A $(M(CO)_3)^+$ (M = ^{99m}Tc, Re, $^{186/188}$Re) LABELLED SYNHTETIC AMINO ACID ANALOGUE TRANSPORTED BY THE LAT1 TRANSPORT SYSTEM INTO TUMOUR CELLS

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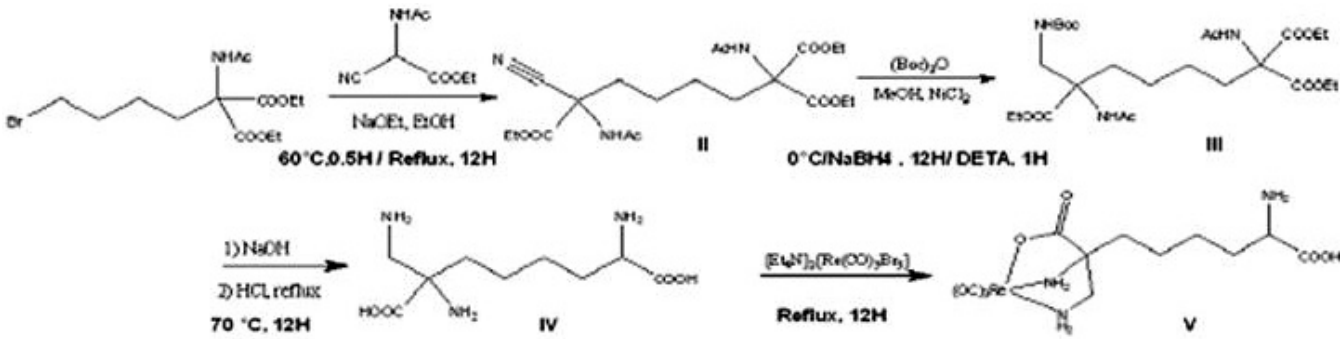
Introduction: The amino acid transport system LAT1, highly over expressed in many human tumour cells, shows a broad selectivity for artificial amino acids with a lipophilic neutral side chain. In view of a further ^{99m}Tc/^{188}Re labelled LAT1 transported tracer this study presents the synthesis and in vitro evaluation of a *fac*-$[Re(CO)_3]^+$ N,N,O tripod complex based pseudo amino acid.

Experimental: The synthesis pathways are represented in figure 1. The intermediate products are II: Triethyl 1,6-diacetami do -6-cyanohexane-1,1,6-tricarboxylate; III: Triethyl 1,6-diacetamido-7- (tert-butoxy carbonyl amino)-1,1,6-tricarboxylate; IV: 2,7-Diamino-2-aminoethyl-octanedioic acid or1,2 diamino 2-carboxyl-6-hexylglycine; V: $Re(CO)_3$ N,N,O tripod-$(CH_2)_4$-L-glycine.The affinity constant Ki was calculated from the inhibition of uptake of [^{3}H]-L-Phe/L-Phe in R1M rhabdomyo sarcoma cells in Na+ free "HEPES"buffer. Transport potency was obtained as stimulation of efflux (%) of [^{3}H]-L-Phe from the cells by the amino acid analogues.

Results and Discussion: In the designed "tube" structure the amino acid tail was enantiomerically pure the L form while the Re(CO)3-tripod has two diastereomers. The tripod N,N,O_{acid} is chosen to neutralize the charge of $Re^+(CO)_3$. The L-amino acid V shows considerable competitive affinity for LAT1 (Ki value of 0.31 m M) and moreover is transported into the cells proven by the stimulated efflux to 13.5%. The influence of the bulkiness of the lipophilic part is shown in table 1 where a dip is found for neopentyl, while an increase of lipophilicity should introduce a higher affinity (cfr Cys-Metyl and Cys-Butyl).This explains the somewhat lower affinity of V due to the cauliflower structure of the N,N,O-$Re(CO)_3$ moiety.

