## P363 PREPARATION OF $^{66}$Ga-CHITOSAN FOR THE ENDORADIOTHERAPY

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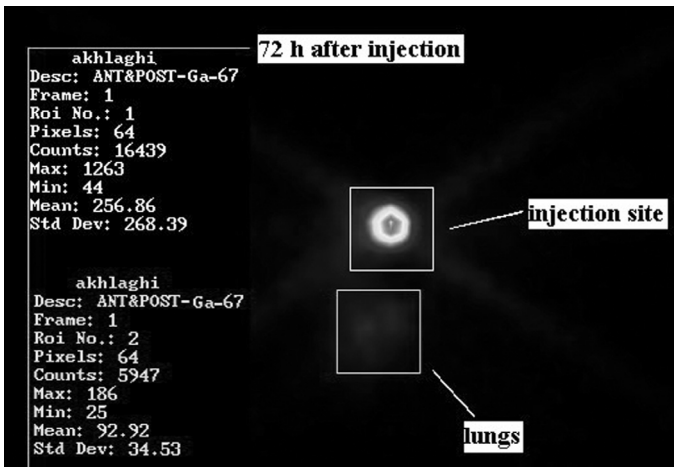
**Introduction:** The purpose of this study was focused on the preparation of gallium-66 chitosan complex ($^{66}$Ga-Chitosan) for internal radiation therapy by administering directly into tumor. Ga-66 radioisotope decay to Zn-66 by EC and $\beta^+$ and can be a candidate for tumor internal radiotherapy.

**Experimental:** Radioisotope $^{66}$Ga ($t_{1/2}$=9.49 h, $\beta^+$: 4.153 (57%) MeV, $\gamma$: 511(114%), 834 (6.03%), 1039 (37.9%), 2752 (23.2%) keV) was prepared by the $^{66}$Zn(p, n)$^{66}$Ga reaction at the Cyclone-30 IBA accelerator. The Ga-chitosan complex was prepared by reacting of aqueous $^{66}GaCl_3$ solution with chitosan solution in 1% acetic acid. Labeling yield and radioanalytical purity of $^{66}$Ga-chitosan complex was measured by R-TLC method using Whatman No.1 chromatography paper and MeOH: AcOH: $H_2O$ (40:40:20) as mobile phase. The effect of pH, concentration of chitosan, molecular weight of chitosan and activity of $^{66}GaCl_3$ on labeling yield was investigated. $^{67}$Ga-chitosan complex was used for in-vivo stability investigation instead of $^{66}$Ga-chitosan complex for its longer half life by direct administration of complex into fibrosarcoma tumor in a mice model.

**Results and Discussion:** The $R_f$ values for $^{66}$Ga-chitosan complex and free $^{66}GaCl_3$ were 0.0-0.1 and 0.9-0.95, respectively. Labeling yield of chitosan was over 99% in pH=3.5-4 and 30mg/3ml chitosan concentration. The $^{66}$Ga-chitosan complex showed high in-vitro stability. Also it showed high in-vivo stability and low leakage even 9 days after injection. Only low molecular weight $^{66}$Ga-chitosan complex leaked from injection site and up took in mice lungs.




**Conclusion:** $^{66}$Ga-chitosan complex can be effective agent for radionuclide tumor internal radiotherapy because of its high in-vivo stability and low leakage from injection site.

Keywords: Chitosan, Gallium-66, Endoradiotherapy

## P364 PREPARATION AND QUALITY CONTROL OF ($^{64}$Cu)-DOTA-ANTI-CD20 FOR TARGETED THERAPY

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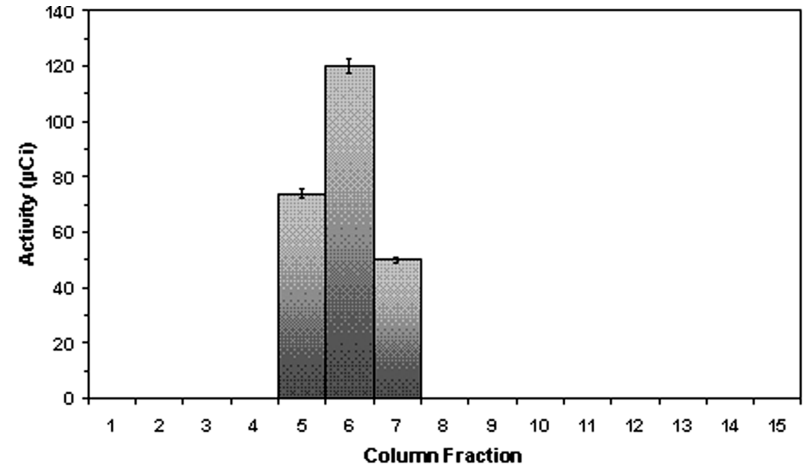
**Introduction:** In order to obtain an anti-CD20 conjugate for use in future therapeutic studies using therapeutic radioisotopes, $^{64}$Cu-labeled antibody was prepared as a model of metal chelated immunoconjugate for preliminary dosimetric and biodistribution studies.

**Experimental:** Copper-64 was produced as a by-product of $^{55}$Co *via* $^{64}$Ni(p,n)$^{64}$Cu by 15 MeV proton bombardment of $^{nat}$Ni resulting in a thick target yield of 5.31 MBq/μAh (143.5 μCi/μAh) and a radiochemical separation yield of 95% (radionuclide purity > 97% after 25 h of the bombardment). Rituximab was successively labeled with [$^{64}$Cu]-$CuCl_2$ using N-succinimidyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA-NHS). DOTA-NHS (0.01-0.1 mg) was prepared at 25°C using DOTA and N-hydroxy succinimide (NHS) in $CH_2Cl_2$ followed by the addition of 1 ml of a Rituximab pharmaceutical solution (5 mg/ml, in phosphate buffer, pH=7.8).

**Results and Discussion:** Radiolabeling was performed at 37°C in 3 h. Radio-thin layer chromatography showed an overall radiochemical purity of 90-95% at optimized conditions (specific activity =30 GBq/mg, labeling efficacy; 82%). The final isotonic $^{64}$Cu-DOTA-rituximab complex was checked by gel electrophoresis for radiolysis/chemolysis control. Radio-TLC was performed to ensure the formation of only one species followed by filtration through a 0.22 μ filter.




