TARGETED RADIONUCLIDE THERAPY OF MELANOMA: ANTI-TUMOURAL EFFICACY OF A NEW IODINE-131 LABELLED AGENT

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Objectives: In recent years, there has been a dramatic worldwide increase in incidence of melanoma. Although localized disease is frequently curable by surgical removal, metastatic melanoma is inherently resistant to most treatments and has a very poor prognosis. In this context targeted radionuclide therapy could be an efficient alternative to conventional therapeutic agents.. At our institution, a class of iodobenzamides has been developed as potent melanoma-seeking agents. After pharmacomodulation study, we selected a quinoxaline derivative molecule (ICF01012) for its high, specific and long-lasting uptake in melanoma with rapid clearance from non-target organs providing suitable dosimetry parameters for targeted radiotherapy. Aim of this study was to investigate the efficacy of [^{131}I]ICF01012 for melanoma radionuclide therapy.

Methods: [^{131}I]ICF01012 was administered i.v into C57BL6 mice bearing the nonmetastatic B16F0 or metastatic B16bl6 cell lines grafted s.c.

Results: Treatment drastically suppressed the growth of both B16F0 ($p=0.001$) and B16bl6 ($p=0.009$) tumors whereas treatment with [^{131}I]NaI or unlabeled ICF01012 was without significant effect. The tumor doubling time after [^{131}I]ICF01012 treatment was significantly increased (+147 to +164%). Histology analysis of residual treated-tumors revealed an increased extracellular melanin content, a decreased number of mitoses and a defect in microvascularization. All these features suggested a loss of aggressiveness. We showed a significant decrease of PCNA expression confirming that proliferation index of resting cells after radionuclide treatment was strongly decreased in comparison of control group. Moreover, after macroscopic analysis, we observed that 55% of the untreated mice bearing B16Bl6 tumours had lung metastases whereas no metastasis was counted on [^{131}I]ICF01012 treated group.

Conclusions: In conclusion, our data demonstrated a strong anti-tumoural efficacy of [^{131}I]ICF01012 for radionuclide therapy on two in vivo melanoma models whatever their dissemination profiles. Results presented here support the concept of targeted radionuclide therapy using a [^{131}I] labelled iodoquinoxaline for an effective melanoma treatment. Further studies will attempt to optimize the therapy protocol by increasing the balance between the anti-tumoral effect and safety on non-target organs.

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TO BE OR NOT TO BE – Tb OR Lu? – BIOLOGICAL EVALUATION OF Tb-161-DOTA-TYR3-OCTREOTATE

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Objectives: Terbium-161 ($t_{½}$=6.91 d) with moderate energy beta emissions of 0.59 MeV and low energy gamma emissions (46-48 and 74 keV) can be produced in a no carrier added form by neutron irradiation of isotopically enriched Gd-160 to produce Gd-161, which rapidly beta decays (3.7 min) to Tb-161. Chemical separation of Tb-161 from the Gd target material yields a high specific activity Tb-161. The aim of the study was to evaluate the in vitro stability and in vivo biodistribution of the somatostatin analog, DOTA-Tyr^3-octreotate (DOTA-Y3-TATE), radiolabeled with Tb-161. Both Tb-161 and Lu-177 have half-lives of ~ 7 days, beta emissions appropriate for therapy (~ 0.5 MeV) as well as gamma emissions that enable dosimetry and pharmacokinetics. The Tb-161 is prepared in high specific activity and its lower gamma emission (74.6 keV) is being investigated as it may result in lower dose to normal tissues than that observed for Lu-177.

Methods: The Tb-161 was produced at MURR and supplied in 0.05 N HCl. The Tb-161 was reacted with DOTA-Y3-TATE and the radiolabeling yield confirmed by TLC and HPLC. The in vitro stability of the formed complex was evaluated in rat serum and hydroxyapatite at 37°C. Receptor binding assays were performed in AR42J cells using ^{111}In-DOTATIDE as the competing ligand. The biodistribution was conducted by intravenous injection into ICRSC-M mice xenografted with the rat pancreatic tumor line AR42J.

