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Radiopharmaceuticals for Targeted Tumor Diagnosis and Therapy

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Abstract: Radiopharmaceutical research and development is carried out by the Center for Radiopharmaceutical Science as part of the PSI Life Science Department, the Department of Applied BioSciences at the Swiss Federal Institute of Technology Zürich and the University Hospital Zürich. The common theme is the search for radioactive-labeled tracer molecules, which bind to specific targets in the body. Such radiopharmaceuticals are applied either systemically into the blood stream or locally to patients. Due to their specific molecular binding properties combined with the emitted radiation, they can be used for non-invasive imaging of tumors and the destruction of tumor cells. In this first of two articles, we will present exemplified topics from the research activities of the groups involved with tumor targeting.

Keywords: Nuclide therapy · Pharmaceutical chemistry · Radioimmunotherapy · Radiopharmaceuticals · ^{99m}Tc · Tumor imaging

Introduction

One in two men and one in three women will get cancer in their lifetime, according to a study presented by the President of the American Association for Cancer Research (AACR), Daniel Von Hoff. A comparable study in the EU indicates that every third European will become a cancer patient. At the time the disease is first diagnosed, already forty percent of patients will present metastatic disease, with unfavorable prognosis. In these cases the systemic application of radiolabeled tumor-seeking substances may allow destruction of disseminated tumors which cannot be reached efficiently by other treatment modalities.

One of the historical challenges of nuclear medicine has been to develop radiopharmaceuticals that will target a specific site while minimizing nontarget uptake. Early work was limited to those elements that had natural affinities for a given organ, such as iodine for the thyroid. Nowadays the efforts focus on developing radiopharmaceuticals with physical characteristics that would dictate their *in vivo* properties. Suitable molecules for imaging and therapy were explored for their ability to be 'radiolabeled'. Systemic delivery of therapeutically effective radiation (α, β) to tumor cells is most advanced in the case of specific antibodies targeting tumor-associated cell surface proteins. This is exemplified by the phase III radioimmunotherapy (RIT) trials in non-Hodgkin's lymphoma patients with high doses of ^{131}I-labeled Lym-1 antibody, which were pursued at the Center for Radiopharmaceutical Science in collaboration with industrial partners.

The clinical success of monoclonal antibodies in imaging and therapy has refocused efforts on the development of bifunctional chelates that could form a bridge between various radiometal isotopes used in imaging and therapy and peptides or proteins optimized for tumor targeting.

In this paper we will present the collaborative effort of the three research groups at the Center for Radiopharmaceutical Science: – the group ***Radionuclide Chemistry***, the group ***Tumor-avid Peptides*** and the group ***Tumor Targeting*** – in the search for optimal agents for RIT. Our research activities cover all stages in drug development from basic chemistry to preclinical studies and finally to GMP-approved clinical trials.

Radionuclide Chemistry

The main task of this group is to design stable radiometal compounds for application in cancer diagnosis and therapy. This research area involves synthetic inorganic, organic and radiochemistry. A new technique for radioactive labeling of biomolecules, developed in our laboratories, using the organometallic aquaion *fac*-$[M(OH_2)_3(CO)_3]^+$ (M = ^{99m}Tc, ^{188}Re in the low oxidation state +I) has been accepted internationally as a powerful alternative to the common technetium (+V) and rhenium (+V) protocols. This is mainly due to the outstanding features of the new '$M(CO)_3$' moiety in terms of size and kinetic inertness and the one step, fully aqueous synthesis of the precursor (Scheme). Another major advantage of this precursor is the broad versatility of appropriate ligand types able to coordinate to the metal-carbonyl center. The flexible choice of a ligand is a prerequisite, if the labeling of biomolecules like small peptides or proteins is to be achieved with one and the same organometallic moiety.

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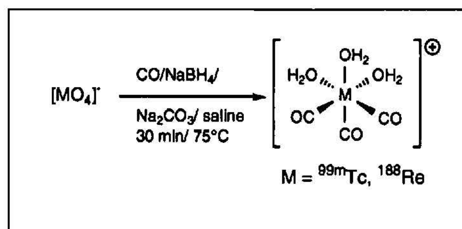
Therefore, we are constantly searching for improved ligand systems to find optimal labeling characteristics (*e.g.* low ligand concentration) and pharmacokinetic behavior (fast clearance from non-targeted tissue and organs). N-heterocycles, thioethers, and thiolates have been



Scheme. Kit preparation of the organometallic precursor *fac*-$[M(OH_2)_3(CO)_3]^+$ (M = ^{99m}Tc, ^{188}Re).

shown to be good ligands for ^{99m}Tc-tricarbonyl. These functionalities correspond to side chains of methionine, cysteine and histidine and are therefore of particular interest in terms of direct labeling of peptides and proteins. Aromatic amines as present in histidine proved to be superior in terms of kinetic stability and labeling efficiency. For labeling of peptides, these results opened a new avenue: a peptide requires only a minor modification at the sequence by the introduction of a histidine at the N-terminus by standard solid-phase peptide synthesis procedures. Results of this labeling method will be exemplified for the peptide neurotensin in the group ***Tumor-avid Peptides***.