**Conclusion:** The method used for the production of $^{64}$Cu from $^{nat}$Ni is suitable where $^{64}$Ni is not available. Total labeling and formulation of [$^{64}$Cu]-DOTA-rituximab took about 60 min, with a yield of 95% while using optimized conditions. The radiolabeled complex was stable in human serum for at least 24 h and no significant amount of free $^{64}$Cu as well as $^{64}$Cu-DOTA was observed. Trace amounts of $^{64}$Cu-copper chloride (”1-3%) were detected by TLC. [$^{64}$Cu]-DOTA-rituximab can be a suitable therapeutic radiopharmaceutical for the treatment of Lymphoma B malignancy in human.

Keywords: Copper-64, Rituximab, Therapy, Conjugation, Quality Control

## P365 TOWARDS A RADIOPHARMACEUTICAL FOR PALLIATIVE TREATMENT OF METASTATIC BONE PAIN: A NOVEL $^{117m}Sn(Sn^{II}$ OR $Sn^{IV})$-POLYMER COMPLEX

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**Introduction:** In an attempt to identify potential radiopharmaceuticals for palliative treatment of metastatic bone pain [1], or at least contribute to the larger understanding of these compounds and their mode of action, we've considered the use of the phosphonate ligand N,N',N'-trimethylenephosphonate-polyethyleneimine (PEI-MP) as delivery agent of $^{117m}Sn^{II}$ and $^{117m}Sn^{IV}$ to bone tumours [2]. PEI-MP is a novel polymer comprising of ethylenediamine-tetramethylenephosphonate (EDTMP) sub-units. $^{117m}Sn$ ($t_{1/2}$=13.6 d) could prove to be a promising therapeutic radionuclide in that it emits mono-energetic Auger and conversion electrons with a discrete range (0.2-0.3 mm) in bone tissue, allowing for larger radiation doses with limited radiotoxicity to bone marrow. It also emits a gamma (159 keV, 86.4%), enabling visualisation of radio-treatment localisation.

An important aspect of the biological activity of tin-based radiopharmaceuticals is the oxidation state of the metal-ion. Bone uptake of tin(II)-ethylenehydroxy disodium phosphonate (Sn(II)-EHDP) exceeds that of the Sn(IV) analogue in mice [3], whereas for methylene diphosphonate (MDP) and diethylenetriaminepenta-acetic acid (DTPA) the inverse was observed [3]. The fate of the drug-complex may depend on the valence stability of the metal-ion *in vivo* – oxidation of the Sn(II) could affect the biodistribution of the complex due to the overall charge. The valence stability of Sn-bisphosphonate complexes was investigated with ligands 1-hydroxyethylene-diphosponate (HEDP) and PEI-MP. With particular interest in the possible interconversion between Sn(II) and Sn(IV), the complexes were monitored with the aid of $^{31}$P-NMR spectroscopy. Slight oxidation of the Sn(II)-complexes was observed upon preparation, beyond which they were stable. Sn(II)- and Sn(IV)-complexes were found to co-exist in solution without change. Further oxidation was induced by the addition of $H_2O_2$, and was partially reversed by the addition of glutathione (GSH). The results suggest that Sn(II)- and Sn(IV)-complexes would be stable under physiological conditions.

**References:** [1] V.J. Lewington, *J. Nucl. Med.*, **46** (1) 38S-47S, 2005, Society of Nuclear Medicine. [2] J.R. Zeevaart, W.K.A. Louw, Z.I. Kolar, E. Kilian, F.E. Jansen van Rensburg, I.C. Dormehl, *Drug Res.*, **54** (6), 2004, 340-347. [3] S.C. Srivastava, G.E. Meinken, P. Richards, P. Som, Z.H. Oster, H.L. Atkins, A.B. Brill, F.F. Knapp, T.A. Butler, *Intern. J. Nucl. Med. Biol.*, **12,** 1985, 167.

## P366 A NOVEL $^{177}$Lu-LABELED PORPHYRIN FOR POSSIBLE USE IN TARGETED TUMOR THERAPY

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**Introduction:** Porphyrin and its derivatives exhibit inherent affinity for localization in tumors. Hence, porphyrin derivatives radiolabeled with suitable therapeutic radionuclides could be envisaged as potential agents for targeted tumor therapy. In this direction, a water soluble porphyrin derivative viz. 5,10,15,20-tetrakis[4-carboxymethyleneoxyphenyl]porphyrin was synthesized and radiolabeled with $^{177}$Lu with an aim to prepare an agent for in-vivo tumor radiotherapy. $^{177}$Lu is presently being considered as a promising radionuclide for the development of targeted radiotherapeutic agents owing to its suitable decay characteristics [$T_{1/2}$=6.73 d, $E_{[beta]max}$=0.49 MeV, $E_{\gamma}$=208 keV (11%)]. The comparatively longer $T_{1/2}$ of $^{177}$Lu provides logistic advantages and high (n,$\gamma$) cross-section (2100 b) of $^{176}$Lu ensures its production with high specific activity.

**Experimental:** $^{177}$Lu was produced by irradiation of enriched $Lu_2O_3$ (64.3% $^{176}$Lu) at a neutron flux of $1x10^{14}$ n/cm$^2$.s for 14 d. For $^{177}$Lu labeling, the porphyrin was coupled to a suitable BFCA, namely, *p*-aminobenzyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid. The coupling was effected in dioxan medium in presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide at room temperature for 20 h. The porphyrin-BFCA conjugate was purified by preparative TLC and characterized by FT-IR and $^1$H-NMR spectroscopy. The $^{177}$Lu labeling was achieved by incubating 50 $\mu$g of the conjugate with $^{177}LuCl_3$ (200 ng Lu) in acetate buffer (pH 5) at 50°C for 1 h. The radiolabeled conjugate was characterized by HPLC and its biological efficacy was studied in Swiss mice bearing fibrosarcoma tumors.

**Results and Discussion:** $^{177}$Lu was obtained with ~550 TBq/g specific activity and 99.98% radionuclidic purity. The $^{177}$Lu labeled porphyrin conjugate was obtained with 99% radiochemical purity under optimized reaction conditions and it exhibited excellent stability upto 7 d at room temperature. Biodistribution studies revealed good tumor uptake (1.66% ID/g) within 30 min post-injection (p.i.). At 3 h p.i., tumor uptake increased to 2.01% ID/g with >94% injected activity exhibiting renal clearance. No significant accumulation was observed in any vital organ/tissue. The tumor/blood and tumor/muscle ratios were 2.89 and 16.80, respectively, at this time point which further increased till 48 h p.i. upto which the study was carried out. Serial scintigraphic images recorded using a gamma camera exhibited significant accumulation of activity in tumor over background at 3 d p.i. and the activity was observed to be retained in the tumor till 14 d.