Results: Complexes remained >93% intact when challenged by serum and hydroxyapatite. An IC_{50} value of 15 nM was determined. The complex exhibited excellent blood clearance with only 0.6 % ID remaining at 1 h and 0.01 % ID at 72 h. The main excretion route was through the urine with 63.3 % ID cleared at 1 h and 92.5 % at 72 h. The compound exhibited high uptake in the tumor 12.6 % ID/g at 1 h which decreased to 5 % ID/g at 72 h. The uptake in the somatostatin-rich receptor tissues was 16.2 % ID/g at 1 h in the pancreas and 3.7 % at 4 h in the adrenals. A blocking study using unlabeled DOTA-Y3-TATE resulted in >97% lower uptake in the tumor, 98% in adrenals and 98% in pancreas indicating that ^{161}Tb-DOTA-Y3-TATE was binding specifically to somatostatin receptors in these tissues. The biodistribution was similar to that of the analogous Lu-177 agent.

Conclusions: The ^{161}Tb-DOTA-Y3-TATE stability and biodistribution is analogous to that of the Lu-177 agent and offers an alternative approach for radionuclide therapy.

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STABILIZED SULFATED CHOLECYSTOKININ-8 (CCK-8) ANALOGUES LABELED WITH IN-111 FOR TARGETING CCK RECEPTOR-POSITIVE TUMORS

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Objectives: Radiolabeled cholecystokinin-8 (CCK8) peptide analogues potentially can be used for peptide receptor radionuclide therapy (PRRT) of small cell lung cancers and medullary thyroid carcinomas, which abundantly express CCK2/gastrin receptors (CCK2R). We have previously shown that sCCK8 (D-Tyr(OSO_3H)-M-G-W-M-D-F-NH_2) has better characteristics for PRRT than gastrin analogues. However, sCCK8 contains an easily hydrolysable sulphated tyrosine residue and two methionine residues which are prone to oxidation. Therefore, we aimed to develop stable sCCK8 analogues. To prevent oxidation, the Met residues were replaced by norleucine (Nle) or homopropargylglycine (HPG). The tyrosine sulphate moiety was replaced by a robust isosteric sulphonate.

Methods: The sulphonate isostere of Tyr sulphate, Phe(p-CH_2SO_3H), was synthesized from L-Tyr by modification of known routes. With this Phe(p-CH_2SO_3H) building block, 3 sCCK8 analogues with either Met, Nle or HPG in positions 3 and 6 were synthesized using Fmoc solid phase peptide synthesis: sCCK8[Phe^2(p-CH_2SO_3H),$Met^{3,6}$], sCCK8[Phe^2(p-CH_2SO_3H),$Nle^{3,6}$] and sCCK8[Phe^2(p-CH_2SO_3H),$HPG^{3,6}$]. All peptides were N-terminally conjugated with DOTA and purified by HPLC. Peptides were labeled with In-111 and stability of the peptides was studied. The receptor binding affinity and internalization was determined by in vitro assays using CCK2R-transfected HEK cells and AR42J rat pancreatic tumor cells. Biodistribution of all peptides was studied in nude mice bearing a sc AR42J tumor.

Results: Oxidation assays and subsequent HPLC analysis revealed that sCCK8[Phe^2(p-CH_2SO_3H),$Nle^{3,6}$] and sCCK8[Phe^2(p-CH_2SO_3H),$HPG^{3,6}$] indeed were resistant to both hydrolysis and oxidation. In vitro receptor binding assays revealed that all peptides showed specific binding and receptor-mediated internalization in AR42J cells. Binding affinities (IC_{50}) of the peptides were all lower than 9.5 nM. Biodistribution studies in AR42J tumor-bearing mice showed that tumor uptake was highest for sCCK8 and sCCK8[Phe^2(p-CH_2SO_3H),$Nle^{3,6}$] (4.78 ± 0.64 and 4.54 ± 1.15 %ID/g, respectively, 2 h p.i.). Tumor accumulation of sCCK8[Phe^2(p-CH_2SO_3H),$Met^{3,6}$] and sCCK8[Phe^2(p-CH_2SO_3H),$HPG^{3,6}$] was significantly lower (2.18 ± 0.18 and 1.92 ± 0.31 % ID/g, respectively). For all compounds tested, the blood, lungs and peripheral soft tissues such as muscle, spleen and small intestine showed similar low uptake. Specific uptake was also found in the pancreas and, to less extent, in the stomach. Kidney uptake of all peptides was lower than 1.5 % ID/g.

