of medicinal products (Official gazette No. 106/07,88/2010, 36/11, 53/11, 136/11, 11/12, 147/13, 27/14, 43/14, 88/15)

3) Rulebook on Good Manufacturing Practices (fully in a compliance with EudraLex Volume 4 Good Manufacturing Practices Annex 1 for Manufacture of Sterile Medicinal Products 01/fully 03/2009, Annex 3 for Manufacture of Radiopharmaceuticals, Annex 11 for Computerized Systems and Annex 15 for Qualification and validation.

4) Law on Ionizing Radiation Protection and Safety (Official gazette No.48/02, 135/07, 53/11, 164/13, 43/14, 149/15)

**Conclusion:** The Law on medicinal products and medical devices of the Republic of Macedonia is completely in compliance with the EU regulatory. But, it is obvious that the requirements used for non-radioactive drugs could not be applied directly to the radiopharmaceuticals. Several special characteristics of the radiopharmaceuticals had to be taken into consideration. Keeping in mind that recently in Republic of Macedonia PET centre was established and we have our own production of radiopharmaceuticals it is necessary to take some steps forward and make the effort to separate radiopharmaceuticals from general legislation for medicinal products. Most requirements concerning documentation of the safety and efficacy of a non-radioactive drug must be also applied to radiopharmaceuticals. But, it must be recognised that radiopharmaceuticals do not have a measurable pharmacodynamic effect. In the same time radiopharmaceuticals have a changing composition with time due to the radioactive decay of the ultra-short lived radionuclides, so sometimes it is impossible to perform quality control. Studies of chronic toxicity would only be required in special cases. Clinical documentation would normally be more limited than for non-radioactive drugs. Therefore, Republic of Macedonia must to establish national registration procedures to obtain official market authorisation for a radiopharmaceutical, taking bearing in mind that we did not have a drug legislation that did also cover radiopharmaceuticals.
PPS50
Optimization of production of $^{11}$C CH$_4$ with Methylator II for synthesis and development of $^{11}$C radiotherapeutics

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PPS50

Aim: University Institute of Positron Emission Tomography Skopje is equipped with the Methylator II (Comeper Spa. Former Veenstra Instruments BV.), a module designed for the production of high specific activity Methylidode (11C CH$_3$I) and/or Methyl Triflate (11C CH$_3$OSO$_2$CF$_3$) and CarbonSyntheon I (Comeper Spa.) for production of simple 11C radiotherapeutics. The synthesis process starts with the production of [11C]CO$_2$ in the cyclotron (GE PETtrace 16.5MeV) via the $^{14}$N(p, n)11C nuclear reaction. The produced [11C]CO$_2$ is delivered into the Methylator, where it first was trapped and subsequently reduced to [11C]CH$_4$ and converted thereafter into [11C]CH$_3$I and/or [11C]CH$_3$OSO$_2$CF$_3$. The trapped [11C]CO$_2$ in the Methanizer was reduced into a [11C]CH$_4$ with hydrogen on a nickel catalyst (Shinwasorb) at a rather moderate temperature 350 $^\circ$C. The next step was the purification of the [11C]CH$_4$ over a Carboxen 1000 column, with the knowledge that the H$_2$ will flow about 7 times faster than [11C]CH$_3$I through carbon packing causing the separation of H$_2$ and CH$_4$. This is one of the most important steps in the production process which affects directly the equilibrium reaction which forms the [11C]CH$_3$I and H$_2$. which is formed in the iodine oven by the reaction of H$_2$ and I$_2$ as well.

Methods: Optimization experiments where performed maximizing the yield of [11C]CH$_4$. By changing the time for switching the valve V04 (see diagram) the effectiveness of the purification was influenced. In ‘Active’ state the formed [11C]CH$_4$ and excess of H$_2$ was directed toward waste, but in ‘Inactive’ state in direction of the Iodine Oven. If the time was too short the reduced [11C]CH$_3$I would not be separated thoroughly enough from the H$_2$, but when the time was too long the produced [11C]CH$_4$ would be lost into waste. The first syntheses were performed with V04 active for 25 sec upon release of the [11C]CH$_4$, after which it was deactivated. Different timings for switching the valve were tested and the different yields were obtained.