**Conclusion:** Bioevaluation studies provide supportive indications towards possible potential of the novel $^{177}$Lu labeled porphyrin for targeted tumor therapy.

**Acknowledgement:** The authors acknowledge IAEA for providing the enriched Lu target.

Keywords: Lu-177, Porphyrin, Targeted Radiotherapy

## P367 RADIOIMMUNOTHERAPY AS A NOVEL MODALITY FOR TREATMENT OF INFECTIOUS DISEASES

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**Introduction:** There is an urgent need of new approaches to antimicrobial therapy in the field of infectious diseases. Radioimmunotherapy (RIT) has proved to be a successful therapy for certain malignancies. Though RIT of cancer exists for more than 30 years - potential of RIT as an antimicrobial treatment strategy has not been developed clinically, which could reflect lack of awareness of the difficult problems in clinical infectious diseases by the nuclear medicine community and of RIT - by the infectious diseases physicians.

**Experimental:** Several years ago we introduced RIT into the realm of infectious diseases by demonstrating its efficacy in treating murine cryptococcosis using a monoclonal antibody to *Cryptococcus neoformans* capsular glucuronoxylomannan labeled with Bismuth-213 or Rhenium-188. Later on we expanded RIT approach to treatment of bacterial (*Streptococcus pneumonia*) and viral (HIV-1) infections.

**Results and Discussion:** Treatment significantly prolonged the survival of infected mice and did not result in acute hematologic toxicity in treated animals. The mechanism of RIT of infection is complex and includes killing of microbial cells by "direct hit" and "cross-fire" effects, promotion of apoptosis-like death, cooperation with macrophages and modulation of the inflammatory response. RIT for infection can be developed for any microbe susceptible to radiation, including bacteria, fungi, viruses and parasites. For many infectious diseases, not every microbial cell needs to be killed by RIT as immune system can "mop up" the remaining post-RIT load. It is also potentially less toxic to normal tissues and organs than cancer RIT as, in contrast to tumor cells, cells expressing microbial antigens are antigenically very different from host tissues and thus provide the potential for exquisite specificity and low cross-reactivity.

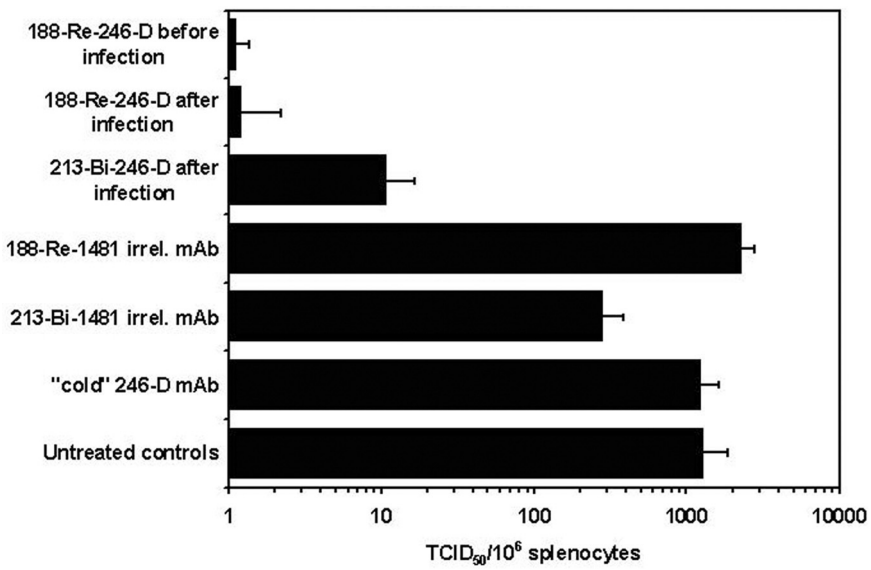


Fig. 1. *In vivo* experiments in HIV animal models. SCID mice injected intrasplenically with HIV-1 infected hPBMCs.

**Conclusion:** We believe that the combination of immune and radionuclide therapy provides an exciting new strategy that may be potentially useful against a variety of infections.

**Acknowledgement:** Funding from the National Institute of Health, AECOM NIH-designated Cancer Center and AECOM Center for AIDS Research (CFAR) is acknowledged.

Keywords: Radioimmunotherapy, Toxicity, Mycoses, Bacterial Infections, Viral Infections

## P368 LABELING OF THE 1H7 ANTIBODY WITH Re-186 AND Re-188 FOR IGF RECEPTOR TARGETING

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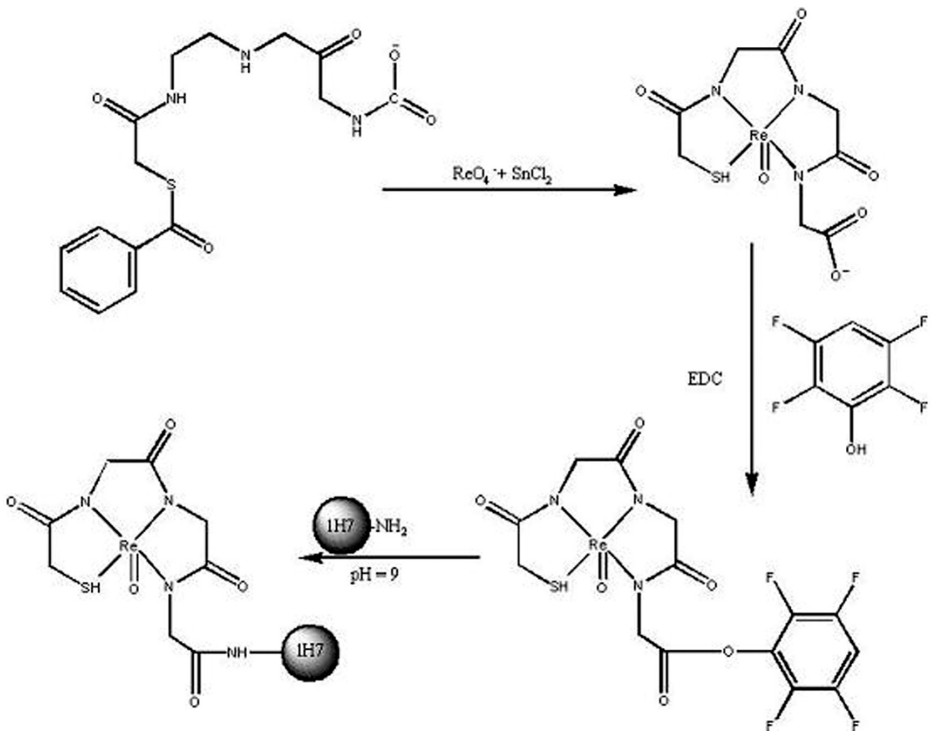
**Introduction:** $^{186}$Re and $^{188}$Re are emerging as a good candidates for targeted radiotherapy. Several groups have reported labeling various antibodies with $^{186/188}$Re. Here we report on labeling of the 1H7 antibody which is specific for the IGF1 receptor. This receptor is known to be overexpressed on many types of cancer cells. In addition, it has been suggested that blockade of this receptor may lead to increased radiosensitivity (Cosaceanu *et al* Cancer Lett. **222**, 173-181, 2005).