Conclusions: We developed CCK-R targeting peptides with improved stability and showed that the acid-labile sulphated tyrosine residue can be replaced by the nonhydrolyzable Phe(p-CH_2SO_3H) without loss in affinity. Also, replacement of the two Met residues by a non-oxidizable Nle did not affect receptor affinity. Therefore, DOTA-sCCK8[Phe^2(p-CH_2SO_3H),$Nle^{3,6}$] will be explored further for peptide receptor radionuclide therapy of CCK2R expressing tumors.

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INORGANIC NANOPARTICLE MONOCLONAL ANTIBODY CONJUGATES

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Objectives: A number of nanoparticles (NP) have been studied as potential carriers of radioisotopes for applications in targeted imaging and therapy [1], however, details of the fate of these constructs are scarce, in part, due to difficulties quantifying NP in vivo. Our aim is to follow the in-vivo fate of targeted CdTe(^{125m}Te) NPs using MAb 201B [2]. Although this model is not directly applicable in patients, it does indicate basic principles of biodistribution of targeted NP in vivo. In our approach, ^{125m}Te ($t_{1/2}$ = 60 d, and similar decay to ^{125}I) is an integral part of the CdTe NP, and was found to be a good marker of NP biodistribution. Our results further demonstrate that, even in very favorable targeting situations in vascular space, NP are rapidly recognized by the reticuloendothelial (RE) system significantly affecting targeting efficiency and retention.

Methods: CdTe NP were prepared with $^{nat.}$Te or ^{125m}Te/ ^{124}Te (stable) mixture [1]. Te metal was dissolved in trioctyl-phosphine. To form 5 nm dia. particles, CdO, tetradecyl-phosphonic acid and Te were reacted with a trioctylphosphine oxide/trioctylphosphine colloid at 250^{0}C for 5m. CdTe NP were capped with ZnS to reduce leaching, and functionalized with mercaptoacetic acid providing an anchor site for antibody conjugation. To evaluate specific targeting, we used MAb201B that binds to murine thrombomodulin expressed in lumen of lung blood vessels. MAb-NP was tested for targeting performance in vivo using SPECT/CT imaging, tissue autoradiography and standard organ biodistribution methods. Biodistribution was also studied in mice depleted of phagocytic cells using clodronate-loaded liposomes.

Results: Cd^{125m}Te/ZnS NP-MAb 201B retained radioisotope and antibody activity and accumulated in lung (>400 %ID/g) within 1h of iv injection. Control antibody-coupled NP did not accumulate in lung (<10%ID/g) but lodged in liver and spleen. microSPECT/CT images and autoradiography document the specific uptake, showing uniform distribution in lung with minor liver/spleen accumulation (Fig 1). Within a few hours, a large fraction of lung-targeted NP redistributed to spleen and liver or was excreted. We hypothesized that phagocytic cells engulf and remove NP from circulation, as confirmed by comparing normal mice vs mice depleted of phagocytic cells. In mice treated with clodronate liposomes at 1h, NP level in liver was reduced 5 fold while accumulation in lung was enhanced by ~50%. By 24h, targeted NP loss from lung was inhibited by several fold while level in liver/spleen levels remained constant. Treated mice had a much larger retention of NP in lungs and decrease in other organs except spleen.

Conclusions: CdTe(^{125m}Te) NPs/ZnS can be targeted with MAb to sites in the lumen of lung vasculature. NP targeting is a competition between effectiveness of targeting agent and natural uptake of NP by RE system. Temporary inhibition of RE system may enhance usefulness of NPs for drug and radioisotope delivery.

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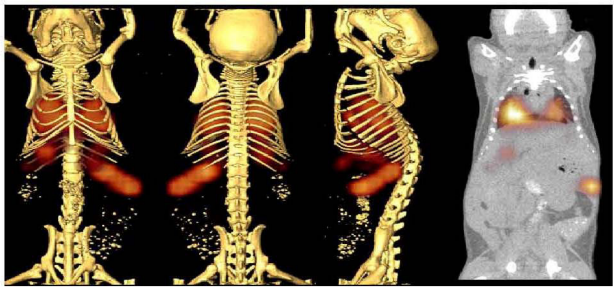
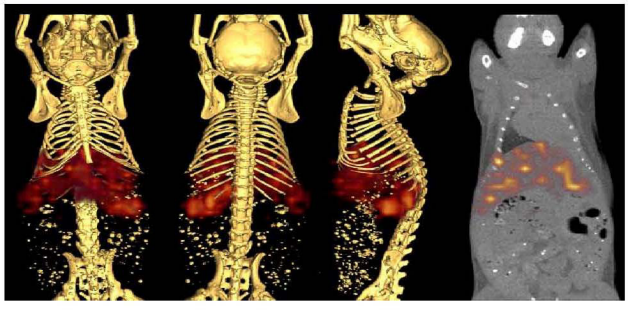
A. MAb 201B targeted NP



