# RADIOSYNTHESIS AND PRELIMINARY EVALUATION OF A FOLIC ACID DERIVATIVE LABELED WITH F-18 FOR IMAGING FOLATE RECEPTOR-POSITIVE TUMORS

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**Introduction**

The so called folate binding protein (FBP), a glycoprotein receptor with high affinity ($K_D$~1 nM) to the oxidized forms of folic acid, has been found to be overexpressed in a variety of tumors of epithelial tissues such as ovarian carcinoma, endometrial cancer, renal cancer, lung cancer, breast cancer and colorectal cancer. As part of our program to develop a PET ligand for visualizing folate receptor-positive tumors in humans, we have synthesized a new folic acid derivative and evaluated its potential as an imaging agent using the high resolution small animal quad–HIDAC PET scanner (NanoPET ™).

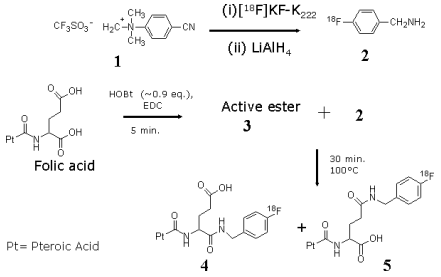
**Methods**

The radiolabeling of the new folic acid derivative with fluorine-18 was accomplished in a three-step reaction sequence as depicted in Scheme 1. The key intermediate, 4-$^{18}$F fluorobenzylamine (**2),** was prepared in two steps adopting an improved version of the method developed by Dolle et al. (1) and coupled to folic acid using in situ activation with HOBt-EDC. Preliminary PET experiments were performed on athymic nude mice subcutaneously inoculated with ca. 5 x $10^6$ KB 31 cells 10-15 days prior to PET experiments. The rodents were maintained on folate-free diet; 5.1 to 18.0 MBq of tracer were injected and the animals (n=4) were scanned for 45 minutes at 30, 75, 120 and 165 minutes post injection (p.i.) using the high resolution quad–HIDAC camera.

**Scheme 1.** Fluorine-18 labeling of folic acid

**Results**

The overall radiochemical yields including purification ranged from 2% to 4%. After HPLC purification the ratio of the two isomers **4 and 5** was 1:4. Whole body PET scans showed a predominance of hepatobiliary and renal clearance of the tracer. The folate receptor-positive tumor masses in all animals were clearly visible and the high resolution of the small animal PET camera allowed to clearly identify a heterogeneous uptake pattern within the tumor.




**Conclusion**

We have developed a method for the $^{18}$F labeling of folic acid. The use of 4-$^{18}$F fluorobenzylamine as a prosthetic group allowed us to perform the labeling directly on commercially available folic acid without expensive and time consuming chemical modification of the native compound. Although the labeling yields are not very high, the radiochemical yields obtained were adequate for animal experiments. For human studies, however, the radiochemical yields would have to be improved.

**Reference**

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Keywords: Folic Receptor, Folic Acid, PET

# DEVELOPMENT OF $^{111}$In-LABELED TUMOR ASSOCIATED ANTIGEN PEPTIDES FOR NON-INVASIVE MONITORING OF DENDRITIC CELL BASED IMMUNOTHERAPY

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**Introduction:** Dendritic cells (DC) are professional antigen-presenting cells. In patients, DC can induce an effective antitumor response by presenting a tumor-derived peptide in the groove of major histocompatibility complexes (MHC) to naïve T-cells. In our ongoing clinical trials, HLA-A2.1$^+$ melanoma patients are vaccinated with mature DC loaded with tumor-derived gp100 peptides. However, little is known about the fate of the MHC-peptide complex after injection of these peptide-loaded DC. Therefore, we investigated the site-specific DTPA conjugation and subsequent $^{111}$In labeling of tumor-derived peptides and their binding to MHC class I.