A similar concept can be used for labeling single chain Fv antibody fragments (scFvs), which have potential for tumor imaging and therapy. As mentioned before, ^{99m}Tc-tricarbonyl forms very stable complexes with imidazole groups in the side chains of histidine, thus, the method directs labeling to the C-terminal 6x-histidine tag commonly used for ease of purification of scFvs by immobilized metal affinity chromatography (IMAC).

As a consequence of this work and experience, we are currently focussing on the development of tridentate chelating systems with the potential to be readily linked to various classes of hydrophilic biomolecules. We were able to synthesize tridentate bifunctional ligands containing an iminodiacetic acid or an amino bis-imidazoyl chelating moiety with a spacer and an additional amine or carboxylic acid group (Fig. 1). The primary amine and/or carboxylic acid group enables the connection to a biological vector

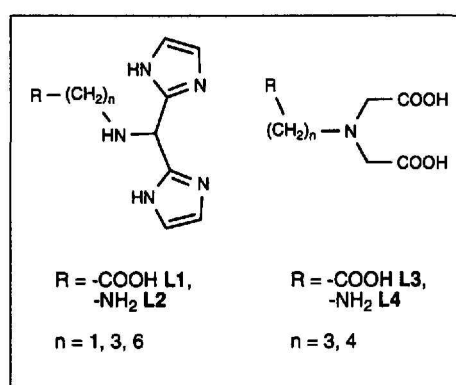


Fig. 1. Bifunctional ligand systems for the derivatization of biomolecules to be labeled with *fac*-$[M(OH_2)_3(CO)_3]^+$.

via a stable amide bond. These ligands produce cationic (L1, L2) or anionic (L3, L4) complexes of high hydrophilicity, which give generally rise to a better *in vivo* clearance. Another relevant advantage of this new group of tridentate ligands is their intramolecular C_s-symmetry which avoids the formation of stereoisomers, when connected to a biomolecule. The rhenium complexes *fac*-[Re(MeL1) $(CO)_3]$ Br and *fac*-[Re(HL4) $(CO)_3]$ could be crystallized and their X-ray structures were elucidated. ORTEP pictures are given in Fig. 2. It is remarkable, that the free $-NH_2$ and the COOMe group, respectively, points right away from the metal center. Steric interactions in a labeled biomolecule should thus, be minimized.

Ligands L2 and L4 were coupled to biotin and the corresponding bioconjugates subsequently labeled with ^{99m}Tc-tricarbonyl and ^{188}Re-tricarbonyl (Fig. 3). The concentration of the biotin derivatives necessary to obtain labeling yields > 95% varied between 10^{-4} M to 10^{-6} M, enabling unprecedented high specific activities up to 1300 GBq/µmol. These specific activities exceed published results by two to three orders of magnitude. Binding affinity for streptavidin was fully retained. These novel organometallic biotin derivatives may be useful for tumor pretargeting protocols, using established three-step approaches, where the first step consists in the application of biotinylated monoclonal antibodies followed by avidin and in a final step the therapeutic biotinylated derivative. Up to now, biotin labeled with generator produced isotope ^{188}Re (β^--emitter) could not be used for cancer therapy due to low labeling yields and the thermodynamic instability of high oxidation state (+III to +V) rhenium complexes. The good results obtained with the novel ^{188}Re(+I)-tricarbonyl labeling method could be pivotal for the future development of therapeutic radiopharmaceuticals.

Collaborations outside of the Center for Radiopharmaceutical Science exist with the group of R. Alberto, University of Zürich, G. Folkers, Federal Institute of Technology, Zürich Switzerland, and B. Johannsen, Forschungszentrum Rossendorf, Germany.