**Experimental:** S-benzoylmercaptoacetlyglycylglycylglycine was synthesized as reported in the literature (Xiuli *et al* J. Radioanal Nucl Chem. **256**, 339-343 2003). The 1H7 antibody was labeled with this preparation as according to Crudo (Int. J. Pharma. **248**, 173-182, 2002). The Re solution was brought to dryness under a nitrogen atmosphere. The Re-$MAG_3$ complex was prepared by the addition of 50μl of a 10 mM solution of s-benzoyl-$MAG_3$ in 60:40 acetonitrile in water and 125 μl of a 60 mM solution of a freshly prepared $SnCl_2$ solution in 0.1 M citric acid buffer pH 5.5. The reaction mixture was sealed under a nitrogen atmosphere and heated to 90° C for 45 min. Yields as determined by TLC were typically >95%. The activated ester was prepared by the addition of 100 μl of a 0.6 M solution of tetrafluorophenol in 9:1 acetonitrile and water and 50 mg of EDC. The mixture was allowed to react at room temperature for one hour. Yields as determined by TLC were typically >95%. The ester was washed on a $C_{18}$ cartridge with water, eluted with acetonitrile and dried under nitrogen. The 1H7 antibody was added, the pH was adjusted to 9 with 0.1 M $Na_2CO_3$ and allowed to react at room temperature for one hour. The final product was purified on a PD-10 column. Yields for the final bioconjugation step were typically ~20-30%.




**Results and Discussion:** The overall yield for the labeling of the 1H7 antibody with $^{186/188}$Re was typically 20-30%. Experiments are currently underway to determine the binding specificity of the labeled antibodies.

**Conclusion:** We have prepared 1H7 antibodies in high radiochemical purity labeled with generator produced $^{188}$Re and reactor produced $^{186}$Re.

**Acknowledgement:** The authors would like to thank Urs Hafeli for the use of the $^{188}$Re generator. This work is funded by NSERC I2I and TRIUMF Life Sciences.

Keywords: Radioimmunotherapy, 186Re, 188Re, IGF Receptor, 1H7 Antibody

## P369 A NOVEL BIO-MAGNETICALLY TARGETED RADIO-IMMUNONANOPARTICLE

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**Introduction:** Cancer is considered as the most dangerous threat to human health. Currently cancer therapy involves chemotherapy, surgery or radiotherapy. Effectiveness of the treatment is directly related to targeting ability of a treatment while affecting as few healthy cells as possible. Bio-magnetically targeted radio-immunonanoparticle could become a potential drug for cancer therapy.

**Experimental:** Amino-functionalized superparamagnetic iron oxide nanoparticles (SPION)were synthesized by coprecipitation method. The particles were characterized by X-ray diffraction (XRD), vibrating sample magnetometer (VSM), scanning electron micrographs (SEM), transmission electron micrographs (TEM) and atomic force micrographs (AFM). The size of the modified particles varied in the range of 10-15 nm (Fig.1)and did not change significantly after modification. Hepama-1, an excellent humanized monoclonal antibody directed against liver cancer, was conjugated to the SPION to prepare immuno-magnetic nanoparticles (IMN). An indirect labeling method was employed to radiolabel IMN with $[^{188}Re(CO)_3]^+$.

**Results and Discussion:** From Fig. 1 and Fig. 2, the immunonanoparticle has good dispersity and can kill markedly liver cancer cell.

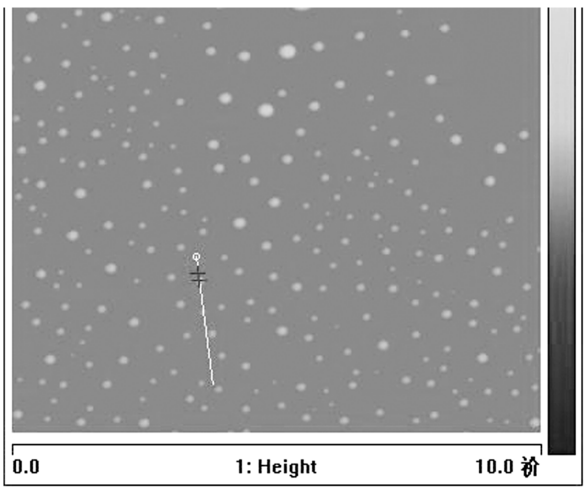


Fig. 1

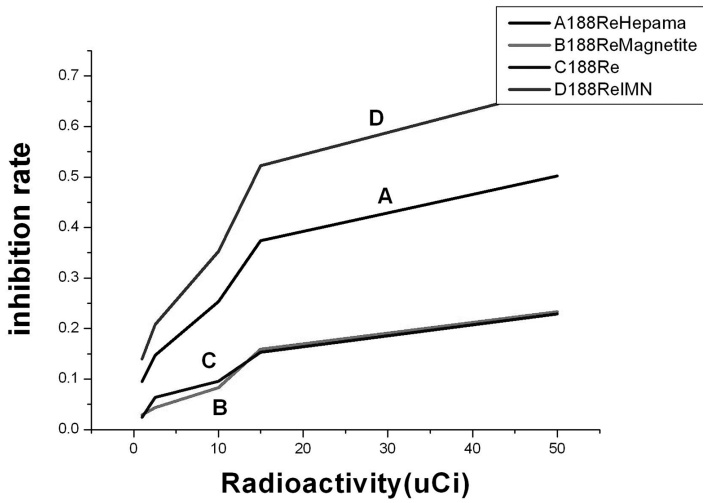


Fig. 2

**Conclusion:** The radiolabeling efficiency was about 90% with good in vitro stability. $^{188}Re$ labeled IMN could markedly kill SMMC-7721 liver cancer cell lines. Such SPIONs might be very useful for bio-magnetically targeted radiotherapy in liver cancer treatment.

