^{111}In-Bz-DTPA-$Z_{HER2:342}$, A CANDIDATE FOR IMAGING OF HER2-EXPRESSION IN MALIGNANT TUMORS

A. Orlova,[1] F. Nilsson,[1] M. Magnusson,[1] L. Gedda,[2] C. Widstrom,[3] A. Wennborg,[1] V. Tolmachev.[2]
[1]Radiopharmacology Unit, Affibody AB, Bromma, Sweden; [2]Unit of Biomedical Radiation Sciences, Uppsala Univeristy, Uppsala, Sweden; [3]Department of Hospital Physics, Uppsala University Hospital, Uppsala, Sweden.

Expression of HER2/neu antigen in breast cancer has well-documented prognostic and predictive values. There are evidences that diagnostically important information may be obtained by detection of HER2 expression in other carcinomas e.g. prostate cancer. The use of molecular imaging of HER2 expression can help to avoid false-negative biopsy results associated with sampling errors of heterogeneity of an antigen expression. A number of conjugates based on anti-HER2 antibodies and their fragments has been proposed and evaluated for non-invasive imaging of HER2-expression *in vivo*.

The goal of this study was to evaluate a labeling of anti-HER2 Affibody molecule $Z_{HER2:342}$ with ^{111}In for single-photon imaging of HER2-expression. Affibody molecule $Z_{HER2:342}$ is a small phage-display selected protein, which binds to HER2 with an affinity of 20 pM. Small size, less than 8 kDA enables quick extravasation and tumor penetration, was well as fast blood clearance.

In this study, a bi-functional chelator, isothiocyanate-benzyl-DTPA was attached to recombinant $Z_{HER2:342}$ by coupling under gentle heating in alkaline condition. Calculated substitution ratio was approximately one chelator per Affibody molecule. One-hour long incubation in acetate buffer with ^{111}In-indium chloride enabled a yield of more than 94%. Final purification on disposable size-exclusion column NAP-5 provided radiochemical purity of more than 98%. *In vitro* characterization of ^{111}In-Bz-DTPA- $Z_{HER2:342}$ demonstrated that indium label is stable, and the conjugate preserves capacity to bind specifically to HER2-expressing SK-OV-3 ovarian cancer cells.

In vivo experiments have shown that ^{111}In-Bz-DTPA- $Z_{HER2:342}$ can target SK-OV-3 xenografts in Balb C nu/nu mice. Tumor uptake of 11.6 ± 2.7 % IA/g was obtained 12 h pi. The tumor binding was specific, since it can be blocked by pre-injecting of large amount of non-labeled $Z_{HER2:342}$ (p<0.001). The only organ besides tumor, which accumulated radioactivity to a high extent, was kidney. Quick blood clearance enabled to obtain high contrast between tumor and blood and other healthy tissues. Data concerning tumor-to-organ rations 4 h pi are presented in Table 1. Gamma-camera study demonstrated clear and high-contrast visualization of SK-OV-3 xenografts in mice 4 h pi.

In conclusion, a combination of Affibody technology with ^{111}In labeling provides a conjugate for efficient early imaging of HER2 expression in vivo.

Tumor-to-organ ratio 4 h after injection of 111In-Bz-DTPA- ZHER2:342 Affibody molecule in Balb C nu/nu mice bearing HER2-expressing SK-OV-3 xenografts.

blood	100,5 ± 25,8
heart	116,0 ± 41,8
lung	20,4 ± 6,4
spleen	20,7 ± 3,5
pancreas	47,7 ± 11,7
stomach	33,5 ± 15,0
intestine	28,7 ± 12,2
salivary gland	22,6 ± 5,2
muscle	185,4 ± 84,3
bone	70,3 ± 69,8

Data are presented as an average (N=4) ± standard deviation

Acknowledgement: This work was partially supported by Swedish Cancer Society (Cancerfonden)