**Methods:** To investigate the effect of the position of the chelator, the HLA-A2.1 binding peptide gp100:154-162mod (KTWGQYWAV) was conjugated with diethylene-pentaacetic acid (DTPA) either at the N-terminus ('*alpha-DTPA*') or at the epsilon amino group of the Lys$^{154}$-residue ('*epsilon-DTPA*') and labeled with $^{111}InCl_3$. The specific activity was determined by RP-HPLC. The $IC_{50}$ of the radiolabeled peptides for the MHC molecule was determined both on a HLA-A2.1$^+$ B lymphoblastoid cell line (JY) and on human DC in a competition-based assay with a FITC-labeled peptide and subsequent FACS-analysis. MHC binding studies with the $^{111}$In-labeled peptides were performed on the same cell lines. Stability of the radiolabeled peptides in PBS and in the presence of DCs was analysed with RP-HPLC. To investigate whether the radiolabeled peptides could induce a cytotoxic T-lymphocyte response, a cytotoxicity assay was performed using $^{51}$Cr-labeled JY cells as target cells.

**Results:** For both *alpha-DTPA* and *epsilon-DTPA* peptide, the maximum specific activity after $^{111}$In-labeling was 13 GBq/μmol and radiochemical purity exceeded 99%. The $IC_{50}$ of the $^{111}$In-labeled *alpha-DTPA* peptide was >1000 μM. The $IC_{50}$ of the $^{111}$In-labeled *epsilon-DTPA* peptide was 3 μM, which was similar to that of the unconjugated peptide. MHC binding studies showed specific binding of the $^{111}$In-labeled *epsilon-DTPA* peptide to the JY cells at 4°C which could be blocked by an excess of unconjugated peptide. Interestingly, no specific binding was observed for the $^{111}$In-*alpha-DTPA* peptide, most likely due to interference of the DTPA moiety. Incubation at 37°C resulted in less efficient binding to MHC, which may be due to instability of the MHC-peptide complex at 37°C. Moreover, RP-HPLC analysis showed partial degradation of the peptides, most likely due to DC-associated (amino)peptidase activity. The cytotoxicity assay revealed that in contrast to the $^{111}$In-*alpha-DTPA* peptide, the $^{111}$In-*epsilon-DTPA* peptide was able to elicit a specific cytotoxic response.

**Conclusion:** We showed that DTPA conjugation can strongly affect the MHC binding and biological activity of the gp100-derived peptides. Conjugation of DTPA to the N-terminal $NH_2$-group resulted in loss of affinity for the MHC. When DTPA was conjugated to the epsilon $NH_2$-group of the Lys$^{154}$-residue, MHC binding of the peptide was preserved. Moreover, cells loaded with the $^{111}$In-*epsilon-DTPA* gp100:154-162mod peptide were still able to elicit a specific cytotoxic response. These studies allow the noninvasive determination of the behavior of MHC-peptide complexes on DC in vivo. This could allow the rational design of vaccination schemes for immunotherapy.

Keywords: Peptide, Indium-111, Immunotherapy

# VASCULAR IMAGING OF SOLID TUMORS IN RATS WITH A RADIOACTIVE ARSENIC-LABELED ANTIBODY THAT BINDS ANIONIC PHOSPHOLIPIDS

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**Purpose:** We recently reported that anionic phospholipids, principally phosphatidylserine, become exposed on the external surface of viable vascular endothelial cells in tumors, possibly in response to oxidative stresses present in the tumor microenvironment [1-3]. The long-lived arsenic isotopes $^{72}$As and $^{74}$As represent interesting positron emitters with potential for PET [4,5]. In the present study, we tested the hypothesis that a monoclonal antibody directed against anionic phospholipids and labeled with radioactive arsenic isotopes can be used for the vascular targeting and molecular imaging with PET of solid tumors in rats *in vivo*.

**Experimental design:** A new chimeric $IgG_3$ monoclonal antibody, ch3G4 (Tarvacin®), directed against anionic phospholipids was raised. A method for the labeling of antibodies with radioactive arsenic isotopes was developed and the radioarsenic labeled antibody was tested for its *in vitro* stability, immunoreactivity and its ability in terms of localization to tumor vessels and imaging qualities *in vivo*.