Table 1. SAR: Side chain-affinity (Ki) - Transport Potency (% Efflux)

Compound	Ki (mM)	% Efflux
L-Cys-S-Metyl	0.11	21
L-Cys-S-butyl	0.016	36
L-Cys-neopentyl	0.48	9.5
BCH	0.15	27
L-Gly-but-N,N,O Re(CO)3	0.31	13.5



Conclusion: $Re(CO)_3$ N,N,O-butyl-L-glycine, amino acid enantiomerically pure L, shows for the first time, that it is possible to combine a small biological moiety such as an L-alfa-amino acid with a bulky $M(CO)_3$ complex while retaining affinity for LAT1 transport.

Acknowledgement: The authors thank Mallinckrodt Medical (Petten) and EUREKA for financial support.

Keywords: Fac-[Re(CO)3]+ N,N,O Tripod Complex, (CH2)4-L-Glycine Analogue, LAT1 Transport, Cancer Cells

^{64}Cu LABELLING AND SMALL ANIMAL PET IMAGING OF A PH-DEPENDENT INSERTION PEPTIDE – ^{64}Cu-DOTA-PHLIP

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Introduction: Many tumors are known to have a significantly lower extracellular pH than that of normal tissues. This phenomenon can be exploited by the spontaneous, pH dependent insertion of a water-soluble peptide, pHLIP – pH (low) insertion peptide. At neutral pH, pHLIP binds weakly to the surface of membrane, but at acidic pH (< 7.0) it inserts across the membrane and forms a transmembrane α-helix. Conjugation of cargo molecules to the N-terminus does not affect the insertion process. We have shown that pHLIP can deliver a variety of fluorescent dyes conjugated to its N-terminus to a tissue with elevated extracellular acidity *in vivo*. Here we report the first attempt to deliver a radionuclide conjugated with pHLIP to a tumor to enable nuclear imaging modalities.

Experimental: pHLIP, a 38 amino acid peptide with a Cys residue on its N-terminus, was prepared by solid-phase peptide synthesis and purified by RP-HPLC. DOTA maleimide was conjugated to the Cys residue on the N-terminus of the peptide, and the construct was purified on a G-10 size-exclusion column and transferred to PBS. Purity was ensured by SELDI-TOF. ^{64}Cu was produced as previously described and diluted in 0.5 M sodium acetate buffer, pH 5.5. 1-5 mCi ^{64}Cu was added to a solution of 1-5 mg pHLIP and incubated for 30 min to 2 h at 25-40°C. Purification was achieved by either C_{18} SepPak Light using 100% ethanol as the eluent or in working buffer by a G-10 size-exclusion spin column, depending on the end use of the compound. Small animal PET/CT imaging studies were performed in male, athymic mice bearing LNCaP tumors at 1, 4, and 24 hours post injection of ~200mCi ^{64}Cu-DOTA-pHLIP.

Results and Discussion: ^{64}Cu-DOTA-pHLIP was labeled successfully in high radiochemical purity (>95%) at a specific activity of 0.5-1.0 mCi/ug (2085-4170 mCi/μmol). Preliminary imaging studies afforded excellent tumor visualization in LNCaP tumor-bearing mice utilizing small animal PET. Tumor to muscle ratios (SUV) were 3.44 ± 0.50, 5.56 ± 0.21, and 6.55 ± 1.98 at 1, 4, and 24 hours, respectively. Further imaging studies comparing tumor models of different known acidities are ongoing.

Conclusion: ^{64}Cu-DOTA-pHLIP represents a novel PET imaging agent with pH-dependent delivery properties that may provide a new approach for diagnosis and treatment of tissues with endogenously low extracellular pH.

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Keywords: PET, Cu-64, Insertion Peptide, DOTA

RADIOLABELED SOMATOSTATIN RECEPTOR ANTAGONISTS MAY BE SUPERIOR TO AGONISTS FOR IN VIVO TUMOR TARGETING

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Introduction: The in vivo imaging and targeted radionuclide therapy of somatostatin receptor positive tumors are established and successful methods in oncology. It was common agreement that agonists are preferable over antagonists as they readily internalise upon G-protein binding which presents an active cell uptake mechanism. We now studied the properties of two sst2 and sst3 selective DOTA-coupled and In-111-labeled peptides in comparison to powerful agonists In-111-DOTA-NOC and In-111-DTPA-TATE.

Experimental: The peptides were assembled on solid support followed by DOTA-coupling, cleavage, deprotection and purification. The binding affinity to all somatostation receptor subtypes were measured by using in vitro receptor autoradiography. Saturation binding affinities were performed on HEK-sst2 or -sst3 cells.