Keywords: Indium-111, Affibody Molecule, HER2

IN VITRO AND *IN VIVO* EVALUATION OF ^{177}Lu-LABELED HUMAN *E. COLI* HEAT-STABLE ENTEROTOXIN FOR SPECIFIC TARGETING OF HUMAN COLON CANCERS

M.F. Giblin,[1,5] G.L. Sieckman,[2] T.D. Shelton,[3] T.J. Hoffman,[2,3] L.R. Forte,[2,4] W.A. Volkert.[1,2,5]
[1]Radiology, University of Missouri-Columbia, Columbia, MO, United States; [2]Research Service, Harry S. Truman Memorial Veterans' Hospital, Columbia, MO, United States; [3]Internal Medicine, University of Missouri-Columbia, Columbia, MO, United States; [4]Medical Pharmacology and Physiology, University of Missouri-Columbia, Columbia, MO, United States; [5]Nuclear Sciences and Engineering Institute, University of Missouri-Columbia, Columbia, MO, United States.

The human *E. coli* heat-stable enterotoxin (ST_h, amino acid sequence N^{1}SSNYCCELCCNPACTGCY^{19}) binds specifically to the Guanylate Cyclase C (GC-C) receptor, which is present in high density on the apical surface of normal intestinal epithelial cells as well as on the surface of human colon cancer cells. GC-C is the cognate receptor for the endogenous peptides guanylin and uroguanylin, which act to regulate ion and fluid homeostasis via activation of cGMP production in the intestine. Analogs of ST_h are currently being used as vectors targeting human colon cancers. Previous studies in our laboratory have focused on development of ^{111}In-labeled ST_h analogs for *in vivo* imaging applications. Here, we extend the scope of this work to include targeting of the therapeutic radionuclides ^{90}Y and ^{177}Lu.

ST_h analogs have been synthesized with pendant N-terminal DOTA moieties and radiolabeled with Yttrium-90 and Lutetium-177. DOTA-F^{19}-ST_h(1-19) was produced by SPPS using standard Fmoc chemistry. The reduced, linear peptide was oxidatively refolded by stirring a 0.1 mg/ml peptide solution in 0.1 M ammonium formate, pH 8.0 at room temperature for periods up to 5 days. The refolded peptide was purified by C18 RP-HPLC and characterized by MALDI-TOF MS. ^{177}Lu conjugates were prepared in high yield (>95%) by addition of $^{177}LuCl_3$ in 0.05 M HCl (0.5 - 1 mCi, 18.5 - 37 MBq) to a solution of DOTA-peptide (30 µg) in 200 ml 0.2 M ammonium acetate, pH 6.0. Reaction mixtures were incubated for 1 hr at 80°C, quenched by addition of 50 µl 2 mM EDTA, and purified by C18 RP-HPLC. The ^{177}Lu-labeled species eluted 1.4 min. earlier than the unlabeled DOTA-peptide-conjugate under the HPLC conditions employed, allowing purification of high specific activity labeled product. The ^{90}Y conjugate was prepared in an analogous fashion, in yield similar to the ^{177}Lu conjugate. The two radioactive species were shown to be inseparable by the RP-HPLC method employed.

Macroscopic amounts of nonradioactive Yttrium- and Lutetium-containing DOTA-peptides were also synthesized, and assayed in an *in vitro* competitive binding assay employing T84 human colon cancer cells. These assays demonstrated IC_{50} values of 4.2 ± 0.9 nM for Y-DOTA-F^{19}-ST_h(1-19) and 2.6 ± 0.1 nM for Lu-DOTA-F^{19}-ST_h(1-19). Such values are similar to the previously determined IC_{50} for the Indium-labeled peptide. *In vivo* pharmacokinetic studies of the ^{177}Lu-labeled peptide were conducted using SCID mice bearing T84 human colon cancer derived tumor xenografts. The labeled peptide demonstrated rapid clearance from the blood pool, primarily by renal/urinary excretion. At 1 hr pi, >90% ID was excreted into the urine, and tumor uptake was 1.86 + 0.91 %ID/g, values in agreement with previous experiments using the ^{111}In-labeled peptide (1). At all time points examined, uptake (%ID/g) in tumor tissue was higher than in any other organ except kidneys. No co-infusion of basic amino acids was performed in these experiments. Scintigraphic imaging studies have also been undertaken to evaluate targeting efficiency of the labeled peptide.

