## P221 IMAGING SOMATOSTATIN 2 RECEPTOR GENE TRANSFER WITH GA-68 DOTATATE AND PET

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**Objectives:** Montoring of gene expression through external imaging may be a useful tool for following gene therapy procedures. In particular, the use of PET may allow quantitative evaluation in these situations. Given the relatively low endogenous expression of the Somatostatin Receptor 2 (SSTR2) and the availability of very efficient positron emitter labeled radiotracers capable of binding this receptor such as 68Ga-DOTA-Tyr(3)-Thr(8)-octreotate (68Ga-DOTATATE), we have evaluated the use of this combination of reporter gene/reporter compound for non invasive monitoring of gene transfer.

**Methods:** Adeno Associated Virus (AAV) vectors encoding the human SSTR2 gene or green fluorescent protein (GFP) as control, under the cytomegalovirus (CMV) or the thyroxin binding globulin (TBG) promoters were constructed. Different sets of C57/BL6 mice received intravenous (IV) AAV-TBG-SSTR2, intramuscular (IM) or inhaled AAV-CMV-SSTR2. Control animals received both IV AAV-TBG-GFP and IM AAV-CMV-GFP. PET imaging was carried out over a 6 month period following the AAV administration in treated and control animals with 1-5 MBq of 68Ga-DOTATATE using a clinical PET scanner.

**Results:** IV AAV-TBG-SSTR2 transduced animals showed markedly increased uptake in the liver, that was several-fold above control levels. IM AAV-CMV-SSTR2 transduced animals showed a 3 to 4 fold increase in muscle uptake compared to controls. In both instances, the increase in 68Ga-DOTATATE uptake was dependent on the amount of administered AAV. Liver transduced animals showed a tendency for a decrease in liver uptake over time whereas transduction in muscle showed a steady increased uptake over the 6 month observation period. Inhaled AAV-CMV-SSTR2 did not yield significant increase in 68Ga-DOTATATE uptake in the lungs.

**Conclusions:** Use of the SSTR2 as reporter gene and repetitive imaging with 68Ga-DOTATATE demonstrates that monitoring is feasible and high uptake levels are maintained for long periods of time. This approach may be useful for quantitative monitoring of gene therapy in animal models.

## P222 MOLECULAR TARGETING OF BREAST CANCER WITH CU-64 LABELED PNA-PEPTIDE CONJUGATES

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**Objectives:** Standard screening misses up to 40% of breast cancers. Breast cancer cells express the novel breast cancer associated glycoprotein mamaglobin A (MamA), which is present in 80-100% of breast cancers and up to 90% of lymph node metastases, but not in normal tissue. Peptide nucleic acid (PNA)s are DNA-like molecules with an artificial backbone which bind to corresponding RNA and DNA with high binding affinity and selectivity, but a carrier is required to deliver PNAs through the cell membrane for intracellular targeting. We demonstrated that a receptor specific peptide can efficiently deliver PNA into cells. It is our hypothesis that an anti-MamA-PNA analogue attached to an IGF1 receptor binding peptide can be created which will allow in vivo and in situ detection of invasive breast cancer.

**Methods:** To test our hypothesis, we 1) synthesized $^{64}$Cu-DOTA-anti-MamA-PNA-cskc, along with appropriate control sequences, at very high specific activities suitable for imaging breast cancer MamA mRNA overexpression in vivo; 2) determined (in ZR-75-1 breast cancer cells, which express both MamA mRNA and IGF1 receptor), that the antisense conjugate has internalization and retention; 3) obtained the biodistribution of the conjugate in ZR-75-1 murine xenografts. Using standard solid phase synthesis, we successfully synthesized the antisense conjugate DOTA-anti-MamA-PNA-cskc as well as the three control chimeras, DOTA-cskc, the peptide mismatch conjugate, and the PNA mismatch conjugate. The antisense conjugate and the peptide have been labeled with $^{64}$Cu in 0.2 M ammonium acetate, pH 5.0, and containing 0.1% Tween-80. The radiolabeled compounds were purified by RP-HPLC. Cell internalization and efflux studies were carried out in ZR-75-1 cells. SCID mice bearing mammary fat pad ZR-75-1 human breast cancer xenografts were administered 10 $\mu$Ci of $^{64}$Cu labeled antisense conjugate for biodistribution studies. Biodistributions were obtained at 1 h and 24 h post-injection.