Adenylate cyclase activity was determined using forskolin-stimulated cAMP scintillation assay. Sst2 and sst3 receptor internalisation was determined by immunofluorescence microscopy. In vivo biodistribution was deternied by using a double tumor nude mouse model expressing sst2 and sst3, respectively.

Results and Discussion: The In(III)-complexed antagonists DOTA-sst3 ODN-8 and In(III)-DOTA[4-NO2-Phe-c(Dcys-Phe-Tyr-DTrp-Lsy-Thr-Cys)-DTyr-NH2] showed high sst3 and sst2 binding affinity, respectively. They did not trigger sst3 and sst2 internalisation. Scatchard analysis revealed that they recognised 75 and and 15 times more binding sites than respective agonists with even higher binding affinity. Biodistribution studies revealed a much higher tumor uptake at all time points from 1h to 72h for the radiolabeled antagonists compared to the agonists.

Conclusion: Somatostatin based radiolabeld antagonists appear to be preferable over agonists for the in vivo targeting os sst3- or sst2-expressing tumors. This may represents a shift in paradigm of high clinical relevance. Antagonist radioligands for other G-protein coupled receptors need to be studied in nuclear oncology.

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Keywords: G-Protein Coupled Receptors, Somatostatin, Tumor Targeting, Antagonists, Indium-111

^{68}Ga-LABELLED RGD-PEPTIDES FOR IMAGING αvβ3 INTEGRIN EXPRESSION

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Introduction: Recently it has been demonstrated by using [^{18}F]Galacto-RGD and PET, that non-invasive determination of αvβ3 integrin expression is possible. Here we compare two ^{68}Ga-labelled RGD peptides containing either DOTA or a new chelator system (p-SCN-Bn-oxo-DO3A; B505) with [^{18}F]Galacto-RGD.

Experimental: ^{68}Ga-Labelling of both peptides was carried out at 80°C using 20 μg of c(RGDfK(DOTA)) or c(RGDfK(B505)) within 7 min. Stability was studied by incubating the compounds in PBS and human plasma. Hydrophilic properties were investigated by determining PBS/octanol partition coefficients. For in vitro uptake, in vivo biodistribution and microPET studies human melanoma M21 (αvβ3 positive) and M21-L (αvβ3 negative) cells were used. Melanoma-bearing nude mice were sacrificed 1 h and 4 h p.i., respectively and activity distribution was measured using a γ-counter. Dynamic PET images were acquired over 90 min p.i.

Results and Discussion: Labelling of DOTA-RGD and B505-RGD resulted in high radiochemical purity (>95%). For B505-RGD HPLC analysis showed 2 peaks in the ratio 1:9. PBS/octanol partition coefficients are -3.9 for [^{68}Ga]DOTA-RGD and -3.4 for [^{68}Ga]B505-RGD. More than 95% intact tracer was found in PBS after 120 min incubation. Protein binding after 120 min was ~20% for [^{68}Ga]DOTA-RGD and >70% for [^{68}Ga]B505-RGD. Analysis of the supernatant revealed >95% intact tracer for [^{68}Ga]DOTA-RGD. In vitro internalization assays using [^{68}Ga]DOTA-RGD showed significantly higher uptake in M21 cells as in M21-L cells which could be blocked using c(RGDfV). One hour p.i. activity accumulation in the αvβ3-tumor was 2.9±0.3% ID/g and in the negative control tumor 0.8±0.1% ID/g with no significant washout from the αvβ3-positive tumor during the observation period of 4 h. For [^{18}F]Galacto-RGD these values are 1.6±0.2% ID/g and 0.4±0.1% ID/g, resulting in very similar uptake ratios (receptor positive tumor/negative tumor) for both compounds. Due to higher activity concentration in blood a variety of tumor/organ ratios were lower as found for [^{18}F]Galacto-RGD (e.g. tumor/blood: 4.0 vs 8.7). However, no organ showed higher [^{68}Ga]DOTA-RGD uptake than the tumor 1 h after tracer injection. MicroPET images confirm the biodistribution data and show superior contrasts for [^{18}F]Galacto-RGD.