(1) Giblin, MF, Gali, H, Sieckman, GL, Owen, NK, Hoffman, TJ, Forte, LR, Volkert, WA. Bioconj. Chem. 15:872-880, 2004.

Keywords: E. coli Heat-Stable Enterotoxin, Lutetium-177, Colorectal Cancer

SYNTHESIS AND EVALUATION OF In-111 DOTA-POLYDIAMIDOPROPANOATE (PDAP) DENDRIMER-PNA-PEPTIDE CHIMERAS FOR NON-INVASIVE IMAGING OF CANCER

N. Amirkhanov,[1] M.R. Aruva,[2] K. Zhang,[2] B. Mathew,[2] M.L. Thakur,[2] E. Wickstrom.[1]
[1]Department of Biochemistry and Molecular Pharmacology, Thomas Jefferson University, Philadelphia, PA, United States; [2]Department of Radiology, Thomas Jefferson University, Philadelphia, PA, United States.

Coupling of dendrimers to receptor specific biological probes allows one to label the probe with multiple radioactive or non-radioactive metal ions, thereby increasing the efficacy of a probe in diagnostic or therapeutic applications. In this work the novel polydiamidopropanoate (PDAP) dendrimers with different numbers of primary amino group residues were extended from peptide nucleic acid (PNA)-peptide chimeras directly on polymer supports by coupling Fmoc-protected diaminopropanoic acid (DAP) monomers and Fmoc-protected aminoethoxyethoxyacetic acid (AEEA) monomers after solid phase synthesis of PNA-peptide chimeras with a C-terminal insulin-like growth factor 1 (IGF1) analog D(Cys-Ser-Lys-Cys). PDAP dendrimer PNA-peptide chimeras, $(H_2N\text{-AEEA})_n$-PDAP^m-$AEEA_2$-**GCCAACAGCTCC**-AEEA-D(Cys-Ser-Lys-Cys)-$C(O)NH_2$, with different generations of PDAP dendrimers (m= 1, 2, 3 or 4) and different numbers of free amino groups on the ends of PDAP dendrimer (n= 2, 4, 8 or 16) were synthesized with a PNA complementary to 12 nt of mutant KRAS mRNA. A protected macrocyclic chelating moiety, 1,4,7,10-tetraazacyclododecane-1,4,7-tris(acetic acid tert-butyl ester)-10-acetic acid (DOTA-3tBu), was activated by O-(7-azabenzo-triazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and coupled to each free amino group of the PDAP dendrimer on PNA-peptide before cleavage from the polymer support. The DOTA-PDAP-PNA-peptide probes with 1, 2, 4, 8 or 16 amino (or DOTA) moieties were cleaved, purified by RP-HPLC, and characterized by MALDI-TOF mass spectroscopy.

The $(DOTA)_{1,2,8,16}$-PNA-peptide probes were labeled with gamma-emitting In-111 for scintigraphic imaging. $(In\text{-}111\text{-}DOTA)_{2,8,16}$-PNA-peptide probes were administered by tail vein into immunocompromised mice bearing human pancreatic cancer xenografts along with increasing concentrations of cold $(Gd\text{-}DOTA)_{2,8,16}$-PNA-peptide chimeras, respectively. Elevated doses of Gd-DOTA-PNA-peptide increased accumulation of In-111-PNA-peptides in tumors, thereby increasing the tumor-to-muscle ratio of radionuclide uptake from 2 to up to 4, and decreased kidney-to-tumor ratio of radionuclide uptake from 120 to up to 20. Thus, these experiments show that PDAP dendrimers with up to 16 DOTA chelators attached to PNA-peptide probes do not perturb tumor uptake of PNA-peptide probes, allowing the use of these chimeras for preclinical magnetic resonance imaging (MRI) experiments. Supported by NCI CO-27175.