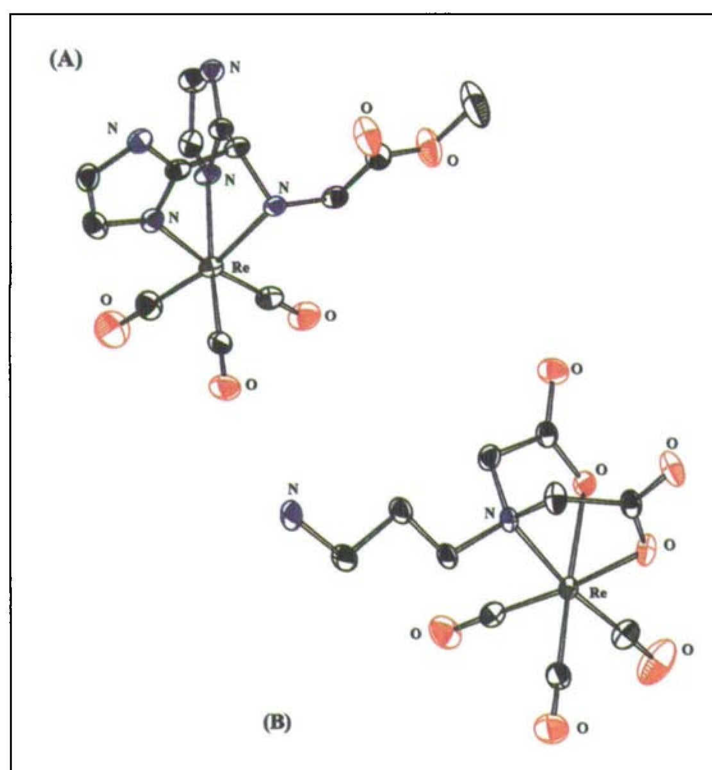


Fig. 2. ORTEP of the X-ray structure of the octahedral complexes (A) *fac*-[Re(MeL1)$(CO)_3]$Br and (B) *fac*-[Re(HL4)$(CO)_3]$. Only heteroatoms are labeled. Hydrogen atoms and counter ions are omitted for clarity.

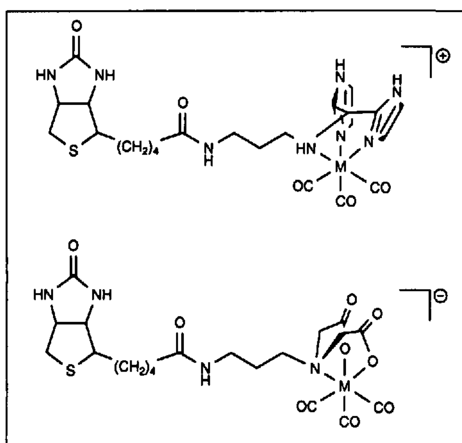


Fig. 3. Structure of two functionalized and Tc/Re-tricarbonyl labeled biotin derivatives.

Tumor-avid Peptides

Since the discovery of peptide receptors and the synthesis of small, biologically active peptides, it has been recognized that these molecules can provide new approaches for radiopharmaceutical development. In many cancers an overexpression of receptors is observed which makes receptor-avid peptides an attractive tool for tumor imaging and therapy. Numerous peptides are active in neuronal and lymphatic tissues and in the endocrine system. These highly potent neuropeptides exhibit a wide range of actions and regulate essential biological processes. One of the first clinical applications consisted in exploiting ^{111}In-somatostatin analogues to localize endocrine-related tumors. However many tumor cells do not express receptors for somatostatin and it is therefore important to search for other tumor markers. As part of an European BIOMED project, we evaluated neurotensin (NT), a 13-amino acid neuroregulatory peptide. Various tumors, such as pancreatic and colon cancer, express high levels of neurotensin receptors and therefore neurotensin is emerging as potential tool for early diagnosis and therapy.

Small peptides can be easily synthesized chemically whereas antibodies have to be derived from a biological source. On the other hand, minor modifications in their structure can result in a substantial loss of the binding affinity. Only site-specific radiolabeling can circumvent this impairment of receptor affinity. For our novel labeling technique using ^{99m}Tc-tricarbonyl, we linked histidine and (N_α-histidine) acetate (N_α-His)Ac to the amino terminus of neurotensin NT(8-13) (Fig. 4). Structure-activity relationships have demonstrated that this truncated peptide of only six amino acids is sufficient for preserving high affinity receptor binding. To ensure that the additional histidine complex does not interfere with binding to the NT receptor we have tested the NT complex on a human colon carcinoma cell line (HT-29) which has high expression of NTS1 receptors. In competition binding studies, the K_d value for the labeled His-NT(8-13) was 0.6 nM which compared well with the native NT(8-13) value of 1 nM. Obviously the addition of the ^{99m}Tc-tricarbonyl moiety to such a small peptide of only six amino acids did not interfere with binding to the receptor.