Keywords: Magnetic Nanoparticles, Magnetic Separation, 188Re, Radiolabeling, Targeted Therapy

## P370 IN VIVO EVALUATION OF THREE $^{177}$Lu-LABELED NOVEL TETRAAZACYCLODODECANE-BASED LIGANDS DIFFERING IN THE NUMBERS OF PERIPHERAL HYDROXYMETHYL GROUPS

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**Introduction:** Three tetraazacyclododecane derivatives differing in the numbers of hydroxymethyl groups were originally designed as new PARACEST contrast agents when complexed with europium. By introducing the hydroxymethyl groups, it was expected that the *in vivo* kinetics of lanthanide metal complexes could be optimized for their biomedical applications. Radiolanthanides have been clinically used for diagnostic and therapeutic radiopharmaceuticals due to their disparate nuclear properties and similar chemical behavior. In this study, $^{177}$Lu complexes of the three ligands were examined as potential lanthanide radiopharmaceuticals.

**Experimental:** The radiolabeling was carried out in 0.1 M $NH_4OAc$ buffer (pH 6.5) at 60°C. The partition coefficients (log P) were determined in octanol/water. The serum stability of the complexes was evaluated by incubating with rat serum in a water bath at 37°C. The solutions were analyzed by radio-TLC out to 24 hr post addition. Normal BALB/c male mice (4-5 wk) were used for *in vivo* evaluation (n = 4). Prior to the first time point of tissue distribution, the blood was collected from the retroorbital sinus for the determination of pharmacokinetic parameters.

**Results and Discussion:** All of the three ligands were successfully labeled with $^{177}$Lu (> 95% RCY) after 1 hr incubation at 60°C. The large negative logP values of the complexes indicated their hydrophilic nature. As expected, their serum stability was considerably enhanced by introducing more peripheral hydroxymethyl groups. Neutral or negatively charged tetraazamacrocyclic complexes were previously reported to have more efficient renal clearance than their positively charged counterparts. However, these tripositively charged complexes exhibited rapid washout at 4 hr p.i. from the blood and low uptake in liver and kidneys. For comparison, the retention of $^{64}$Cu-DOTA ($2^-$ charge) in these major organs was significantly higher even at 24 hr p.i. This is likely due to the presence of peripheral hydroxymethyl groups. The *in vivo* kinetics of all the complexes in the blood could be described by the one-compartment open model. Surprisingly their elimination half-lives and other kinetic parameters are nearly identical.

**Conclusion:** Introducing peripheral hydroxymethyl groups has been demonstrated as an effective way to enhance the *in vitro*/*in vivo* stability and optimize the biodistribution profile of metal complexes of tetraazacyclododecane-based ligands.

Keywords: Tetraazamacyclics, Lanthanide Radioisotope, Biodistribution, Metal Chelator, Pharmacokinetics

## P371 EVALUATION OF RADIONUKLIDTHERAPY WITH p-($^{131}$I)IODO-L-PHENYLALANINE IN COMBINATION WITH EXTERNAL BEAM RADIOTHERAPY AS A NOVEL THERAPEUTIC OPTION FOR BRAIN TUMORS

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**Introduction:** Despite aggressive treatment protocols, patients with high grade gliomas still experience poor outcome. Therefore, new adjuvant therapeutic options have been studies. Initial studies demonstrated the efficacy of radionuklidtherapy with p-[$^{131}$I]iodo-L-phenylalanine (IPA) in C6-glioma xenografts. The present study examines the effectiveness of IPA in combination with external radiation as a novel approach in treatment of malignant gliomas.

**Experimental:** N.c.a. IPA was prepared by a iododestannylation method with 90 ± 5% radiochemical yield and > 99% radiochemical purity after HPLC. The primary human T3868 and A1207 glioblastoma cells were exposed for 6–48h to 0.01–1 μCi/ml of n.c.a. IPA, ionising radiation (2–20 Gy), and combined treatment. Cell viability was determined at 6, 24 and 48h by colony assays or by fluorescence microscopy. In addition, T3868 and A1207 cells ($10^6$) were implanted intracerebrally into RNU rats to induce glioblastomas. Nine and 18 days later, IPA (20 MBq) was administered intravenously into nine rats. A second group with nine animals was irradiated (Tx) with 4 Gy. A third group (n = 9) was treated with both IPA and Tx. Another 6 tumor-bearing rats served as controls. The following parameters were compared: median survival time, tumor size and histology.

**Results and Discussion:** In vitro, IPA induced markedly radiotoxicity against glioblastoma cells. Cell survival rate was 50 ±10% and 20 ±10% after incubation with 0.5 μCi/ml of IPA for 24 and 48h, respectively, and decreased (<5%) after additional external radiation with 5 Gy. At higher doses cell survival decreased dramatically, amounting to 1–2% after 24h. 5/6 untreated rats with T3868 glioblastoma died within 24d. None of the control rats with A1207 glioblastoma remained alive at day 30 after cell implantation. All had large tumors and/or hadrocephalus. At day 90, 44–55% of the rats treated with IPA alone were still alive. While only 22–33% of the animals remained alive at day 50 after external radiation with 4 Gy. In comparison, 66–77% survival rates were registered after combined treatment (IPA + Tx) at day 90 after therapy.

**Conclusion:** These results indicate that in particular the radionuclidtherapy with p-($^{131}$I)iodo-L-phenylalanine in combination with external beam radiotherapy is a very promising and effective therapy for high grade gliomas, warranting further in vivo studies to confirm this findings and, potentially, to further increase the efficacy of the novel treatment option.

Keywords: Brain Tumors, Human Glioma Models, Amino Acid Transport-Based Endoradionuklide Therapy, Radiotherapy

## P372 BIODISTRIBUTION OF A $^{131}$I LABELLED THERMORESPONSIVE DRUG DELIVERY SYSTEM IN MICE

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**Introduction:** Thermoresponsive (thermosensitive) drug delivery systems have recently attracted much attention as carriers of conventional pharmaceuticals [1,2,3]. They are water-soluble at room temperature, but at certain point (still bellow the body temperature) the hydrophobic interactions between polymer molecules prevail hydrophilic interactions with water molecules, and the polymer precipitates completely in very narrow temperature interval. If injected, precipitation occurs immediately on the site of application. In addition, the molecules of thermoresponsive polymers can be modified in order to become labellable by wide spectrum of radionuclides. Such polymers are thus promising drug delivery systems for local administration of therapeutic radionuclides.

