Radiopharmaceuticals: From Molecular Imaging to Targeted Radionuclide Therapy

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Abstract: The research and development of smart radiodrugs is the goal of the Center of Radiopharmaceutical Science of ETH, PSI, and USZ. Positron Emission Tomography (PET) allows the non-invasive visualization of biochemical processes within the body. Radiolabeled PET-tracers allow the study of neurophysiological diseases like Alzheimer, Parkinson's disease or the imaging of metastatic tumors. PET-techniques are nowadays an important part of routine nuclear medicine diagnosis. Tumor-cell targeting biomolecules (*e.g.* antibodies or peptides) coupled to therapeutic radionuclides can sterilize the malignant cells while sparing healthy tissue. This so-called targeted radionuclide therapy has made tremendous progress in the recent years and the first approved radiotherapeutics are available for clinical use.

Keywords: Molecular Imaging · PET-tracer · Radioimmunotherapy · Radionuclide therapy · Radiopharmaceuticals

Introduction

One of the historical challenges of nuclear medicine has been to develop radiopharmaceuticals that will target a specific molecular site (*e.g.* neuronal, myocardial or tumoral) in the human body while minimizing any uptake in a non-target organ. Early work was limited to those elements that had high natural affinities for a given target organ, such as iodide for the thyroid. Depending upon the radiation characteristic of the chosen isotope of radioiodine it is possible to either image (with ^{123}I) the uptake and entrapment of iodide into the thyroid ($\rightarrow$ molecular imaging) or to destroy the tumorous thyroid cells by using therapeutic ^{131}I with its large local deposition of the β^- energy ($\rightarrow$ targeted radionuclide therapy).

For molecular imaging we have nowadays a very powerful non-invasive technique – positron emission tomography (PET) – to monitor pharmacokinetic and functional processes in the intact organism at physiological concentrations (*e.g.* for neuroreceptor ligand interaction nM–pM) and with an image spatial resolution from 1 mm^3 (animal) to 64 mm^3 (man). Biological active molecules can be labeled with 'biological' radioisotopes such as ^{11}C (physical half live 20.3 min) for the inactive ^{12}C, which are chemically indistinguishable from the endogenous non-radioactive biomolecules. This technique allowed the study of biodistribution and biochemical fate from outside the body with a so-called PET scanner. Our PET-tracer research projects focus on the visualization of molecular functions in the brain and tumors, some examples are given in the section on 'Molecular Imaging' below.

The second main focus of our research group concentrates on developing radio therapeutics against tumor cells for systemic application. A study of the EU-commission revealed that at the time a cancer is first diagnosed, 40% of the patients will already present metastatic disease with unfavorable prognosis. In these cases the systemic application of tumor-seeking molecules labeled with therapeutic radionuclides may allow destruction of disseminated tumors, which cannot be reached efficiently by other modalities. Systemic delivery of therapeutically effective radiation (*e.g.* so-called β^- emitters) to tumor cells is most advanced for specific antibodies against tumor-associated cell surface proteins (in 2002 the FDA approved Zevalin® as the first antibody for radioimmunotherapy). Our research strategy focuses on the investigation of interesting tumor target structures (antibody antigens, peptide receptors, and enzymes), specific tailoring of promising target molecules (intact and fragmented antibodies, peptides, and enzyme substrates), the development of suitable bifunctional chelators for therapeutic radionuclides and the characterization and application of novel radio conjugates. Examples from our most exiting research results are given below in the sections 'Radionuclide Chemistry', 'Tumor-avid Peptides', and 'Radioimmunotherapy'.