In general, small peptides distribute more uniformly and penetrate more readily in tissues and clear more rapidly from the circulation than do antibodies. Hydrophilicity enhances renal clearance whereas more lipophilic peptides show substantial hepatobiliary excretion. Radiolabeling can result in important changes in lipophilicity and charge, with consequences for biodistribution and kinetics. Eleven NT(8-13) analogues have been synthesized and characterized (Table 1). A tendency to better *in vivo* properties is observed if N_α-(his)Ac is used instead of His for labeling. This was confirmed with the matched pairs NT-I, NT-II and NT-IV, NT-VI. An explanation for this phenomena could be that Tc-tricarbonyl has three positions to bind to a ligand. The (N_α-His)Ac can occupy all three positions whereas when Tc-tricarbonyl is coordinated bidentately to His-NT(8-13), one position is left non-occupied (occupied by a substitutionally labile water molecule respectively). This free position can probably interact with any possible donor which is present. NT-III and NT-IV showed high accumulation in both kidney and liver, reaching in the kidney 90% of the injected dose per gram of tissue for the latter analogue. As a consequence all new peptides, NT-V to NT-XI, have been synthesized with a (N_α-His)Ac tag.

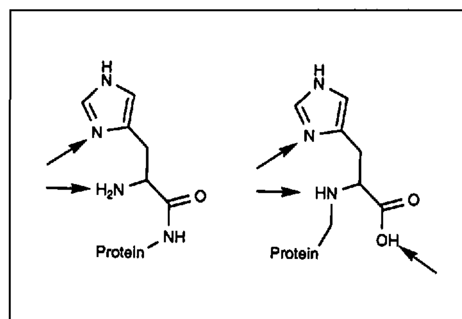


Fig. 4. The bidentate and tridentate ligands histidine and (N_α-histidine) acetate which are linked to the amino terminus of the neurotension analogues. The arrows are indicating the donor atoms forming the complex with the Tc-tricarbonyl moiety.

Due to fast excretion and metabolism by peptidases, the plasma half-life of many neuropeptides is less than a few minutes. Rapid metabolism or excretion of the radiolabeled peptide decreases the potential for the radiopharmaceutical to accumulate at the target site; on the other hand, reasonably fast clearance enhances the target to non-target ratios. Therefore it is crucial to design new stabilized derivatives with still high binding affinities and improved biodistribution (*e.g.* low liver and low kidney accumulation and high tumor uptake). In neurotensin the main proteolytic fragments 1–10 and 11–13 have been observed. This enzymatic destruction can be inhibited by molecular modifications as substitution of D-amino acids for L-amino acids, or the insertion of unusual amino acids or side chains. Amidation is another way to inhibit proteolytic degradation. A number of altered peptides were tested for their *in vitro* and *in vivo* characteristics. Binding assays and internalization studies were performed *in vitro* with HT-29 cells. All labeled peptides showed a high affinity for the neurotensin receptor with K_d values in the nanomolar range (between 0.2 and 3 nM, Table 2). After interaction of neurotensin with its receptor, peptide-receptor complexes are rapidly internalized and the rate of internalization is similar for all analogues tested. In all cases about 80% of the peptide was internalized within the first 30 min and remained trapped inside the cell for at least 2 h.

Biodistribution studies in nude mice with xenografted tumors showed increased tumor uptake for the stabilized peptides. To date the best results were obtained with NT-VIII for which we found at 1.5 h post injection tumor/blood ratios of 11.3 and a tumor uptake of 4.5% I.D./g tissue. A blockade experiment with unlabeled NT-II showed significant reduction in tumor uptake and confirmed the specificity of the binding (Fig. 5). In order to confirm these results we introduced a second cell line, the human prostate adenocarcinoma cell line PC3, which also has high expression of NTS1 receptors. The image of a nude mouse with xenografts originating from HT-29 and PC3 cells taken 1.5 h post injection of ^{99m}Tc labeled NT-VIII nicely demonstrates the potential of this compound (Fig. 6).

In conclusion, we believe that the most promising approach for the clinical application of peptide radiopharmaceuti-

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Table 1. Overview of the synthesized NT analogues: NT(8-13) binds to the receptor, His and (N_α-His)Ac in the place of Pro form the metal binding complex (bold: site of stabilizing effect).