Keywords: Indium-111 DOTA, PNA, Dendrimers, Cancer Diagnostics, Imaging

SYNTHESIS OF ^{123}I-RADIOPHARMACEUTICALS WITH THE LABEL IN A BRIDGEHEAD POSITION

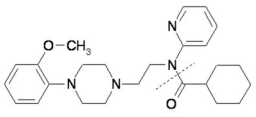
J.D.M. Herscheid,[1] J. Verbeek,[1] J. Booij.[2]
[1]Nuclear Medicine and PET Research, Location Radionuclide Center, VU University Medical Center, Amsterdam, Netherlands; [2]Nuclear Medicine, Academic Medical Center, Amsterdam, Netherlands.

Most common mechanisms for *in vivo* deiodination of a radiopharmaceutical are nucleophilic substitution (S_N2) and β-elimination. In order to reduce deiodination, the radioiodine atom is normally attached to an sp2 carbon atom in an aromatic or vinylic moiety.

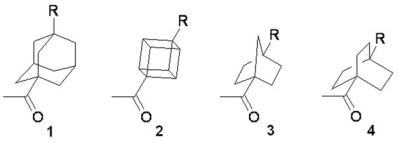
A possible alternative is to place the radioiodine atom on a bridgehead carbon atom. Shielding by the ring structure prevents a backside S_N2 attack, while β-elimination is unlikely because the ring is too much strained to accommodate an sp2 carbon atom. An additional advantage is that introduction of an Iodine atom on a bridgehead carbon atom often yields no additional chiral centers, and in the case of cubane never.

WAY-100635 in its ^{11}C-labelled form is used world-wide to image the serotonin-5HT$_{1A}$ receptor using Positron Emission Tomography (PET).

We have prepared WAY-derivatives with, instead of the cyclohexyl group, an adamantane(**1**), cubane(**2**), bicycloheptane(**3**) or bicyclooctane(**4**) moiety and tested them *in vitro* on RBHS1AM membranes containing the human 5-HT$_{1A}$ receptor. The cubane derivative with Iodine on the opposite bridgehead position was found to exert a comparable *in vitro* activity (EC50 = 0.9 nM) as the native WAY-100635 (EC50 = 0.4 nM).



WAY-100635



R = I, ^{123}I, Br

Surprisingly, labelling of the cubane moiety by an ^{123}I for Iodine or for Bromine exchange was found to be the most facile (see Table).

^{123}I-labelled bridgehead WAY-derivatives

Precursor	Product (R=^{123}I)	RC yield
1 R=I	**1**	40 %
2 R=I	**2**	85 %
3 R=I	**3**	0.5 %
4 R=I	**4**	0 %
1 R=Br	**1**	10 %
2 R=Br	**2**	70 %
3 R=Br	**3**	0 %
4 R=Br	**4**	0 %

Applied reaction conditions: 2mg of precursor and 0.1 mg of Cu(II)triflate dissolved in 100-200 µl of acetonitrile containing radioiodine (obtained according to AH Braker et al, Appl.Radiat.Isot., **57**, 475 (2002)) is heated in a closed vial for 30 minutes at 140 °C.

The results of biodistribution and replacement studies in rats, as well as images made with our animal SPECT-scanner will also be presented.

We feel that the above approach, *i.e.* making use of the iodocubane moiety, might be applicable for a large variety of radiopharmaceuticals.

Keywords: Iodine-123, Bridgehead-Labelling, WAY-Derivatives