Conclusion: Our data indicate that [^{68}Ga]DOTA-RGD allows non-invasive monitoring of αvβ3 expression. However, the high plasma protein binding results in inferior imaging characteristics compared to [^{18}F]Galacto-RGD. Unfortunately, the new chelator system did not improve the protein binding properties. Further in vitro and in vivo studies with [^{68}Ga]B505-RGD will supply more detailed informations.

Keywords: Ga-68 Labelling, Alpha(v)beta3 Integrin, Angiogenesis, PET Tracer, RGD-Peptides

SITE-SPECIFIC LABELING OF ANNEXIN-V FOR IN VIVO IMAGING OF CELL DEATH

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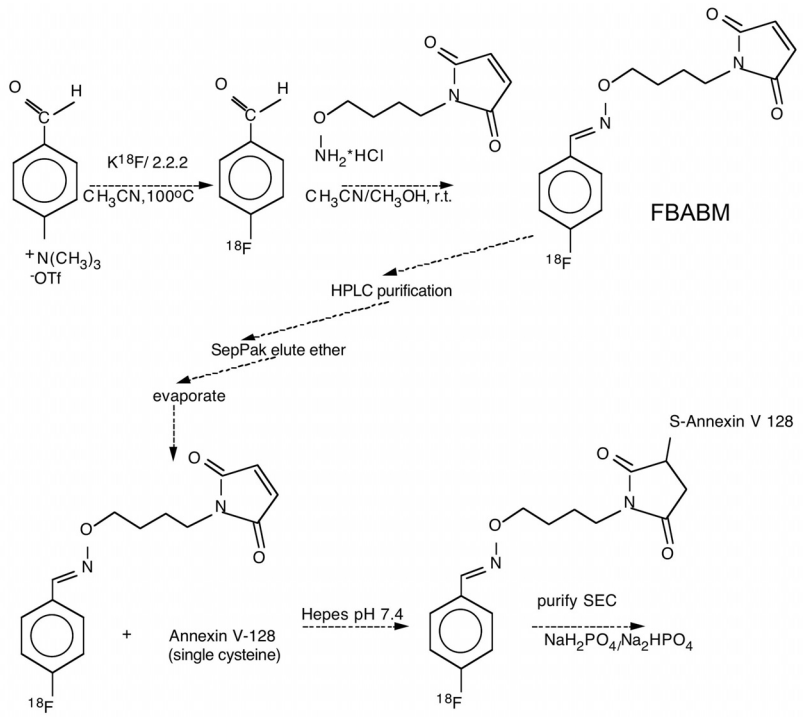
Introduction: Radiolabeled annexin V is being tested for detection of early stage cell death [1]. A number of radiolabeled Annexin V-based probes have been made, mostly by targeting NH_2 groups. However, labeling multiple sites on annexin can decrease phosphatidyl serine binding [2]. We have labeled annexin V-128, a single cysteine mutant [2], with thiol-reactive radiotracer N-{4-[(4[^{18}F]fluorobenzylidene)-aminooxy]butyl}maleimide (FBABM) [3].



Experimental: The annexin V-128 is labeled as shown in figure 1. Synthesis of FBA through FBABM [3] is done in one pot in 30 min. Purification of [^{18}F]-FBABM is critical to remove a diamine side product and is done using C18 (Prodigy 250 x 10mm 5μm, 45% EtOH/H_2O at 50°C). [^{18}F]-FBABM is coupled to annexin V-128 (0.5-1 mg) in Hepes buffer (pH 7.4) at RT for 15 min and purified using size exclusion HPLC (BioSep-SEC-S2000 300 x 7mm, 0.15M Na phosphate pH 7.4). Membrane-binding affinity of the product was tested in an in vitro cell assay using human red blood cells that express high levels of phosphatidyl serine [2].

Results and Discussion: [^{18}F]-FBABM is obtained in 12% decay corrected yield >99% radiochemically pure and < 10 nmole mass. [^{18}F]-annexin V-128 was obtained in 28% decay corrected yield from FBABM. Cell binding pK was 30.0±2.2, similar to that for fluorescent labeled wild-type annexin (30.1±2.20 [4]), or fluorescent labeled annexin V-128 (30.8±1.0 [2]), and better than HYNIC amino-modified wild-type annexin V, pK = 21.3±2.2 [2].