**Experimental:** A labellable polymer system was prepared by copolymerizing N-isopropylacrylamide with N-methacryloyl tyrosinamide (0.7 wt. %, molar weight 23 800). The polymer was labelled with $^{131}$I at pH = 7.5, using Chloramine-T as oxidizing agent, in 88% yield (ca 1 MBq/mg). The labelled polymer was lyophilized and then dissolved in DMSO (a surplus of non-labelled polymer was added, so that the resulting solution was 1,8 wt. %). Volume activity of the solution was 4.4 MBq/ml. The solution was then administered into the femoral muscle of Bulb/C male mice in amount 50 μl per mouse. Biodistribution of radioactivity always in 6 animals peer time point was checked at 1 h, 8 h, 24 h, 3 and 7 days after the administration.

**Results and Discussion:** Preliminary results confirmed high retention of $^{131}$I in the site of application. Still 7 d post administration, 78.7±3.5% of radioactivity was found in the femoral muscle of the animals. Roughly one half of the released $^{131}$I was excreted via urine, and the other half was distributed over the rest of the body. No organ-specific deposition of the released $^{131}$I was observed, including thyroid. Lyophilized polymer in dry state was found to be stable over 5 half-lives of $^{131}$I.

**Conclusion:** A new thermoresponsive drug delivery system as a carrier of therapeutic radionuclide $^{131}$I has been developed. The encouraging results of its biodistribution after the local administration and its stability proved its potential in local radiotherapy.

**Acknowledgement:** Authors gratefully thank the Grant Agency of the Czech Academy of Sciences (grant no. IAA400480616) for financial support.

**References:** [1] Le Garrec D et al.: *J. Drug Targeting* **10**(5), pp. 429-437, 2002. [2] Chytry V and Ulbrich K: *J. Bioactiv. Compat. Polym.* **16**(6), pp. 427-440, 2001. [3] Wright ER and Conticello VP: *Adv. Drug. Delivery Rev.* **54**(8), pp. 1057-1073, 2002.

Keywords: I-131, Thermoresponsive Drug Delivery System, Radionuclide Therapy

## P373 $^{198}$Au AND $^{64}$Cu LABELLED POLYMERS – NEW POTENTIAL NANOCARRIERS FOR LOCAL RADIOTHERAPY

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**Introduction:** Radiolabelled polymers are believed to be promising materials for both radiotherapy and radiodiagnostics [1]. We have synthesized, characterized and radiolabelled several moieties bound to a water-soluble polymer that are suitable for therapeutical radionuclides *$^{198}$Au (T = 2.7 d.)* and *$^{64}$Cu (T = 12.7 h.).* The polymer bearing a DOTA moiety served as s a reference. The other types tested were thiosemicarbazone, dipicolylamine, phosphine and Ag-ionophore II ligands.

**Experimental:** Functionalized polymers have been generally prepared by the modification of a water-soluble biocompatibile polymer, poly{[*N*-(2-hydroxypropyl)methacrylamide]-*co*-glycidylmethacrylate}, $M_w$ = 120 kDa, by epoxide ring opening reactions. The thiosemicarbazone polymer has been prepared directly by copolymerization of allylthiosemicarbazide with this polymer. All the polymers were fully characterised. Carrier added *$^{198}$Au* and *$^{64}$Cu* have been prepared by activation of corresponding metal in a nuclear reactor (LVR-15, ÚJV Reď, Czech). Separation of macromolecular carriers has been performed by gel permeation chromatography (PD-10 desalting columns, GE, USA). Radiochemical yields were determined by the measurement of collected fractions on a proportional counter (Thermo-Eberline, Germany).

**Results and Discussion:** Labelling yields are summarized in table 1. Nevertheless the reported disadvantage of the DOTA functional group (targeting to kidneys [2]), yields were good both for *$^{198}$Au* and *$^{64}$Cu*. The best labelling yield was obtained with thiosemicarbazone polymer for *$^{198}$Au* and dipicolylamine polymer for *$^{64}$Cu*.

Table 1. Labelling yields

| Polymer type | $^{198}$Au | $^{64}$Cu |
|---|---|---|
| DOTA | 99% | 67% |
| thiosemicarbazide | >99% | 37% |
| dipicolylamine | <5% | 93% |
| phosphine | 15% | <5% |
| Ag ionophore II | >99% | 14% |

**Conclusion:** We have prepared polymers labelled with *$^{198}$Au* and *$^{64}$Cu*. DOTA and also alternative functional groups can be used to obtain moderate yields.

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Keywords: Polymer, 198Au, 64Cu, Radiotherapy

## P374 SYNTHESIS OF HIGHLY AFFINE CLOSO-BORANE CONJUGATED TYR3-OCTREOTATE-DERIVATES FOR THE BNCT

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**Introduction:** Boron neutron capture therapy (BNCT) provides a way in theory to destroy cancer cells without damaging healthy tissue. It's based on the nuclear reaction between $^{10}$B-atoms and thermal neutrons. Hence, the reaction of a neutron with a $^{10}$B-atom yields two charged particles $^{7}$Li and $^{4}$He, each of which is able to destroy tumor cells due to its high linear energy transfer. However, BNCT in practice is still limited because of the lack of boron containing tumor targeting compounds, which selectively deliver boron to cancer cells. Since many neuroendocrine tumors show an overexpression of the somatostatin receptor, it was the aim of this work to synthesise octreotate based compounds, which are highly affine towards this receptor and contain a high number of boron atoms.

**Experimental:** The synthetic peptide octreotate was selected as the tumor targeting vector, which should be connected to the boron compounds. It was our intention to develop a synthetic route, by inserting a spacer between peptide and the closo-borane containing molecule (linker), since prior results with DOTA-octreotrate-compounds showed a loss of affinity if the voluminous chelating agent was connected directly to the N-terminus of the peptide. The partition into the synthesis of octreotate, spacer, linker and subsequent coupling of these compounds shall ensure an easy and fast synthesis of many different products. The different linker molecules were synthesised starting from benzoic acid-derivates and decaborane. Using coupling reagents, the various systems were synthesised and purified by HPLC.

**Results and Discussion:** Starting from 3 different benzoic acid derivates various linker molecules containing 10/20 boron atoms were synthesised in 3 steps and yields of up to 66%. The sarcosine spacers with chain length of 2/4/6 were synthesised via coupling reagents with yields of up to 70%. The combination of these various systems with the peptide, synthesised on solid phase, gave 7 different closo-borane-octreotate derivates with yields of up to 22%. The obtained in vitro affinities showed excellent values (low nM) for the compounds containing linker and spacer units.

**Conclusion:** Via the developed synthesis strategy linker molecules containing up to 60 boron atoms could be obtained. Using the successful building-block chemistry, various products can be synthesised fast and easily with good yields. Some octreotate targeting vectors compounds showed high affinities (low nM) towards hsstr2. By measurement of the in vitro binding affinities, reliable structure-affinity correlations were obtained. With the most promising compounds, the potential of closo-borane-octreoate systems for BNCT will be evaluated in further experiments.