NT(1-13)	pGlu-Leu-Tyr-Gly-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu
NT(8-13)	Arg-Arg-Pro-Tyr-Ile-Leu
NT-I	His-Arg-Arg-Pro-Tyr-Ile-Leu
NT-II	(N_α-His)Ac-Arg-Arg-Pro-Tyr-Ile-Leu
NT-III	His-**(N-CH_3)**-Arg-Lys-Pro-Tyr-Tle-Leu
NT-IV	His-Lys-**(ΨCH_2NH)**-Arg-Pro-Tyr-Ile-Leu
NT-V	(N_α-His)Ac-Arg-**(ΨCH_2NH)**-Arg-Pro-Tyr-Ile-Leu
NT-VI	(N_α-His)Ac-Lys-**(ΨCH_2NH)**-Arg-Pro-Tyr-Ile-Leu
NT-VII	(N_α-His)Ac-Asp-Lys-**(ΨCH_2NH)**-Arg-Pro-Tyr-Ile-Leu
NT-VIII	(N_α-His)Ac-**(N-CH_3)**-Arg-Lys-Pro-Tyr-**Tle**-Leu
NT-IX	(N_α-His)Ac-Gly-Lys-**(ΨCH_2NH)**-Arg-Pro-Tyr-**Tle**-Leu
NT-X	(N_α-His)Ac-Arg-Arg-Pro-Tyr-**Tle**-Leu
NT-XI	(N_α-His)Ac-Lys-**(ΨCH_2NH)**-Arg-Pro-Tyr-**Tle**-Leu

Table 2. Pharmacological data of $^{99m}Tc(CO)_3$ NT-analogues

		Plasma	%ID/g organ (24 h)		
	Kd [nM]	Stability[a]	Tumor	Kidney	Liver
NT-I	0.6	-	0.4	2.8	4.8
NT-II	0.3	0.1	0.1	0.5	0.7
NT-III	1.5	-	-	11.6	13.6
NT-IV	0.3	-	-	89.0	9.0
NT-V	0.5	-	-	8.0	3.7
NT-VI	0.5	0.15	0.5	4.3	0.4
NT-VII	1.3	-	-	18.8	5.4
NT-VIII	1	> 24	0.7	1.4	0.5
NT-IX	3	> 24	1.3	7.3	1.0
NT-X	0.2	2	0.2	0.8	1.2
NT-XI	0.4	> 24	1.4	6.2	0.7

[a] hours with 50% of intact peptide remaining in plasma

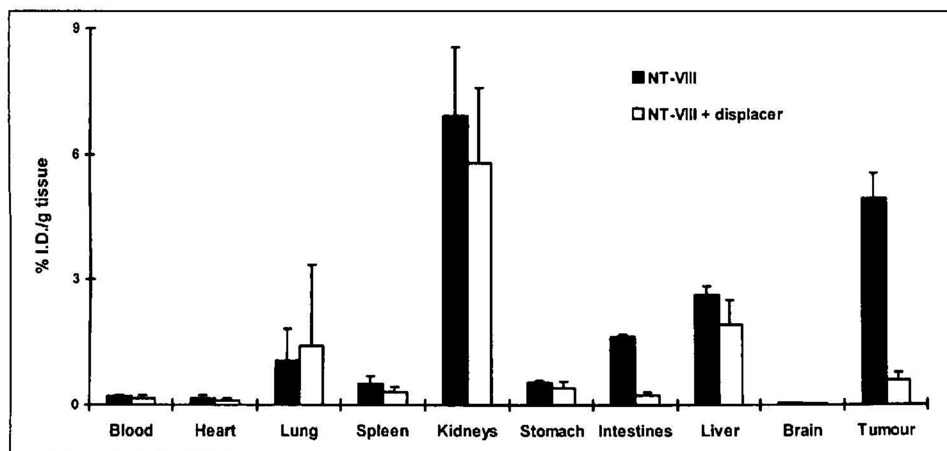

Fig. 5. Biodistribution 1.5 h post injection of ^{99m}Tc-labeled NT (VIII) and ^{99m}Tc-labeled NT (VIII), coinjected with unlabeled NT (II) in tumor-bearing nude mice demonstrates specific binding of the tracer in intestine and tumor.

cals is the synthesis of stabilized peptide analogues with the metal chelating sequence (N_α-His)Ac incorporated at a position in the molecule not essential for receptor binding. This eliminates the need for inefficacious procedures for conjugation of bifunctional chelates, protection and deprotection of functional groups and the laborious purification steps. Our novel labeling method combines the advantage of highest specific activities with minimal functionalization of peptides under retention of biological affinity.

Collaborations outside of the Center for Radiopharmaceutical Science exist with the groups of D. Tourwé, Free University of Brussels, Belgium, J.-C. Reubi, University of Bern, Switzerland, A. Beck-Sickinger, University of Leipzig, Germany and A. Bischof-Delaloye, University of Lausanne, Switzerland.