Conclusion: This method provides an alternative labeling scheme for Annexin V with promising in vitro characteristics. Further study in vivo is warranted.

References: [1] Lahorte CM, et al. Eur J Nucl Med 14:887-919, 2004. [2] Tait JF, et al. J Nucl Med 47:1546-53, 2006. [3] Toyokuni T, et al. Bioconjugate Chem 14:1253-59, 2003. [4] Tait JF, et al. J Biol Chem 279:40351-57, 2004.

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Keywords: Annexin V-128, Thiol Labeling, [^{18}F]-Maleimide, Cell Binding Affinity

DPA-714 A NEW TRANSLOCATOR PROTEIN (18kDa) LIGAND: SYNTHESIS, RADIOFLUORINATION AND PHARMACOLOGICAL CHARACTERISATION

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Introduction: Radioligands for the translocator protein (18kDa) (TSPO), formerly known as the peripheral benzodiazepine receptor (PBR), can measure microglial activation and hence active disease in the brain. Pyrazolopyrimidines represent a promising set of tools for visualising TSPO density *in vivo*. This study details the synthesis, radiofluorination and pharmacological evaluation of a new TSPO pyrazolopyrimidine DPA-714.

Experimental: The affinity of DPA-714 for the TSPO was measured including its ability to increase pregnenolone synthesis. ^{18}F-Radiolabelling of DPA-714 was achieved by reacting the tosylate precursor with ^{18}F-fluoride using the TRACERlab $MX_{FDG\ synthesiser}$ (Fig 1). Biodistribution of [^{18}F]DPA-714 was determined in baboon using PET. Blocking and displacement studies were conducted using PK11195 (1.5 mg/kg) and DPA-714 (1 mg/kg) respectively (Fig 2). [^{18}F]DPA-714 was also assessed in a quinolinic acid lesioned rat model of activated microglia.

Results and Discussion: DPA-714 displayed high affinity for the TSPO (Ki = 7.0 nM) and stimulated pregnenolone synthesis at levels of 80% above baseline. [^{18}F]DPA-714 was prepared in 16% RCY with s.a. 270 GBq/μmol. PET studies demonstrated good uptake in primate brain while pre-treatment with PK 11195 inhibited uptake by 70% at 20 min p.i. Injection of cold DPA-714 20 min post [^{18}F]DPA-714 administration resulted in rapid radioligand washout. These results illustrated the specificity of [^{18}F]DPA-714 binding. Rat studies indicated an 8 fold higher uptake of [^{18}F]DPA-714 in the lesioned side of striatum compared with its non lesioned counterpart demonstrating radioligand sensitivity. Uptake in the lesioned side was selective for the TSPO as it was abolished to the level of the non lesioned side in the presence of PK11195.

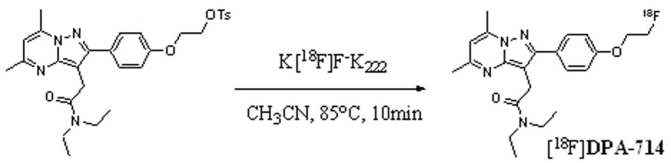

Fig. 1

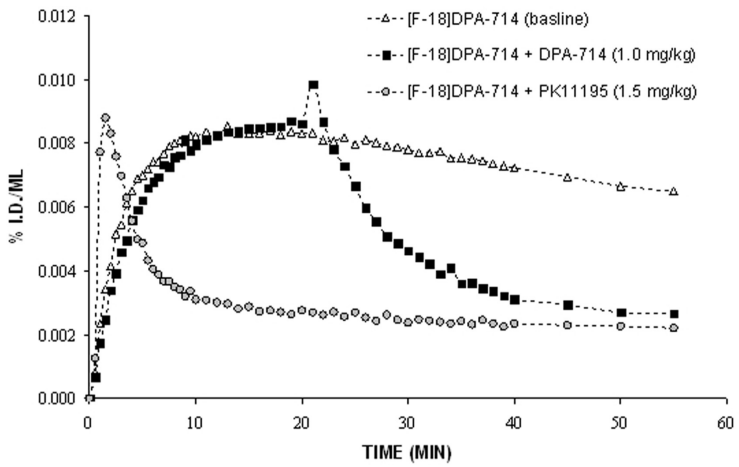

Fig. 2

Conclusion: [^{18}F]DPA-714 is a promising PET radioligand for the TSPO. In addition DPA-714 is a potent agonist of neurosteroid production and has therapeutic potential in treating anxiety related disorders.