**Results:** The mab ch3G4 recognized anionic phospholipids on the external membrane of R3327 Dunning prostate solid tumors in male Copenhagen rats. The tumors could be imaged with planar scintigraphy techniques and with Positron Emission Tomography *in vivo*, showing excellent and antigen-specific localization. The imaging results could be verified with *ex vivo* biodistribution. In addition, the experiments proved the concept of the radiochemical separations applied and the developed labeling chemistry and demonstrated the potential benefits of the use of arsenic radioisotopes for the molecular imaging of antibodies.

**Conclusion:** The mab ch3G4 localized specifically to anionic phospholipids on the surface of vascular endothelial cells in Dunning prostate tumors in rats. The biomedical use of radioactive arsenic isotopes was exemplified for the first time in a multi-modality molecular imaging approach *in vivo*.

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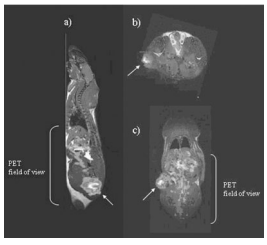
Figure 1: R3327 Dunning prostate AT1 tumor bearing rats were injected with 10 MBq of $^{74}$As[SATA]ch3G4 in 500 µl of PBS (ph 7.4, 1 mMol EDTA) in the tail vein. The graphic shows representative images obtained with a Small Animal PET camera 48 h p.i. in frontal, sagittal and transversal orientation (a-c). The PET images were overlayed with Small Animal MRI Images after 3D reconstruction for better visualization.




Keywords: Phophatidylserine, Arsenic Isotopes, Antibody

# TARGETING PEPT1 FOR DETECTING PANCREAS CANCER WITH [$^{11}$C]GLY-SAR: MICROPET IMAGING OF NUDE MICE BEARING ASPC-1, CAPAN-2, and MPANC-96 XENOGRAFTS

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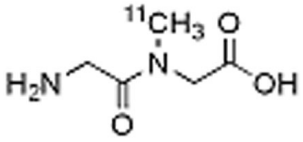
**Background**: Pancreatic cancer is a devastating disease, with over 30,00 patients affected in the USA in 2004 [1] and only a 3-5% two-year survival. Early detection and accurate staging are believed to be the only way to improve prognoses, yet diagnosis remains challenging. Many PET tracers have been used for imaging tumors by targeting specific features that discriminate tumor from normal cells [2], but none are very effective for pancreatic cancer. Recent studies reported amplification of peptide transporter PepT1 in tumors, including human pancreatic cancer cell lines AsPC-1 and Capan-2 [3]. In normal tissue, PepT1 and its homolog PepT2 transport di- and tri-peptides and many peptidomimetic drugs into cells. A radiotracer for PepT1 could be useful for identification and staging of pancreatic cancer.

**Objectives**: Evaluate PepT1 as a potential pancreas tumor imaging target using [$^{11}$C]Glycylsarcosine (Gly-Sar), a radiotracer recently reported by our laboratory for imaging PepT1 and PepT2 transporters [4].

**Methods**: AsPC-1, Capan-2 and mPanc-96 immortalized human pancreatic adenocarcinoma cell lines were grown as subcutaneous tumor xenografts in athymic mice (*nu/nu*, n=3 for each tumor line). Dynamic whole body imaging was performed using Concord Microsystems microPET R4 and P4 for 60 minutes immediately post injection with 0.4-0.5 mCi of [$^{11}$C]Gly-Sar. After allowing prompts rate to decay below 10,000/sec, animals were imaged for 60 more minutes with a similar dose of [$^{18}$F]FDG. Time-activity curves for each radiotracer were generated from volumes of interest drawn manually over the entire tumor.




[$^{11}$C]Gly-Sar

**Results**: All three tumor lines were easily visualized on the [$^{11}$C]Gly-Sar images. Overall [$^{11}$C]Gly-Sar uptake peaked around 7-9 min, retaining about 57-75% id/cc tissue at 55 min post injection. The *in vivo* retention of [$^{11}$C]Gly-Sar radioactivity, but not of [$^{18}$F]FDG, was consistent with in vitro PepT1 expression determined by Western blot analysis. The mPanc-96 tumor exhibited highest PepT1 expression and retention. AsPC-1 and Capan-2 showed about similar PepT1 expression and retention.