B. Control MAb targeted NP



SITE-SPECIFICALLY 89ZR-RADIOLABELED MONOCLONAL ANTIBODIES FOR IMMUNOPET IMAGING

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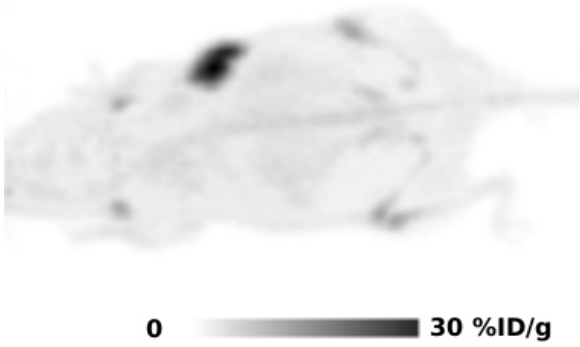
Objectives: ImmunoPET couples the high sensitivity of positron emission tomography PET with the high specificity of monoclonal antibodies (mAbs). This allows immunoPET the ability to track and quantify therapeutic mAbs in vivo. Imaging with intact antibodies (~150 kDa) provides maximum target-to-background ratios two to six days after administration, which requires the use of radioisotopes with corresponding half lives, such as ^{89}Zr ($T_{1/2}$ 3.3 d). Two bifunctional reagents based on desferrioxamine B (Df) were developed for the coupling of ^{89}Zr to mAbs via lysine amino groups. Since random modification of lysine residues provides heterogeneous products and may decrease the immunoreactivity of the conjugate, we investigated the site-specific modification of mAbs with Df. Recently, a method (PHESELECTOR) was reported to identify the optimal amino acid position for substitution to cysteine. The resulting antibody (ThioMab) can be site-specifically conjugated to a cytotoxic drug or a bifunctional chelator while retaining binding affinity and maintaining antibody scaffold stability[1]. In order to obtain site-specific ^{89}Zr-radiolabeled ThioMabs for immunoPET, we developed a novel thiol-reactive reagent Df-SMCC and evaluated the resulting anti-HER2-^{89}Zr-ThioMab (^{89}Zr-4D5ThioMab) for imaging HER2 expression in vivo.

Methods: Desferrioxamine B was conjugated with the heterobifunctional linker succinimidyl 4-[N-maleimidomethyl] cyclohexane-1-carboxylate (SMCC) to obtain the chelator decorated with maleimide group in 43% yield. The subsequent site-specific conjugation to the two engineered cysteines of 4D5ThioMab was completed in 60 minutes. The product was purified by size exclusion chromatography and characterized by mass spectrometry. The ^{89}Zr-Df-SMCC-4D5ThioMab was obtained by incubating 1 mg of Df-SMCC-4D5ThioMab with ^{89}Zr in 0.5 M HEPES buffer for one hour.

Results: The product yield was 77%, with a specific activity of 130 Ci/mmol. Approximately 0.1-0.4 mCi of ^{89}Zr-radiolabeled probe in isotonic solution (100-130 mL) was administered to mice bearing subcutaneous xenograft tumors (BT474). PET imaging was performed at 24, 96, and 144 hours post-tracer injection. The tumor uptake peaked at 96 hours (Figure) reaching 21 %ID/g and the tumor to blood ratio equal to 5.0. The stability of the ^{89}Zr-Df-SMCC-4D5ThioMab was evaluated in vitro by incubation in mouse serum.

Conclusions: In conclusion, we have developed a novel thiol-reactive bifunctional chelator Df-SMCC for radiolabeling proteis with ^{89}Zr. The reagent was used for site-specific radiolabeling of HER2 specific monoclonal antibody (4D5ThioMab) and was evaluated in vitro and in vivo.

References: 1. Junutula, J. R.; et al Nat Biotechnol 2008, 26, 925-32.