Keywords: BNCT, Tyr3-Octreotate, Closo-Borane, Tumor-Therapy

## P375 SYNTHESIS, BIODISTRIBUTION AND METABOLITE ANALYSIS OF DIFFERENT ($^{86}$Y)DOTA-L-RNA OLIGONUCLEOTIDES IN RATS

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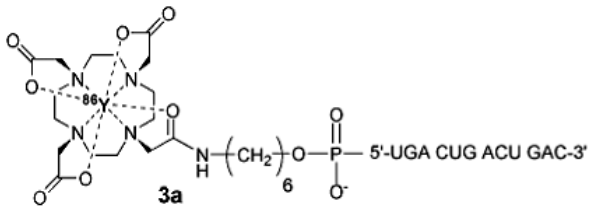
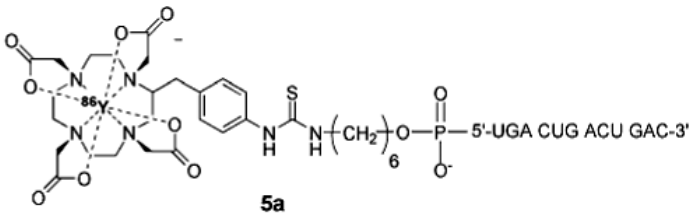
**Introduction:** Mirror-image oligonucleotides are constructed of an L-configured ribose or deoxyribose. This modification leads to an extraordinary high metabolic stability against enzymatic degradation in biological environments. The radiolabeling of an L-oligonucleotide with the positron emitter $^{86}$Y ($t_{1/2}$ = 14.7 h) is done via the bifunctional chelator approach. The influence of two different [$^{86}$Y]DOTA chelates on the radiopharmacological properties of [$^{86}$Y]DOTA-L-oligonucleotides was investigated with an L-RNA 12mer **1** (sequence: 5'-Aminohexyl UGA CUG ACU GAC-3' MW 3975). Both $^{86}$Y-labeled L-RNAs **3a**, **5a** were characterized by a comparative biodistribution study in Wistar rats and metabolite analyses.

**Experimental:** The amine functionality of the 5'-hexylamine modified L-RNA **1** was used for conjugations with DOTA-NHS ester **2** and (*S*)-*p*-SCN-bz-DOTA **4**, accomplished in the formation of a selective amide bond **3** and thiourea bond **5**, respectively. After $^{86}$Y-labeling of **3** and **5** biodistribution studies were performed for each compound **3a**, **5a** with eight Wistar rats.




**3a**




**5a**

**Results and Discussion:** Both DOTA-modified L-RNAs **3**, **5** were radiolabeled with $^{86}$Y(III) (QSA Global, Germany) with radiochemical yields of 76% and 85%, respectively. High renal excretions were found for both $^{86}$Y-labeled L-RNAs **3a**, **5a** whereas differences in the retention were observed for the radiolabeled compounds in the kidneys and the adrenal glands. The standardized uptake value (SUV) of compound **3a** in the kidneys reached 10 ± 2.0 after 5 min and decreased to 6.1 ± 0.53 after 60 min. For compound **5a** the SUV in the kidneys increased from 13 ± 1.6 after 5 min to 14 ± 1.1 after 60 min. Remarkably high SUVs (3.5 ± 0.48 and 3.2 ± 0.33) were also observed in the adrenal glands after 60 min for both compounds. The SUVs in other organs were below 1.0 after 60 min for **3a** and **5a** in this study. Over 85% intact **3a** and **5a** were found in urine samples of Wistar rats 60 min after application of **3a**, **5a** as confirmed by Radio-HPLC.

**Conclusion:** Differences in the kidney excretion profile of **3a** and **5a** indicate an influence of the different chemical attachments of the [$^{86}$Y]DOTA chelates to L-RNA **1**. The high metabolic stability of the L-RNA suggests the potential of L-oligonucleotides as molecular probes for PET.

**Acknowledgement:** This work was supported by the EU-FP6 integrated project BioCare contract no.: 505785.

Keywords: 86Y, DOTA, L-RNA, Oligonucleotides

## P376 COMPARATIVE EVALUATION OF LIPOSOMAL RADIUM-223 IN MICE AND DOGS

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**Introduction:** Cancer therapy using alpha-particle emitting radionuclides are under investigation by several research groups. Nearly all radionuclides need a carrier compound, i.e., a vector targeting the radionuclides to, or nearby the cancer cells. Pegylated liposomes have the ability to change the pharmacokinetics and improve the tissue distribution of the active component and could therefore, by carrying cytotoxic radionuclides, be an interesting vehicle for internal radionuclide therapy [1–4].

**Experimental:** Liposomal $^{223}$Ra was administered intravenously, and biodistribution was investigated in Balb/C mice, nude mice with osteosarcoma xenografts, and in dogs with spontaneous osteosarcoma. Comparative biodistribution studies with cationic $^{223}$Ra were conducted in mice and dogs as well.

**Results and Discussion:** Blood clearance of liposomal $^{223}$Ra was relatively slow with $t_{1/2}$ ~28 h and ~40 h in Balb/C mice and dogs, respectively. In contrast, cationic $^{223}$Ra cleared from the blood with half-lives much shorter than an hour in the two species. In the xenograft model there was generally a higher retention of activity in the tumor vs. soft tissue. In dogs the uptake was considerably higher in both calcified and non-calcified tumor metastases of different organs, than in normal tissue.

**Conclusion:** The favourable biodistribution and tumor uptake observed in both mice and dogs support more extensive studies with liposomal $^{223}$Ra.

**Acknowledgement:** This work was financially supported by the Norwegian Research Council Grant E53544/110 and the company Algeta ASA, Oslo, Norway.

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Keywords: Alpha Emitting 223-Radium, Pegylated Liposomes, In Vivo Evaluation, Mice, Dog

## P377 FORMATION OF ϕX174 DNA STRAND BREAKS BY AUGER ELECTRONS FROM THE VICINAL $^{99m}$Tc-SPECIES

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**Introduction:** $^{99m}$Tc is one of the most useful imaging radionuclides in nuclear medicine due to its emitting 141 keV γ-ray. However, it also emits an average of 1.1 conversion electrons and 4 Auger electrons per decay. The ability of $^{99m}$Tc Auger electrons induce DNA-strand breaks, an indicator of cytotoxicity, is of interest to be investigated. We have adapted ϕX174 DNA to study Auger electron-induced strand breaks with the various $^{99m}$Tc species.