Keywords: Translocator Protein (18kDa), Peripheral Benzodiazepine Receptor, Pyrazolopyrimidine, Fluorine-18

A POTENTIAL RADIOPHARMACEUTICAL FOR IMAGING OF BREAST CANCER: INDIRECT RADIOLABELING OF A NEW ANTI-MUC1 MONOCLONAL ANTIBODY AND ITS QUALITY CONTROL

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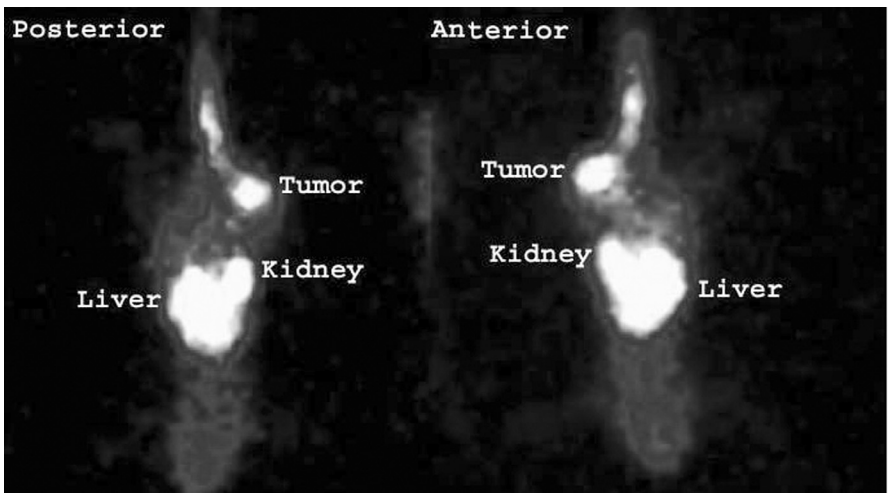
Introduction: Human epithelial mucin, MUC1, is commonly over expressed in adenocarcinoma that includes more than 80% of breast cancers and represents a useful target for radioimmunoscintigraphy studies. The PR81 is a new anti-MUC1 monoclonal antibody that reacts strongly with some MUC1 positive cell lines. This study was performed to develop an indirect labeling of this antibody with ^{99m}Tc in order to imaging of breast tumor in mouse model.

Experimental: A 20 molar excess of dissolved HYNIC was added to a solution of the antibody. The resulting conjugate was labeled with ^{99m}Tc using tricine as a co-ligand. The labeling efficiency was determined by ITLC. Stability of labeled product was determined in human serum by gel filtration chromatography (FPLC) over 24 hr. The integrity of labeled MAb was checked by means of SDS-PAGE. Cell-binding assay was used to test binding ability of ^{99m}Tc-HYNIC-PR81 to MCF-7 cell line. Biodistribution was studied in normal BALB/c mice at 4 and 24 hr post-injection. The tumor imaging was performed in BALB/c mice with breast xenograft tumors at 24 hr after the radiopharmaceutical injection.

Results and Discussion: The labeling efficiency was 89.2%±4.7. *In vitro* stability was 72.6±6.7 in human serum over 24 hr. There was no significant Ab fragmentation due to labeling procedure. Both labeled and unlabeled PR81 were able to compete for binding to MCF 7 cells. Biodistribution studies in normal BALB/c mice showed that there was no significant accumulation in any organ. The radioimmunoscintigraphy studies showed definite localization of the preparation at the site of tumors with high sensitivity and specificity (Figure1).



Conclusion: The ability of the preparation to localize in breast tumors with high sensitivity and specificity in mouse models shows that the new radiopharmaceutical is a promising candidate for imaging of human breast cancer in nuclear medicine.

Keywords: Radioimmunoscintigraphy, Breast Cancer, Radiopharmaceutical, Monoclonal Antibody