Results
Our result presented in the Table showed that yield of [11C] CH$_3$I and [11C] Choline is purification time depended. By increasing the time of purification (from 20 to 37 seconds) obtained trapped [11C] CO$_2$ is more than four time higher and harvested [11C] CH$_3$I as well. After 37 seconds we obtained 41% of [11C] CH$_3$I that is directly reflected to the yield of [11C] Choline (34.6), fitting with our protocol for synthesis of [11C] Choline.

Conclusion: The module and software give us a big opportunity and flexibility for testing and optimization of the production achieving a better yield, and also the development of new 11C radiotherapeutics.

Fig. 1 (abstract PP50). See text for description

| Purification time [s] | Trapped [11C]CO$_2$ [GBq] | Harvested [11C]CH$_3$I [GBq] | Yield [11C]CH$_3$I dc [%] | Yield [11C] Choline dc [%] |
|-----------------------|--------------------------|-----------------------------|--------------------------|--------------------------|
| 20                    | 4.5GBq@17:47             | 0.8 GBq@17:59               | 27                       | 22                       |
| 25                    | 7.2 GBq@1280s            | 1.25 GBq@2500s              | 35                       | 25                       |
| 37                    | 19.564GBq@12:32          | 6.280GBq@12:39              | 41.4                     | 34.6                     |

PPS51
Formulation and Characterization Studies of Radiolabeled, Active Folate Targeted Theranostic Co-Delivery Liposomes for Non-Small Cell Lung Cancer

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PPS51

Aim: Cancer is the main cause of the deaths in the worldwide. Among cancer types, lung cancer is the most common one and it is currently the leading cause of cancer death. 80% to 85% of lung cancers compose non-small cell lung cancer (NSCLC) [1]. Combination therapy in cancer has many advantages including reducing the toxic effects exists in monotherapy, minimizing multidrug resistance and ensuring synergistic effect for clinical response in comparison with monotherapy [2]. Hybrid diagnostic imaging modalities like SPECT/CT can provide both metabolic, functional and anatomic information about the diseases. Using specific agents is essential for sufficient functional information, better diagnosis and effective therapy of NSCLC. Passively or actively targeted systems such as liposomes have different interests for either therapy or diagnosis. Recent studies generally depend on the development of new delivery systems, theranostics, in which diagnosis can be managed together with cancer therapy by evaluating therapeutic effect.

Methods: Theranostic liposomes were formulated by polyethylene glycole (PEG)ylated, either passive or actively (folate conjugated) targeted, $^{99m}$Tc labeled for SPECT/CT imaging and vinorelbine and paclitaxel encapsulated for NSCLC therapy. Their characterization studies were conducted.

Results: Both passive and active targeted liposomes showed proper characterization with about 10% encapsulation efficiency of vinorelbine and 60% encapsulation efficiency of paclitaxel; around 100 nm particle size of passive targeted liposomes and around 120 nm particle size of active targeted formulations. All formulations designated a zeta potential around -10 to -13 mV and phospholipid efficiency of 85%. All formulations designated a stable radiolabeling with $^{99m}$Tc.

Conclusion: Promising characterization profiles were obtained with both nanosized, PEGylated passive targeted and active targeted co-delivery theranostic liposomes for both diagnosis and therapy of NSCLC. Our studies are continuing.

Funding: This study was supported by the grant of H.U. BAB, Project No: 12852. Paclitaxel is obtained from Bristol-Meyers Squibb as a generous gift for our non-clinical research.

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PPS52
HPLC method for analysis of DOTA-TOC

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PPS52
Aim: Main impurities in the 90Y-DOTA-TOC labelling process, apart from the “free” yttrium-90, are DOTA-TOC complexes with other metals that could be present in the solution. The most common method for determination of radiochemical purity (RCP) of labeled peptides is RP-HPLC. However, the standard gradient HPLC methods may not allow determination of the DOTA-TOC complexes with “cold” metals. The information about presence of cold metal complexes might be very important in particular for investigation of reasons of radiolabelling failure and poor RCP. The aim of the study was to develop a new HPLC method that would be suitable for determination of RCP as well as for identification and determination of cold metal complexes that could be present in 90Y-DOTA-TOC.