COPPER-64 LABELING OF LIPOSOMES: POST-LABELING METHOD USING BIFUNCTIONAL LIGAND (BFL)

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Objectives: Non-invasive liposomal studies have been intensively exploited with SPECT. However, PET has rarely been applied in such studies although PET has advantages for pharmacokinetics and dynamics. We have developed a surface chelation labeling method, using Cu-64 (half life = 12.7 h) by simply adding Cu-64 to preformed liposomes which contain benzylTETA-lipids. Although this method provides fast and simple labeling of Cu-64, several limitations, such as limited buffer choice, prescribed pH during liposome formation, and lower labeling yield for targeted liposomal formulations, are disadvantages. In order to overcome these limitations, we designed and synthesized a bifunctional ligand (BFL), which could be conjugated to preformed liposomes at room temperature (Figure).

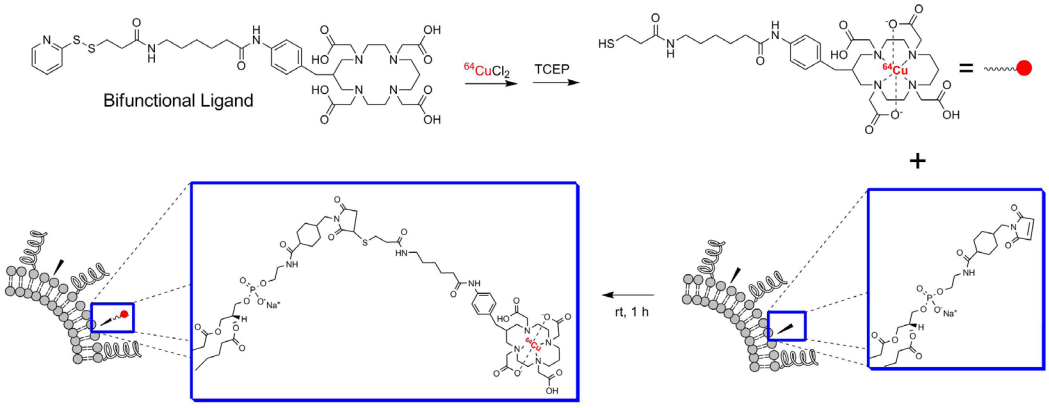

Figure. Post-labeling of liposome with BFL

Methods: Synthesis of bifunctional ligand: In brief, bifunctional ligand was synthesized from 4-nitrobenzyl bromide within six steps, which resulted in 12% overall yield. Liposomal formulation: Liposomes (10 mg) were formulated with HSPC (56 mol%), cholesterol (39 mol%), DSPE-PEG2K (5 mol%), and maleimide lipids of different molar ratios (0.1, 0.5, 1, and 5 mol%) at 60 °C within 10 min by a mini-extruder (100 nm membrane filter) in 0.12 M phosphate buffer (pH 7.0). Labeling: Cu-64 (0.75 mCi) was added to a solution of bifunctional ligands (6 nmol, 60 μL of 0.1 mM in 0.1 M ammonium citrate buffer, pH 5.5). After incubation for 30 min at 30 °C, freshly prepared 1.0 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP) solution (60 nmol, 60 μL) in 0.12 M phosphate buffer (pH 7.0) was added to bifunctional ligand solution. The mixture was incubated for 10 min at room temperature. Finally, the mixture was added to a liposome (5 mg, 0.2 mL) solution and incubated for 1 h. Labeled liposomes and free ligands were separated by a size exclusion column.

Results: Titration assay of Cu-64 (S.A. = 0.64 mCi/nmol) with BFL showed that 6 nmol of BFL ligand incorporated 99% of 0.75 mCi. Excess of TCEP (> 5 times vs BFL) reduces the pyridyldithio group required to generate free thiols within 10 min. Liposomes containing varied mol percent of maleimide (0.1%, 0.5%, 1.0%, and 5.0%) reacted with activated BFL, which resulted in 3%, 19%, 28%, and 83% labeling yield, respectively. Mole ratios of maleimide lipid vs BFL ligand (mol of maleimide lipid/mol of BFL ligand) were 0.55, 2.83, 5.83, and 28.5.

Conclusions: We have obtained 84% labeling yield when 5 mol% of maleimide (172 nmol) liposomes were reacted with Cu-64 chelated BFL (6 nmol). A key parameter is the molar ratio of maleimide lipids and bifunctional ligands. In vivo stability will be tested with mice to compare the stability of liposomes.