Molecular Imaging with PET Tracers

Molecular imaging is a term that is widely used in conjunction with imaging modalities that provide signal detection at the molecular level. Positron emission

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tomography (PET) is one such imaging modality. PET imaging technique has established itself as a clinically relevant technology for non-invasive imaging in neurology, oncology and cardiology. In recent years, the development of small animal PET scanners has made possible the investigation of early proof-of-concept of drug efficacy in animal models whereby dose-finding regimen and treatment strategies are most crucial. The development of new PET ligands for the various neurotransmission systems remains a challenge. Although some general rules exist, predictability of ligand success is problematic. Much, however, remains to be accomplished since no specific PET ligands exist for the majority of neurotransmission systems including the glutamatergic system. At the Center for Radiopharmaceutical Science of ETH, PSI, and USZ, our molecular imaging activities focus on the development of high affinity and specific PET ligands for the imaging of metabotropic glutamate receptors and thymidine kinase substrates for monitoring gene therapy. A further research portfolio in close collaboration with the pharmaceutical industry involves the use of surrogate markers such as ^{18}F-FDG (glucose metabolism), ^{18}F-FMISO (hypoxia) and other routinely established PET ligands to assess drug efficacy in animal models of human diseases. A special research tool for accomplishing these research goals is the recently acquired small animal QUAD HIDAC PET scanner with a uniform resolution of 1 mm. A unique advantage of the small animal PET scanner is the possibility to perform fast, dynamic studies and also to repeat measurements in the same animal which means that only a small number of animals are required. Fig. 1 and 2 show PET images obtained in mice which demonstrate the potential and the performance of the small animal QUAD HIDAC PET scanner [1][2]. Fig. 1 shows coronal sections through the striatum of mice injected with the D_2 receptor ligand ^{18}F-fallypride (left) and the dopamine transporter ligand ^{18}F-FECNT (right). Both left and right striata are clearly visualized and are also very well delineated from extra-cerebral structures. Fig. 2 shows a whole body PET image of ^{18}F-FMISO uptake. ^{18}F-FMISO accumulation was heterogeneous within the B16-F1 melanoma tumor (encircled with an arrow) and high radioactivity uptake was evident in the abdominal regions.

Radionuclide Chemistry

Many types of cancer cells have a great affinity for the vitamin folic acid or folate. One reason is that the ability of a cell to synthesize DNA is dependent upon the vitamin folate in order to grow and divide [3]. In or-
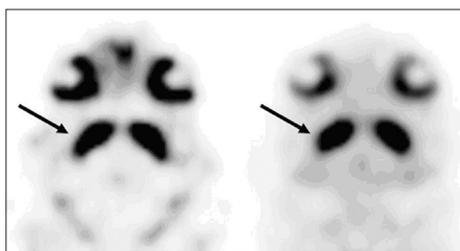

Fig. 1. PET images of ^{18}F-fallypride (left) and ^{18}F-labelled FECNT (right) acquired with the small animal QUAD HIDAC PET scanner with arrows indicating the right striatum
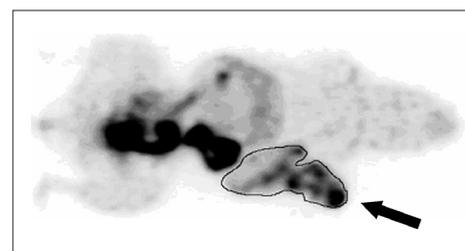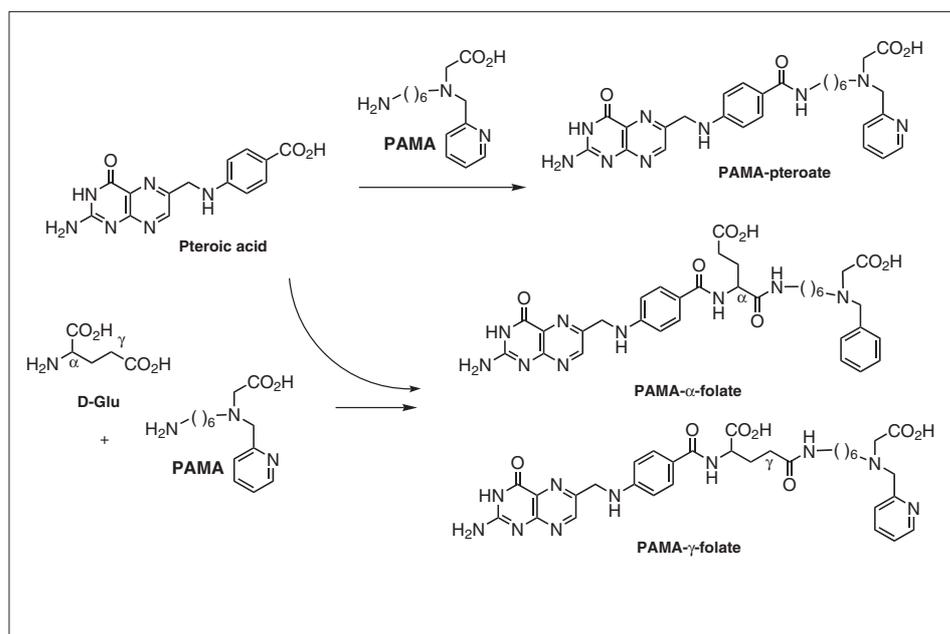