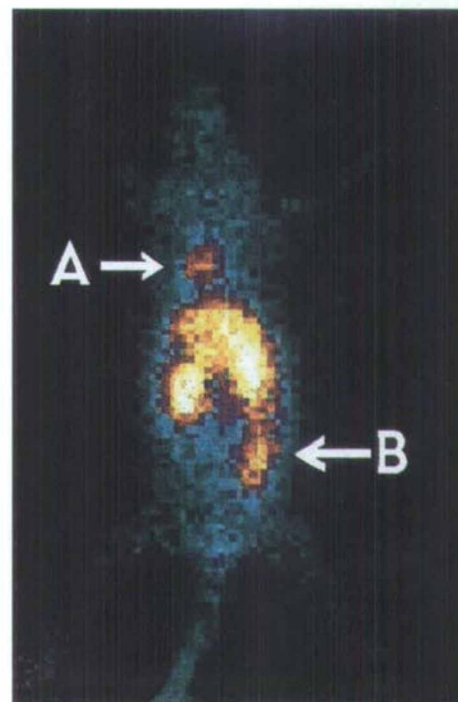

Fig. 6. Scintigraphic image 1.5 h post injection of ^{99m}Tc-labeled NT (VIII) of a mouse bearing PC3 tumors (A) and HT-29 tumors (B). Both tumors depicted by the arrows express NTS1 receptor.

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Tumor Targeting

The Tumor Targeting Group is engaged in the preclinical and clinical evaluation of radioimmunoconjugates derived from tumor-specific monoclonal antibodies and 'designer' molecules such as fragments, multimeric fragments and single-chain antigen binding proteins. These proteins are labeled with β^--particle emitting nuclides for application in RIT. Clinical situations favorable for systemic RIT are hematologic malignancies, particularly non-Hodgkin's lymphoma, or those which are associated with the presence of disseminated small radiosensitive tumor manifestations, such as found for instance in neuroblastoma. These clinical situations are particularly appropriate, because the β^- particle emitting nuclides we are interested in, such as ^{131}I, ^{67}Cu or $^{186/188}$Re, are cytotoxic over only a few cell diameters and deposit their energy within a distance of 1.8 to 11 mm. Radiolabeled antibodies for therapeutic applications have two components: a tumor-targeting vector (monoclonal antibody (mAb) or fragments thereof) and the radiolabel. To date, ^{131}I has been the radionuclide of choice due to its availability and simplicity of labeling: however, its volatility, *in vivo* dehalogenation, and γ-emission at 364 KeV (suboptimal for tumor imaging, and contributing to the whole body dose) are disadvantages. These concerns have led us to investigate other β^--emitters such as ^{188}Re (commercially available, generator produced) and ^{67}Cu, which is produced in house at the PSI with a 72 MeV cyclotron. At the present time, PSI is the only center worldwide which produces ^{67}Cu of sufficiently high specific activity for preclinical and clinical studies. In the past years, a series of preclinical studies of the tumor targeting group with ^{67}Cu-labeled antibodies has shown that in addition to the physical advantages of this nuclide compared with ^{131}I, an added benefit consists in the biological properties of radiocopper-labeled immunoconjugates. Metabolites of radiocopper labeled antibodies accumulate in the target tissue, thereby improving the therapeutic index. Work has progressed to a clinical level, where we collaborated in a diagnostic study in fifteen bladder cancer patients. Results indicated that intravesical administration of ^{67}Cu-labeled anti Muc-1 mAb C595 is a promising method for the treatment of superficial bladder cancer and consequently a dose escalation study of ^{67}Cu-C595 antibody in bladder cancer patients is scheduled to start this year.

Intact mAbs are at the present time the best tumor targeting vehicles available due to their maximal uptake and retention in tumor. Disseminated and radiosensitive tumors such as lymphomas or neuroblastomas are recognized today as the most suitable targets for systemic RIT. Radionuclide therapy with ^{131}I-metaiodobenzyl guanidine (MIBG), a low molecular weight catecholamine analogue which is taken up into neuroblastoma cells, is an established therapy for neuroblastoma. However in recurrent patients metastases appear, which do not take up MIBG and escape current therapies. Our high affinity internalizing anti-neuroblastoma antibody chCE7, directed against the L1-CAM protein, is being studied at the present time in a clinical collaboration, to address the problem of tumor heterogeneity in recurrent neuroblastoma patients. So far the results of an ongoing sequential imaging study of recurrent neuroblastoma patients (to date seven patients) with ^{131}I-MIBG and ^{131}I-labeled mAb chCE7 illustrate the heterogeneity of neuroblastoma and a complementarity of targeting with MIBG and mAb chCE7. Fig. 7 illustrates a case of a patient with recurrent neuroblastoma, where some metastases take up MIBG, whereas other metastases only take up mAb chCE7. Obviously, such cases would be candidates for RIT with ^{131}I- or ^{67}Cu-labeled mAb chCE7. In order to assess the therapeutic efficacy of ^{131}I-labeled chCE7, a study was performed in an animal model. Nude mice bearing neuroblastoma xenografts were treated with ^{131}I-MIBG, ^{131}I-mAb35 as a non-specific control, and ^{131}I-mAb chCE7. Results in Fig. 8 show rapid growth of tumors in controls without treatment, some growth delay with the nonspecific antibody and anti-tumor effects of MIBG and chCE7. Therapy with ^{131}I-chCE7 resulted in complete suppression of tumor growth, and the better efficacy of the treatment with ^{131}I-labeled chCE7 antibody compared with ^{131}I-MIBG can be explained by the very different pharmacokinetics of the two radiopharmaceuticals. The antibody maximizes tumor uptake and retention due to its longer half life in the blood and consequent availability for tumor binding. The data obtained in this study strongly support that RIT with chCE7 appears as a promising alternative in the cases of MIBG-negative neuroblastoma and may be a useful tool in treating residual neuroblastoma after MIBG therapy. Based on these results on therapeutic efficacy in the animal model, more patients will be imaged in order to select patients for a phase I therapy study with ^{131}I-mAb chCE7.