**Conclusions**: PepT1 can be used as a marker for imaging tumors exhibiting elevated levels of this peptide transporter. Such a PepT1 radiotracer could be useful for differentiating tumor from normal tissue, evaluating peptidomimetic anticancer drugs and for identifying patients who might benefit from such chemotherapy.

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Keywords: Pancreatic Tumor, PET, [$^{11}$C]Gly-Sar

# CORRELATION OF [1-$^{11}$C] ACETATE UPTAKE TO FATTY ACID SYNTHASE EXPRESSION IN PROSTATE TUMORS

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Although $^{18}$F-FDG is routinely used for the detection of many malignancies, visualization of prostate cancer has been troublesome with its relatively slow tumor growth and metabolism and excretion through the nearby bladder. Within the last few years, 1-$^{11}$C-acetate has been employed for the reliable imaging of prostate cancer using PET (1,2). Prostatic epithelial cells convert from citrate-producing to citrate-oxidizing once they become malignant leading to an increase in the metabolism of acetate.

Fatty acid synthase (FAS) is a multi-functional enzymatic protein involved in many stages of fatty acid biosynthesis. Fatty acids are generally supplied by dietary uptake and therefore FAS is expressed at low levels in most normal tissues except liver, adipose tissue, fetal lung, and the lactating breast. Although it is minimally expressed endogenously, FAS has been found to be over-expressed in prostate carcinomas as well as other cancers (3-5). An increased level of FAS has been found to be indicative of aggressive and late-stage prostatic adenocarcinomas (6). Carbon-14 labeled acetate is routinely used for the monitoring of FAS production in vitro by analysis of its incorporation during lipid biosynthesis (7). The research presented here proposes the use of 1-$^{11}$C-acetate as a surrogate marker for FAS expression in vivo through PET imaging.

Carbon-11 labeled acetate was prepared by the reaction of $^{11}$C-labeled carbon dioxide with a Grignard reagent as previously described (8). Three prostatic carcinomas (CWR22, LAPC-4, and PC-3) were implanted subcutaneously into male athymic mice and allowed to grow until palpable. Small animal PET imaging (MicroPET®, CTI-Concorde Microsystems LLC) was performed 20 minutes post i.v. injection of 350-450 μCi 1-$^{11}$C-acetate. Post imaging, the mice were euthanized and the tumors excised for subsequent western blot analysis. Regions of interest (ROI) were drawn on the images around the tumors and standardized uptake values (SUVs) were calculated to normalize these values to the injected activity per animal as well as body weight. Tumor tissue was homogenized, the cells were lysed, and BCA analysis was performed to accurately determine protein concentration of the samples. Aliquots were then analyzed by gel electrophoresis and western blot analysis for FAS expression.

Prelimary data shows that SUVs for CWR22 tumors have a 2-fold increase over LAPC-4 tumors, with no visible uptake in the PC-3 tumors which are known to have minimal expression of FAS. This trend was mirrored qualitatively in the intensity of FAS expression by western blot analysis. Further experimental data will serve to validate 1-$^{11}$C-acetate as a surrogate marker of FAS expression and therefore as a potential prognostic indicator.

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Keywords: Fatty Acid Synthase, microPET, [$^{11}$C]acetate