Fig. 2. Coronal whole body ^{18}F-FMISO PET image of a mouse bearing a B16-F1 melanoma tumor

der to cope with the increased appetite, cancer cells overexpress a cell surface receptor with high affinity for folic acid; the so-called folate receptor (FR) [4][5]. FR-overexpression can be found in a variety of neoplastic tissues such as ovarian, breast and colorectal tumors, but is highly restricted in most normal tissues except the kidneys (kidney proximal tubule cells express the folate receptor on their membranes that face the side of urine collection in order to transport folate back into circulation). Since the folate receptors on cancer cells recycle, a large number of folate molecules (20–60 million) can be delivered into each cancer cell. This can result in greater therapeutic responses. Since folate is so essential and is carried into the cell for consumption, modified folate conjugates do not reach *e.g.* the lysosomes, where they are prone to destruction and cell externalization like other drugs [6]. Thus, folate is an effective 'Trojan horse' for the selective delivery of drugs in tumor tissue for diagnostic and therapeutic purposes.

In our group we are interested in radioactive-labeled folate derivatives based

on the diagnostic radionuclide technetium-99m ($t_{1/2}$ = 6 h; γ-energy 141 keV) and the therapeutic radionuclide rhenium-188 ($t_{1/2}$ = 17 h; β⁻-energy 2.1 MeV) because they are both relatively inexpensive and readily available *via* a generator system. In particular we are developing low-valent, organometallic and isostructural folate derivates of ^{99m}Tc and [188]Re with improved *in vitro* and *in vivo* stability. For this purpose folic acid is functionalized with *e.g.* a picolylamine monoacetate (PAMA) [7] chelating system (Scheme 1) at the α- respectively γ-carboxyl group of folic acid. In addition the metal chelating system has been directly attached to pteroic acid, a truncated form of folate (Scheme 1). The folate and pteroate derivatives were radio-labeled with the organometallic precursor [M(CO)$_3$(H$_2$O)$_3$]⁺ (M = [99m]Tc, [188]Re) developed in our laboratories with high specific activity and high radiochemical purity (Scheme 2) [8–12]. *In vitro* experiments of these novel radioconjugates, performed in tumor cells overexpressing the FR such as KB-cells (a human nasopharyngeal carcinoma cell line) showed excellent cell bind-

Scheme 1. Schematic syntheses of PAMA pteroate and folate derivatives for radioactive labeling with Tc-99m and Re-188