To improve the efficacy of RIT, additional therapeutic benefit is expected when small antibody fragments which internalize into tumor cells are used. ^{67}Cu-labeled internalizing F(ab')$_2$ fragments of mAb chCE7 combine prolonged retention time at the tumor site with more rapid clearance from the blood. In the future, recombinant divalent fragments of mAb chCE7 will replace the F(ab')$_2$ fragments employed at the present time. A drawback of radiometal-labeled antibody fragments consists in the unwanted accumulation of their metabolites in the kidney. Labeling procedures with ^{67}Cu use macrocyclic chelators which are attached covalently to an antibody and readily form strong copper complexes with high *in vivo* stability. Both the charge of the macrocycle and the linkage groups to the protein have important effects on the biodistributions of ^{67}Cu-labeled antibody fragments. When we investigated the effect of a tripeptide linkage group between a positively charged CPTA macrocycle and a negatively charged DO3A macrocycle and mAb chCE7 F(ab')$_2$ (Fig. 9), we found that the negatively charged macrocycle combined with the triglycine linker significantly improves the biodistribution of the resulting immunoconjugate (Table 3). The novel peptide-linked copper complexes minimize the toxicity of ^{67}Cu-labeled F(ab')$_2$ to normal organs while improving their uptake by the tar-

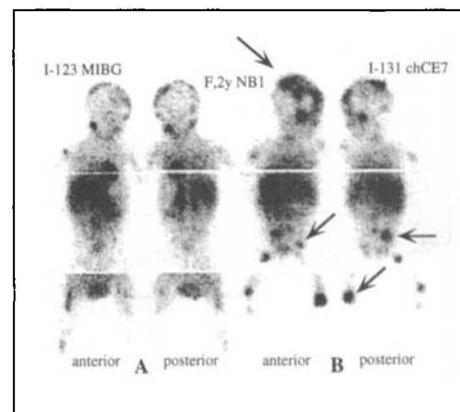

Fig. 7. Comparative scintigraphy with ^{123}I-MIBG (A) and ^{131}I-mAb chCE7 (B) in a two-year-old girl with recurrent neuroblastoma in the abdomen and the right femur. While ^{123}I-MIBG scintigraphy shows pathological concentration in the abdominal recurrence, right orbit, right humerus, right femur and right tibia, ^{131}I-mAb chCE7 scintigraphy reveals pathological accumulation in the skull, the right orbit, right humerus, pelvis, right femur, right tibia and most intensely above the left knee. No mAb chCE7 uptake is visible in the abdominal mass and the central right femur shaft.