# MULTIMERIZATION OF INTEGRIN ANTAGONISTS ALLOWS HIGH CONTRAST IMAGING OF $\alpha_v\beta_3$ INTEGRINS

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**Background:** Cyclic pentapeptide integrin antagonists (c(RGDXY)) have become an interesting class of tracers to investigate angiogenic processes. With the aim to further optimize the binding characteristics and in vivo properties of radiolabeled $\alpha_v\beta_3$ antagonists, we developed and evaluated homo- and heteromultimeric ligand constructs. In this study we investigated multimeric c(RGD)-systems and compared these ligands with their "monodentate" counterparts bearing only one single RGD, but otherwise RAD sequences. **Methods:** c(RGDfE) and c(RADfE) (negative control) were prepared by SPPS using Fmoc-protocol. Homomultimers were generated by SPPS of the entire multimeric backbone (aminooxy acetyl(AOA)-diamino propionyl(Dpr)-lysinyl-heptaethylene glycol(HEG)) and subsequent coupling of c(RGDfE) moieties. In the case of heteromultimeric peptides the c(RADfE) units were coupled as fragments to a core with a remaining protected arm. After deprotection of the remaining unit c(RGDfE) was coupled to the multimeric compound. Finally the peptides were cleaved from the resin followed by deprotection. Radiolabeling was carried out using chemoselective oxim ligation. **Results:** 4-[$^{18}$F]FB-CHO was prepared by $^{18}$F-fluorination of 4-formyl-trimethyl-annilinium triflate (DMSO, 5min, [K/2.2.2]$^{+}$$^{18}$F$^{-}$, 90°C, 25 min, RCY 60-75 %.). For the monomer c(RGDfE)HEG-Dpr-AOA (**M**), as well as the dimers (c(RGDfE)HEG)$_2$-K-Dpr(AOA) (**D**), (c(RGDfE)HEG)-(c(RADfE)HEG)-K-Dpr-AOA (**D$_m$**) and tetramers ((c(RGDfE)HEG)$_2$-K)$_2$-K-Dpr-AOA (**T**), (((c(RGDfE)HEG)(c(RADfE)HEG)-K)((c(RADfE)HEG)$_2$-K))-K-Dpr-AOA (**T$_m$**), ((c(RGDfE)HEG)$_2$-*k*)$_2$-*k*-Dpr-AOA (**dT**) formation of the corresponding N-(4-[$^{18}$F]fluorobenzyliden)oximes ([$^{18}$F]FBOA) were obtained at low peptide concentration (2.0 mM) within 10 min at 60 °C at pH 2-3 (approx. RCY 75 %, overall RCY 50-56 %). To avoid the consumption of educt by reaction of the aminooxy groups with impurities (aldehydes and ketones) during educt purification, we also investigated a one step deprotection and in situ-oxime formation under acidic conditions. In vitro binding studies with the corresponding N-(4-iodobenzyliden)oximes revealed significantly higher $\alpha_v\beta_3$ integrin binding of the multimeric compounds (**M** ($IC_{50}$ = 20 nM), **D** ($IC_{50}$ = 3 nM), **T** ($IC_{50}$ = 0.2 nM)). This trend was confirmed within a series of fluorinated ligands with double heptaethylene glycol spacers, consisting of a monodentate dimer ($IC_{50}$ = 12 nM), a bidentate dimer ($IC_{50}$ = 3.3 nM), a tetradentate tetramer ($IC_{50}$ = 0.75), and an octadentate octamer ($IC_{50}$ = 0.12 nM). The affinity was not influenced by changing the branching from *L*-lysine to *D*-lysine. Furthermore, the affinities of **D$_m$** ($IC_{50}$ = 12 nM) and **T$_m$** ($IC_{50}$ = 25 nM) are similar to the affinity of **M** ($IC_{50}$ = 20 nM). Tumor uptake (%ID/g) 120 min p.i. in mice bearing M21 melanomas reached 0.5±0.2 % for **M**, 2.1±0.4 % for **D$_m$**, 2.0±0.4 % for **D**, 0.7±0.2 % for **T$_m$**, 2.0±0.4 % **T** and 4.5±0.2% for **dT**. Tumor/background-ratios increased in the series **M**, **D$_m$**, **T$_m$**, **D, T** (tumor/muscle: 7.4; 8.3; 10.8; 11.8; 17.1 and tumor/blood: 8.9; 8.5; 16.7; 13.5; 19.0). Metabolite analysis showed high in vivo stability for **M** and **dT** and moderate stability for **D = D$_m$ > T = T$_m$**, indicating low metabolic stability of the *L*-lysine bridging. **Conclusion:** This study demonstrates, that multimerization is a valuable method to improve affinity and tracer uptake. Using the chemoselective labeling strategy, even complex unprotected peptide structures can be labeled in one step. This strategy is currently adapted to the radiometallation of our constructs.

Keywords: Integrin Antagónist, Multimeric Ligand, Angiogenesis