ing (50–60 % of total activity, 1 h post incubation) and cell internalization rates between 15–20% of total activity (Fig. 3). In the presence of excess unlabeled folic acid the radioactive derivatives could be completely displaced, which proves the specificity of these novel folate derivatives. The conjugates exhibit comparable affinities for the FR (α-derivative: $\sim 9 \times 10^{-8}$ M; γ-derivative: $\sim 10^{-7}$ M) as the natural ligand folic acid ($IC_{50} \sim 5 \times 10^{-8}$ M). From these *in vitro* tests it can be concluded that the organometallic modification of folic acid does not alter the binding properties nor does the glutamate moiety seem to be critical for FR binding.
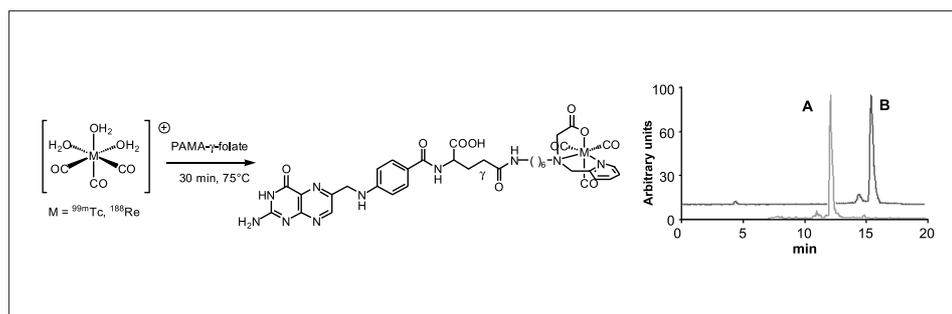
In vivo experiments were preformed in athymic nude mice bearing human tumor xenografts (KB cells). Here, significant differences between the folate and pteroate derivatives could be observed. The folate derivatives revealed tumor uptake and retention up to 2% of injected dose per gram (% ID/g) and kidney and liver uptake between 12–15% ID/g and 2% ID/g respectively 4 h post injection (Fig. 4). The target-to-organ and tissue ratios achieved with this first series of folate derivatives are comparable with those reported for Ga-67 and In-111 radiolabeled folate complexes [13][14]. On the other hand, the pteroate derivative showed reduced radioactivity in the kidney (<5% ID/g) but also reduced tumor uptake (< 1% ID/g, 4 h p.i.), which gives rise to low target-to-organ and tissue ratios. Thus, the results obtained up to now suggest that ^{99m}Tc/^{188}Re-folate derivatives rather than pteroate derivatives should be used for targeted radionuclide diagnosis and therapy. For a potential therapeutic application of these and future Re-188 complexes the kidney uptake is a critical issue which needs to be carefully addressed and significantly reduced, because of the nephrotoxic effects of the particle emission associated with the radioactive decay of this radionuclide.

Radioimmunotherapy

L1 and its soluble form L1s, which is released from tumor cells and deposited in the surrounding extracellular matrix, are tumor-associated proteins and potential markers for tumor staging. We make use of the cell surface bound form of L1 as a target for antibody-based radiopharmaceuticals (radioimmunotherapy) and we showed previously that in neuroblastoma patients it can serve to target the high affinity monoclonal anti-L1 antibody chCE7 for radioimmunodiagnosis of metastatic neuroblastoma [15]. In the past years it emerged from our work and from the work of others that the L1 protein is also highly expressed in tumors other than neuroblastoma such as renal carcinoma [16], melanoma [17] and ovarian and endometrium carcinoma [18]. In addition we recently found evidence that L1 plays a role in the biology of renal cancer, because HGF (Hepatocyte Growth Factor), a physiological factor which is involved in invasion and metastasis of renal tumors stimulates the release of L1s from renal carcinoma cells [19]. At the present time, we are investigating at the preclinical level the efficacy of ^{67}Cu-labeled anti-L1 mAb chCE7 and engineered fragments of this mAb [20] for radioimmunotherapy of L1 expressing tumors. After a scale-up of the production of the radiocopper nuclides ^{67}Cu (therapeutic beta particle emitter) and ^{64}Cu (PET nuclide) at the PSI cyclotron we have now access to up to 12 GBq of ^{67}Cu for therapy studies as well as ^{64}Cu for PET imaging. In collaboration with the PET group we use high-resolution PET imaging to analyze antibody distribution within tumors and changes in tumor metabolism induced by radioimmunotherapy with chCE7 antibody. Fig. 5 shows a dual tracer study in a B16F1 mouse melanoma tumor, generated from transfectant cells expressing human L1. Antibody uptake is measured with