**Results:** The radiochemical purity of labeled conjugates was nearly 100% after purification. The internalization of $^{64}$Cu-DOTA-anti-MamA-PNA-cskc in ZR-75-1 cells increased from about 65% at 5 min to 80% at 4 h of incubation. The retention of this conjugate was dropped from about 80% at 1min to 30% at 4 h of incubation. Biodistribution showed that the mammary tumor uptake of this conjugate was 0.45 %ID/g at 1 h and 0.44 %ID/g at 24 h post-injection.

**Conclusions:** The antisense conjugate showed internalization and retention in the MamA- and IGF1-postitive ZR-75-1 breast cancer cell line. Mammary tumor uptake of this conjugate in the mouse model was above uniform distribution after excretion and showed specific targeting. We are currently redesigning the antisense probe to increase retention in breast cancer cells and uptake in mouse mammary fat pad xenografts.

# P223 SYNTHESIS AND BIOLOGICAL EVALUATION OF C11-LABELED β-GALACTOSYL TRIAZOLES AS POTENTIAL PET TRACERS FOR IN VIVO IMAGING OF LACZ REPORTER GENE EXPRESSION

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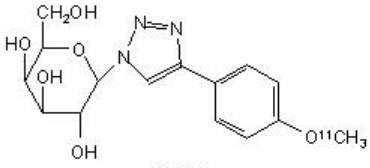
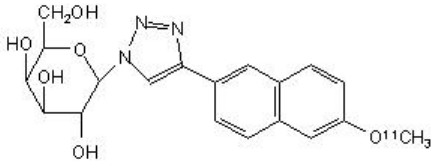
**Objectives:** Gene therapy holds great promise for treatment of various diseases. A prerequisite for widespread implementation is the reliable monitoring of the transduction and expression of the therapeutic gene in space and time. Many promising methods are being developed to image gene expression by including a reporter gene in tandem with the therapeutic gene. One of the most widely used reporter genes is the LacZ gene, which encodes the bacterial β-galactosidase enzyme (β-gal). For its in vitro detection, several probes are available. The ability to image β-gal expression in vivo would further extend the use of this reportersystem. In this study two carbon-11 labeled β-galactosyl triazoles, i.e. [$^{11}$C]-6 and [$^{11}$C]-13, were synthesized and evaluated as potential reporter probes for in vivo visualization of LacZ gene expression using positron emission tomography.



[$^{11}$C]-6



[$^{11}$C]-13

**Methods:** The two precursors for the radiolabeling and the non-radioactive reference compounds (6 and 13) were synthesized using a Cu(I)-catalyzed 1,3-dipolar cycloaddition between acetylated β-galactosyl azide and the corresponding terminal alkynes. Radiolabeling with C11 was done by alkylation of the precursors with [$^{11}$C]methyl iodide in DMF at 70 °C in the presence of NaOH. Purification was done by preparative HPLC on an XTerra $C_{18}$ column eluted with mixtures of 0.1 M $NH_4OAc$ and EtOH. The affinity for β-gal and the uptake in LacZ expressing human embryonic kidney (293T) cells were determined in vitro. The biodistribution of the tracers after i.v. injection in mice and their in vivo stability in plasma and urine were studied.

**Results:** In vitro incubation of non-radioactive 6 with β-gal in the presence of the chromogenic substrate ONPG showed that the triazole acts as inhibitor of β-galactosidase. The log P values were -0.1 and 1.4 respectively for [$^{11}$C]-6 and [$^{11}$C]-13, the latter therefore being a good candidate for increased cellular uptake via passive diffusion. Biodistribution studies in mice showed rapid clearance from blood for both tracers. HPLC analysis of murine plasma and urine revealed high in vivo stability. In vitro evaluation in lentiviral vector transduced 293T cells showed an increased uptake for the more lipophilic [$^{11}$C]-13. However, there was no statistically higher uptake in LacZ expressing cells compared to control cells.