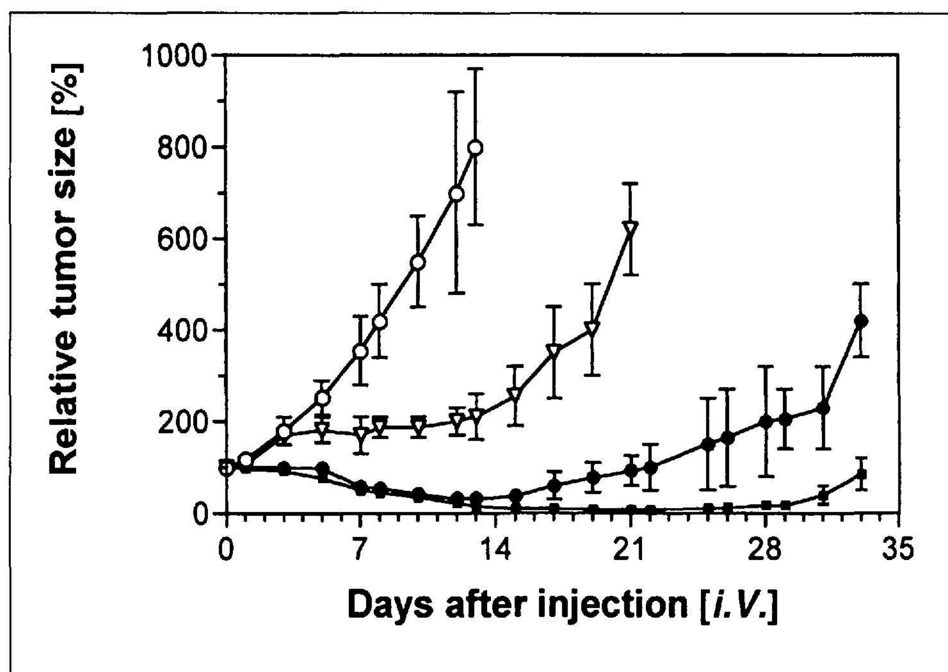

Fig. 8. Tumor growth inhibition in nude mice bearing neuroblastoma xenografts after *i.v.* therapy with ^{131}I-labeled radiopharmaceuticals. Animals received saline (open circles), 17.3 MBq irrelevant control mAb 35 (triangles), 110 MBq MIBG (closed circles) or 17.8 MBq mAb chCE7 (closed squares). Tumor sizes are expressed as mean percentage of the pretreatment value. (M. Rutgers, *et al.*, 'Advances in Neuroblastoma Research', Bath, UK, **1998**.)

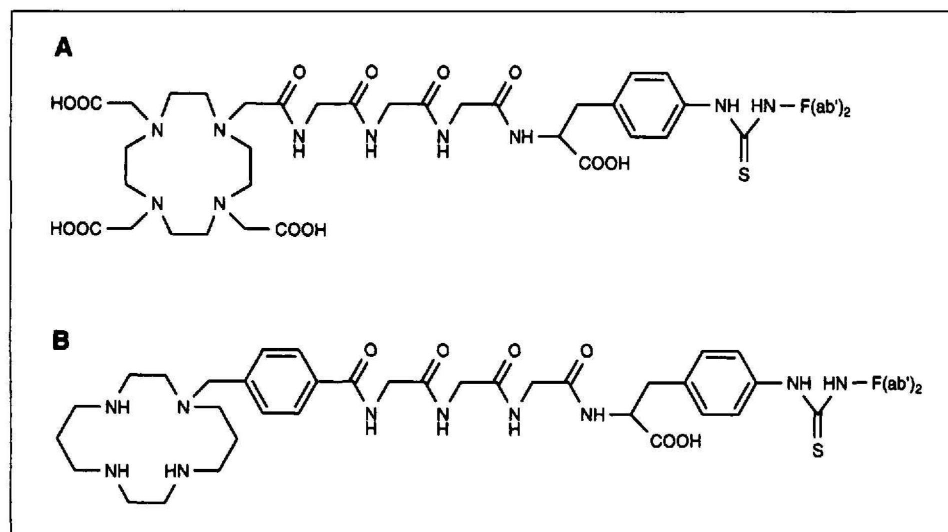

Fig. 9. Structures of the triglycine-linked DO3A- and CPTA-F(ab')$_2$ conjugates. A. Triglycine-linked DO3A; B. Triglycine-linked CPTA.

Table 3. Uptake of ^{67}Cu-chCE7 F(ab')$_2$ immunoconjugates in tumor and normal tissues measured 48 h post injection in nude mice bearing neuroblastoma xenografts. Data are expressed as percent injected dose per gram of tissue (%ID/g).

%ID/g	CPTA	CPTA-trigly	DO3A	DO3A-trigly
Blood	0.4 ± 0.09	0.6 ± 0.06	0.8 ± 0.10	1.8 ± 0.10
Kidney	76.0 ± 20.40	20.4 ± 6.30	21.5 ± 0.50	12.3 ± 1.80
Liver	6.1 ± 1.80	4.5 ± 0.09	11.4 ± 3.20	9.3 ± 0.90
Tumor	3.3 ± 0.08	4.7 ± 0.70	10.2 ± 1.90	15.5 ± 2.90

get (tumor) tissue. These results demonstrate the importance of optimizing the *in vivo* behavior of radiometal-labeled immunoconjugates and efforts are being extended to Tc- and Re-tricarbonyl labeled antibody fragments.

Collaborations outside of the Center for Radiopharmaceutical Science exist with the groups of A. Pluckthun, University of Zürich, K. Chester, CRC Targeting & Imaging Group, London, UK, C.A. Hoefnagel, The Netherlands Cancer Institute, Amsterdam, Holland, A. C. Perkins, University Hospital Nottingham, UK.

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