Scheme 2. Representative radiolabeling conditions of PAMA-γ-folate with the organometallic precursor $[M(CO)_3(H_2O)_3]^+$ (M = ^{99m}Tc, Re) with (A) corresponding HPLC trace of the PAMA-γ-folate (UV, 254 nm) and (B) the corresponding Tc-99m complex (γ-trace).
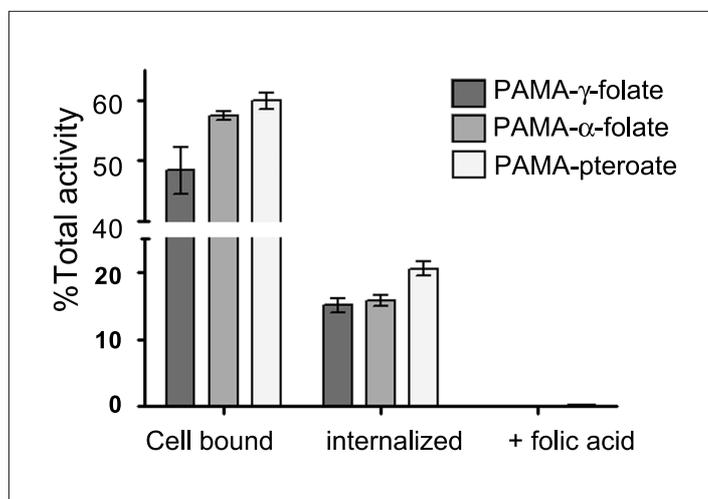

Fig. 3. *In vitro* cell binding (KB cells) of ^{99m}Tc folate and pteroate derivatives
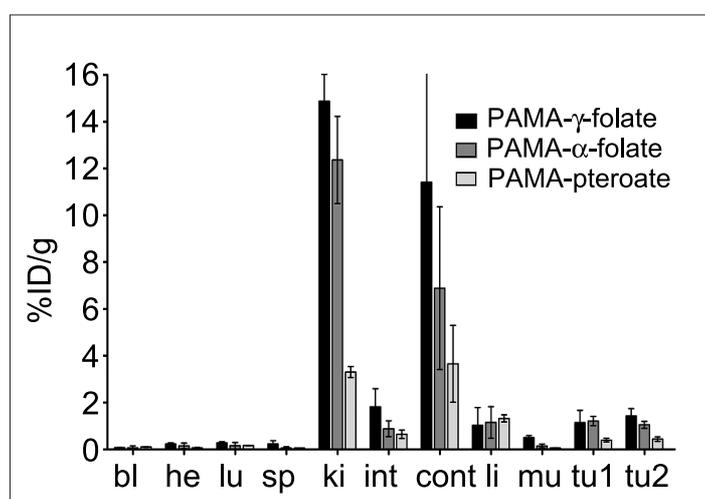

Fig. 4. *In vivo* biodistribution of organometallic ^{99m}Tc folate and pteroate derivatives in nude mice bearing two human tumor xenografts, 4 p.i.; bl = blood, he = heart, lu = lung, sp = spleen, ki = kidney, int = intestines, cont = content of intestines, li = liver, mu = muscle, tu1&2 = tumor 1&2
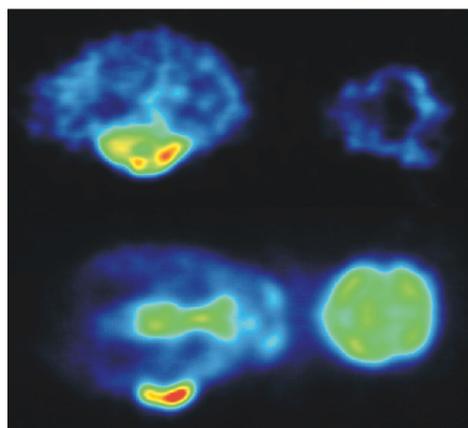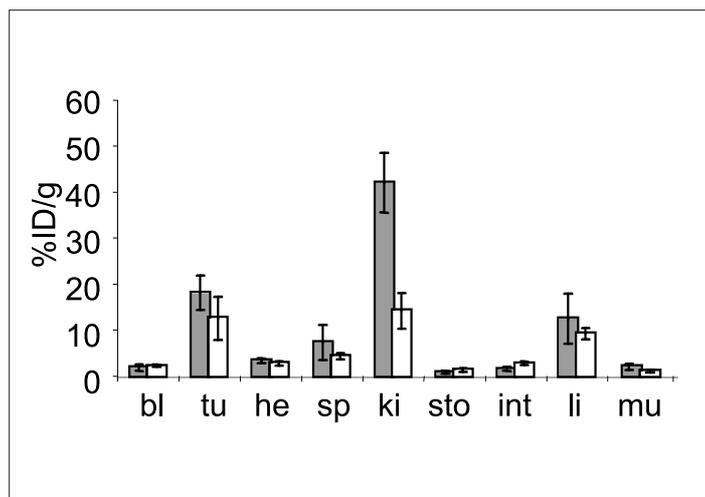
Fig. 5. Sequential PET imaging of a mouse with a B16F1/huL1 melanoma (arrow). Top: imaging with ^{64}Cu-CPTA-chCE7 antibody, below: imaging with ^{18}FDG.