**Conclusions:** An efficient and convenient chemical and radiochemical synthesis of two 4-substituted 1,2,3-triazolyl β-D-galactopyranosides was developed. Although cell uptake experiments in LacZ expressing and control 293T cells revealed increased cell uptake for the naphthylic [$^{11}$C]-13 compared to the phenylic triazole [$^{11}$C]-6, no difference in uptake was observed between LacZ and control cells. This is presumably due to the decreased sensitivity using radiolabeled inhibitors instead of substrates. Development of lipophilic $^{11}$C- and $^{18}$F-labeled β-galactosyl triazoles with a stronger inhibition effect may lead to higher cell uptake ratios and better in vivo imaging contrasts.

## P224 EVALUATION OF NOVEL BIODEGRADABLE VECTORS FOR IMPROVED CELLULAR UPTAKE OF RADIOLABELED OLIGONUCLEOTIDES

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**Objectives:** Oligonucleotides are being increasingly investigated for molecular imaging applications. The development of suitable imaging probes is however hindered by low stability and bioavailability of oligonucleotides in vivo. Whereas stabilization in vivo has been achieved by different chemical modifications, effective approaches to enhance intracellular delivery are still lacking. In this study we report on the initial characterisation of novel synthetic vectors for improved cellular uptake of a fluorine-18 radiolabeled antisense oligonucleotide targeting CDKN1A.

**Methods:** A fully phosphorothioated 20-mer oligonucleotide (PS-ODN) complementary to CDKN1A was radiolabeled with fluorine-18 by using the thiol reactive prosthetic group 2-bromo-N-[3-(2-[$^{18}$F]-fluoropyridin-3-yloxy)propyl]acetamide ([$^{18}$F]PyBrA). Vectorization was achieved by micelle formation with two novel biodegradable polyamine grafted poly(ethylene oxide)-block-poly(ε-caprolactone) based copolymers, grafted with spermine (PEO-b-P(CL-g-SP)) and tetraethylenepentamine (PEO-b-P(CL-g-TP)) and a commercially available liposome formulation. Cell uptake studies were performed in human colon carcinoma cell lines with enhanced (HCT116) or deficient (80S4) CDKN1A induction at 37°C and 4°C, including co-incubation with excess random or antisense sequence and with poly-(L)lysine (PLL) and heparin sulfate.

**Results:** [$^{18}$F]FPyBrA-PS-ODN was prepared with radiochemical yield of 51.4-74.4% and specific activity of 0.77-4.44 GBq/μmol after purification. Preparation of vectorized radiolabeled PS-ODN complexes at a polymer:ODN ratio of 8:1 with PEO-b-P(CL-g-TP) and 16:1 with PEO-b-P(CL-g-SP) resulted in ODN binding >90% as confirmed by gel electrophoresis. Cell uptake of non-vectorized [$^{18}$F]FPyBrA-PS-ODN was efficiently blocked by co-incubation with excess of non-radiolabeled random or antisense PS-ODN, reducing the uptake by >80%. For vectorized [$^{18}$F]FPyBrA-PS-ODN we found an increase of the uptake of up to 20-fold with the liposome formulation and up to 3-fold with PEO-b-PCL based copolymers. Temperature had only a minor effect on the uptake of vectorized formulations. Uptake was increased by co-incubation with PLL and reduced with heparin sulfate.

**Conclusions:** Cell uptake studies revealed considerable differences between naked and vectorized [$^{18}$F]FPyBrA-PS-ODN, whereas no significant difference was observed between HCT116 and 80S4 cells. For PEO-b-PCL based copolymers a cytosolic rather then nuclear delivery pathway can be expected (1). The data provides mechanistic insights and parameters to consider for the evaluation of radiolabeled naked and vectorized oligonucleotides in vitro.

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**Reference:** (1) Xiong XB, Uludağ H, Lavasanifar A. Biomaterials 2009, 30: 242-53.