**Experimental:** $[^{99m}Tc(CO)_3(OH_2)_3]^+$ was prepared by using the Isolink kit (Mallinckrodt). *N*-(2-aminoethyl)-*N*'-pyrene-1-methylethane-1,2-diamine (APMED) was synthesized according to the literature. $^{99m}$Tc-APMED was prepared by reaction of $[^{99m}Tc(CO)_3(OH_2)_3]^+$ with $10^{-2}$M AMPED in water under anaerobic condition at 90° for 30 min. Supercoiled ϕX174 DNA (300 ng, Invitrogen) was then incubated with $^{99m}TcO_4^-$, $[^{99m}Tc(CO)_3(OH_2)_3]^+$, and $^{99m}$Tc-APMED at 3.6 MBq, 2.0 MBq, and 1.6 MBq, respectively. The periods of incubation time were 6 h, 12 h, 15h, 18 h, 21 h, and 24 h, respectively and the sample at each time point underwent agarose gel electrophoresis. The gels were scanned with UVP BioDoc-It™ System and analyzed by SynGene™ software.

**Results and Discussion:** The results are summarized in Figure 1. The percentage of open circular DNA formed along with the incubation time is plotted with the total accumulated decays estimated. The blank test without adding $^{99m}$Tc was found to maintain at 20% open circular DNA. The increase of the fraction of open circular DNA owing to strand breaking of the supercoiled DNA incurred from $^{99m}$Tc is prominent for the incubation either with $^{99m}$Tc-APMED or with $[^{99m}Tc(CO)_3(OH_2)_3]^+$. In contrast, the increase of the fraction of open circular DNA resulted from the incubation with $^{99m}TcO_4^-$ becomes much less.

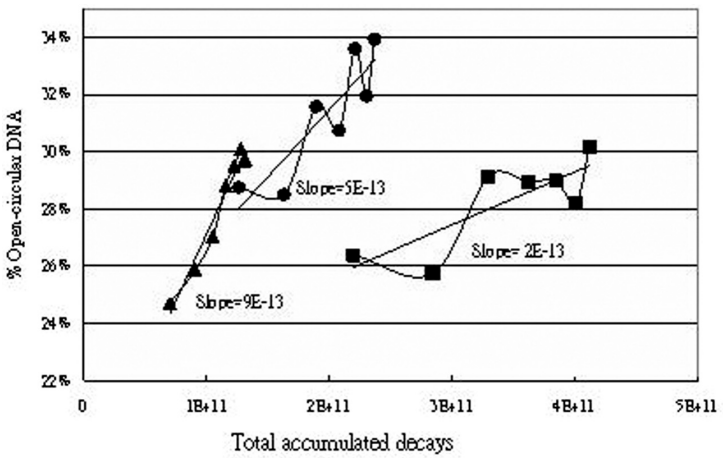


Figure 1. Percentage of open-circular DNA formed after incubation of ϕ-X174 DNA with the various $^{99m}$Tc species (▲, $^{99m}$Tc-APMED; ●, $[^{99m}Tc(CO)_3(OH_2)_3]^{3+}$; ■, $^{99m}TcO_4^-$).

**Conclusion:** The formation of DNA-strand breaks may be caused by the $^{99m}$Tc emitting Auger electrons from both of the $^{99m}$Tc species located at the near vicinity of DNA, $[^{99m}Tc(CO)_3(OH_2)_3]^+$ being able to localize in DNA as 1,2-intrastrand adduct between the N7 atoms of two adjacent purine residues, whereas $^{99m}$Tc-APMED being able to intercalate in DNA due to its pyrene moiety. $^{99m}TcO_4^-$ may not be closely located in the DNA so that the occurrence of strand breaks become relatively not obvious, although the radioactivity added being much higher.

Keywords: DNA-Strand Breaks, Auger Electrons, 99mTc, ϕX174 DNA

## P378 PREPARATION AND EVALUATION OF ASTATINE-211 LABELLED CD20- AND CD33-ANTIBODIES

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**Introduction:** The short range of alpha particles and the high specificity for tumor-cells make 211At-labelled tumour-affine antibodies promising agents for radionuclide immunotherapy, especially against systemic malignancies like lymphoma and leukemia. Rituximab binds to CD20-positive cells present in B-cell lymphoma, whereas gemtuzumab and Mylotarg® bind to CD33-positive leukemia cells. Therefore coupling of 211At to these antibodies may further enhance their toxicity to make them suitable agents in radioimmunotherapy. The current research intends to evaluate their affinity, specificity, and stability in cell-binding experiments.

**Experimental:** 211At was produced at the MHH-cyclotron and linked via m-211At-succinimidylbenzoate (SAB) to the antibodies. The labelled antibodies were purified by gel chromatography. Their stability was determined in murine serum for three half-lifes (21h) by gel-electrophoreses. CD20-positive CI-1 and CD33-positive HL-60 cells were incubated with different amounts of the 211At-labelled antibodies (0.03 to 9.29 kBq) in order to determine binding-kinetics and -specificity. For comparison the binding to the antigen-negative cells was evaluated in each case. Unspecific binding was obtained by preventing the specific binding of different amounts of the 211At- antibodies by a constant excess of the particular unlabelled antibody. Inhibition experiments were carried out using different quantities of unlabelled antibodies (3 to 167 nmol/L) in the presence of a constant amount of the particular 211At-antibody for determination of the IC50 values.

**Results and Discussion:** The labelling yield of all antibodies after purification was 30% with respect to the starting activity of 211At. The stability of 211At-labelled antibodies in murine serum was better than 85% at 37°C. Binding of 211At-rituximab to CD20-positive CI-1 cells reached 31%, binding of 211At-CD33-antibodies to the antigen-positive HL-60 cells achieved 27%. Binding to the particular antigen-negative cells fell below 1% and corresponded to the unspecific binding to the antigen-positive cells. Because of the lower antibody binding sites on HL-60 cells the maximal bound mass for 211At-rituximab to CI-1 cells was 10fold higher when compared with 211At-CD33-antibodies to HL-60 cells. The inhibition experiment revealed an IC50-value of 11 nM for 211At-rituximab and 3 nM for 211At-CD33-antibodies.

**Conclusion:** The results of the study demonstrate that adequate labelling yields of 211At-antibodies can be achieved and that labelling does not compromise the binding affinity to the antigen-positive cells. The high specifity to antigen-positive cells and the stability in serum warrants further evaluation for radioimmunotherapy.

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Keywords: 211 Astatine, Antibodies, Specificity, Stability