Fig. 6. Biodistributions (% injected dose/g tissue) in nude mice with human neuroblastoma xenografts (n=3) 21 h post injection of recombinant ^{177}Lu-DOTA-R1-F(ab')$_2$ (grey bars) or ^{67}Cu-DOTA-R1-F(ab')$_2$ (white bars); bl = blood, he = heart, sp = spleen, ki = kidney, int = intestines, li = liver, mu = muscle, tu = tumor.

^{64}Cu-CPTA-labeled chCE7 [21][22], and tumor glucose metabolism is visualized with ^{18}FDG. The high-resolution imaging resolves intratumor heterogeneity both in cellular antibody uptake and in cellular metabolism, which are important factors influencing therapeutic efficacy. The studies in nude mice with human tumor cell xenografts focus on a comparison of the two beta particle emitters ^{67}Cu and ^{177}Lu. Whereas ^{131}I and ^{90}Y are already established nuclides for clinical RIT, ^{67}Cu and ^{177}Lu nuclides emit beta particles with shorter path lengths than ^{90}Y and may be more effective for the treatment of small tumor masses. Fig. 6 shows biodistributions in xenografted mice of recombinant Fab$_2$ constructs of mAb chCE7 labeled with the two nuclides, using the triglycine-linked DOTA chelate we developed for $^{67/64}$Cu-labeling [23]. In contrast to intact antibody molecules, F(ab)$_2$ fragments show more than 5 fold increase in tumor/blood ratios 21 h post injection and are superior to the intact mAb in this respect. The ^{177}Lu F(ab)$_2$ conjugate was found to lead to higher levels of radioactivity in the kidneys and appears to be less favorable in terms of the therapeutic index tumor/kidney than the ^{67}Cu-F(ab)$_2$ conjugate. At the present time studies are underway measuring radiation doses to tumor and normal organs delivered by the ^{67}Cu and ^{177}Lu nuclides linked to a number of engineered chCE7 constructs with different pharmacokinetics.

To obtain optimal tumor targeting, biodistribution and clearance properties, antibody fragments of intermediate molecular weight can be designed to have the proper combination of high tumor uptake and rapid clearance from normal tissue. An interesting concept makes use of the ribonuclease barnase (12 kDa) and its inhibitor barstar (10 kDa) which form a very tight complex with a $K_d = 10^{-14}$ M. To form multimeric anti-p185HER-2 scFv antibody

constructs with improved stability, fusion proteins of 4D5 scFv with barnase, with dibarnase and 4D5 scFv with barstar were prepared. By mixing 4D5-barnase with 4D5-barstar, dimeric and trimeric constructs were prepared. The monomeric, dimeric and trimeric constructs were labeled at their polyhistidin-tag with ^{99m}Tc-tricarbonyl. The high functional avidity and the high stability of the multimeric constructs led to improved biodistribution in human tumor xenografted mice when compared to the multimerized antibody fragments achieved by the use of self-associating peptides. The results show that the barnase-barstar system can be used to produce multimeric miniantibodies which localize efficiently to tumor xenografts and show improved pharmacokinetics [24].