Methods: The new isocratic RP-HPLC method (Phenomenex Kinetex 150x4.6mm column and isocratic flow of 80% 0.1% TFA in water and 20% of 0.1% TFA in acetonitrile, 1mL/min) has been developed and validated. Accuracy, repeatability, precision, linearity and range of the method was checked. DOTA-TOC complexes with “hot” yttrium-90 and lutetium-177 and “cold” complexes with indium, lutetium, yttrium, gallium, zinc, copper, iron and lead were evaluated. The formation of complexes has been confirmed by LC-MS.

Results: A very good chromatographic separation of the individual DOTA-TOC metal complexes has been achieved. Each of the metal complexes can be easily identified by their characteristic retention times and could be quantitated using estimated molar absorption coefficients. The method enabled quantitative determination of unlabeled peptide in the radioactive preparation in the range of linearity from 10 mcg to 100 mcg of DOTA-TOC (R2 = 0.999).

Conclusion: The developed isocratic HPLC method is suitable for RCP determination of radiolabeled DOTA-TOC. In single HPLC run both the RCP and the contribution of DOTA-TOC complexes with cold metals can be assessed, thus reflecting the specific activity of the radiolabeled peptide. Validation confirmed that the method is accurate, repeatable and precise.

PPS3
Radiochemical purity determination of 68Ga-labelled radiopharmaceuticals. Are the TLC and HPLC results complementary?

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EJNMMI Radiopharmacy and Chemistry 2018, 3(Suppl 1):PPS3

Aim: European Pharmacopoeia monograph for Gallium (68Ga) edotreotide [1] recommends HPLC and TLC as complementary methods for radiochemical purity (RCP) determination. In TLC [68Ga]gallium in colloidal form is determined while HPLC separates the free [68Ga]gallium(III) ion and [68Ga]gallium edotreotide. Mobile phases used in these methods have different pH values. Being aware that gallium speciation in aqueous solution depends on pH and temperature [2], the intrinsic questions arise: (1) what 68Ga species are really determined, (2) are the TLC results matching the HPLC results? Herein we are answering these questions based on our experience with 68Ga-labelled radiopharmaceutical preparations.

Methods: TLC and HPLC methods for RCP determination of 68Ga-DOTA-TOC and 68Ga-DOTA-TATE were based on Ph.Eur. [1], for 68Ga-PSMA on the draft monograph [3]. Other TLC mobile phases were also used: 1M ammonium acetate, 1M sodium acetate/methanol, 5M sodium acetate/methanol, citrate buffer pH 5.5. Reference solutions of 68Ga were prepared [2], in HCl (prepared by addition of 1.5mL of H2Ot to 1mL of 68Ga eluate in 0.1M HCl) and in sodium acetate (24mg/ml). RCP in all samples was assessed by HPLC and TLC followed by calculation of 68Ga recovery.

Results: In HPLC the differences in recovery of 68Ga(III) and 68Ga in colloidal form, 107%±1.7% and 106.2%±1.3%, respectively, were not statistically significant. In 68Ga-PSMA11 (n = 12) both HPLC and TLC revealed very similar results of free 68Ga: 1.64%±0.59% (HPLC) and 1.55%±0.65% (TLC). Similar observations were made in 68Ga-DOTA-TOC and 68Ga-DOTA-TATE.

Conclusion: Depending on pH of mobile phase, the non-bound 68Ga may exists in various ionic forms. At pH around 2, HPLC analysis of 68Ga reference sample revealed significant transition to Ga3+ or Ga(OH)2+ cations, allowing quantitation of free 68Ga with mobile phase containing 0.1%TFA. TLC results vary depending on mobile phase composition. Hence, we suggest that TLC is not to be used as complementary to HPLC but as alternative method for free 68Ga determination, even though when using 1M sodium acetate/methanol (50/50) the results of free 68Ga were consistent with HPLC. It should be stressed that methods for determination of non-bound 68Ga in radiopharmaceutical preparations do not reflect gallium speciation in aqueous solutions.

Funding: This work was partly financed by grant No. POIR/01.02.00-00-0041/15 from the National Centre for Research and Development

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