Tumor-Avid Peptides

Neuropeptide receptors are overexpressed in a number of human cancers. Radiolabeled somatostatin (sst) analogs have been successfully applied for the imaging and therapy of sst receptor-positive tumors such as neuroendocrine tumors [25][26]. However, since not all tumors express enough sst receptors (*e.g.* exocrine pancreatic cancer), the research has also focused on the development of analogs of other regulatory peptides as potential radiopharmaceuticals such as bombesin (BBS) and neurotensin (NT), two peptides which we studied in our laboratory [27–32]. In this report, we will show some results with NT derivatives. In exocrine pancreatic cancer, an overexpression of NT receptors has been found [33][34] and they are interesting targets for diagnosis and therapy of this type of tumor. The major problem with NT which limits its clinical application is the rapid degradation by endogenous peptidases. A series of NT analogs, based on the fragment 8-13, has been synthesized. Some of them have been already reported

[29–32]. Our aim is to design new stabilized analogs with high affinity and good biodistribution properties (high tumor uptake and low kidney and liver accumulation). Here we present four analogs with different stabilizations (Table). All include a tridentate ligand, (NαHis)Ac, for labeling with the ^{99m}Tc/^{188}Re-tricarbonyl technique [35]. The analogs showed high affinity for NT1 receptors, comparable to that of the natural NT, although for NT-XIX was a little lower (Table). The modifications increased strongly the plasma stability, especially for the bi- and the tri-stabilized analogs which were mostly intact after 24 h (Table). The increase in stability led to an increase in tumor uptake. A decrease in kidney and liver accumulation was also observed for the most stable analogs. It was more remarkable in the case of NT-XIX for which the highest uptake was found in tumor at all time points tested (Fig. 7). A minimal affinity would be necessary for binding to the receptors; however, the longer stability seems to play a more important role in the *in vivo* biodistribution. This could explain the better tumor uptake obtained with the stable analogs. However, the reduction in kidney and liver accumulation found for NT-XIX remains unclear. Stable analogs seem to be more suitable for clinical application and NT-XIX is an excellent candidate.

Conclusion

The Center for Radiopharmaceutical Science develops new generations of radiolabeled molecules and technologies for molecular delivery of radioisotopes to the target-sites with a high degree of precision, recognition, and target selectivity. Miniaturized PET technology facilitates molecular imaging and mapping of metabolic organ function, visualizing the molecular biology of cell function, and zooming in on gene function for delineating dif-

Table. Sequence, affinity in HT-29 cells and plasma stability of several NT(8-13) analogs

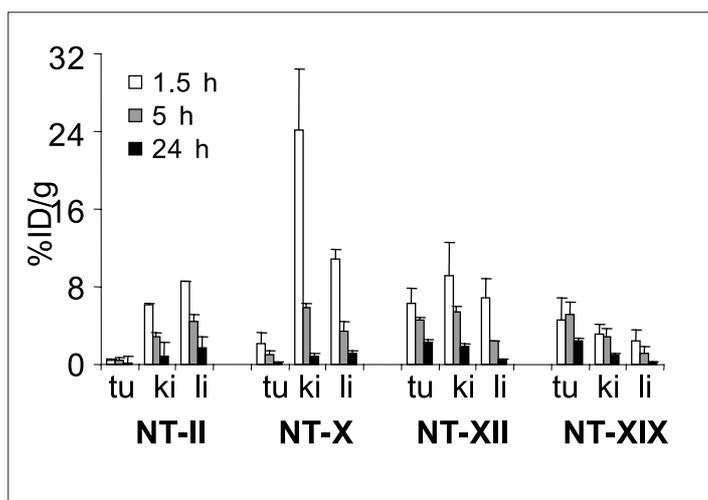|	Sequence	K_d (nM)	Plasma $t_{1/2}$ (h)
NT-II	(Nα-His)Ac-Arg-Arg-Pro-Tyr-Ile-Leu	0.3	0.1
NT-X	(Nα-His)Ac-Arg-Arg-Pro-Tyr-**Tle**-Leu	0.5	4
NT-XII	(Nα-His)Ac-Arg-**(N-CH₃)**-Arg-Pro-Tyr-**Tle**-Leu	2.0	>24
NT-XIX	(Nα-His)Ac-Arg-**(N-CH₃)**-Arg-Pro-**Dmt**-**Tle**-Leu	15.0	>24

Fig. 7. Uptake in tumor (tu), kidney (ki) and liver (li) for the four NT(8-13) analogs (1.5, 5 and 24 h post-injection)

ferences in molecular biology of normal health from disease in humans. Thus, radiopharmaceutical science will advance our understanding of biology and medicine and will help to treat efficiently cancerous diseases through noninvasive molecular tools.

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