[Frontiers in Bioscience 14, 872-886, January 1, 2009]

Radiolabelled RGD peptides and peptidomimetics for tumour targeting

Roland Haubner[1], Clemens Decristoforo[1]

[1]Clinical Department of Nuclear Medicine, Medical University Innsbruck, Austria,Anichstrasse 35, A-6020 Innsbruck, Austria

TABLE OF CONTENTS

1. ABSTRACT

Imaging techniques allowing non-invasive monitoring of tumour angiogenesis have attracted great interest over the last years. The integrin alpha(v)beta3 is overexpressed during tumour spread and metastasis and therefore is an attractive target for monitoring angiogenetic processes. This review summarizes attempts to develop radiolabelled peptides based on the Arg-Gly-Asp (RGD) sequence and related peptidomimetics with high affinity and selectivity for the alpha(v)beta3 integrin for tumour targeting. Most developments were based on cyclic RGD peptides radiolabelled with ^{18}F, ^{64}Cu, ^{68}Ga for PET, ^{99m}Tc for SPECT or ^{177}Lu for therapeutic applications. To enable fast elimination from non target tissue and rapid excretion of the radiolabelled peptides pharmacokinetic modifiers such as sugar amino acids have been evaluated. Out of these developments [^{18}F]Galacto-RGD has shown high tumour-to-background ratios preclinically and has been evaluated in a number of clinical studies, showing the possibility for non invasive imaging of alpha(v)beta3 in tumour patients. To improve targeting efficiency multimeric constructs were reported revealing improved targeting properties in preclinical models. These developments still have to be transferred into the clinical setting.

2. ANGIOGENESIS AND TUMOUR GROWTH

Angiogenesis is the process that results in the formation of new vessels by sprouting of existing vessels into avascular tissue. Angiogenesis is involved in numerous biological processes, such as embryogenesis, tissue remodelling, female reproductive cycle, and wound healing. However, also numerous disorders are characterized by an imbalance or up-regulation of the angiogenic process. Best known are rheumatoid arthritis (1), psoriasis (2), restenosis (3), diabetic retinopathy (4) and especially tumour growth (5). Thus, the field of angiogenesis research is one of the most rapidly growing biomedical disciplines. The interest in this research is based on the potential aimed at developing anti-angiogenesis drugs as novel therapeutics in particular for treatment of tumours.

Growth and metastasis of solid tumours depend on the formation of new blood vessels which originate from the existing vascular system. These blood vessels grow into the tumour and provide the necessary nutrients and growth factors for tumour progression. Moreover, the newly formed blood vessels allow tumour cells to disseminate and form metastases in distant organs. Normally, vascular homeostasis is regulated by a balance between angiogenic

and antiangiogenic factors (6, 7). Tumour-induced angiogenesis is mainly sustained by the production and secretion of angiogenic factors originating from tumour and stroma cells. It is a multistep process characterized by the chemotactic and mitogenic response of endothelial cells to angiogenic growth factors, proteolytic degradation of extracellular matrix, and modulation of endothelial cell interaction with extracellular matrix proteins mediated by a variety of receptors including the integrins (8-10).

3. TARGETING INTEGRIN EXPRESSION IN TUMOURS

Integrins play a key role during migration of endothelial cells in the basement membrane (11, 12). They are not only involved in endothelial cell adhesion, they are also important regulators of endothelial cell growth, survival and differentiation. One member of this class of receptors is the integrin alpha(v)beta3, which has been shown to play an essential role in the regulation of tumour growth, local invasiveness, and metastatic potential (13, 14). Moreover, alpha(v)beta3 is also highly expressed on activated endothelial cells during angiogenesis (15). Based on several knock-out experiments there is evidence that alpha(v)beta3 and alpha(v)beta5 are rather anti-angiogenic or negative regulators of angiogenesis than pro-angiogenic (16). Inhibition of alpha(v)beta3-mediated cell–matrix interactions has been found to induce apoptosis of activated endothelial cells. However, alpha(v)beta3 antagonists can induce apoptosis not only of endothelial cells but also of alpha(v)beta3-positive tumour cells, resulting in a direct cytotoxic effect on these cells (17). Thus, the use of alpha(v)beta3 antagonists is currently being evaluated as a strategy for anticancer therapy (18). However, the aim of such a treatment is prevention of metastasis and disease stabilization rather than reduction of tumour mass during a relatively short period of therapy ("response rate"). Therefore conventional monitoring schemes do not fit for this kind of therapy and new biomarkers are needed for planning and monitoring of treatments targeting the alpha(v)beta3 integrin. Furthermore such techniques may also supply information about the angiogenic process in an individual patient.

One approach to monitor alpha(v)beta3 expression is based on nuclear medicine tracer techniques, which allow non-invasive determination of radioactive compounds in the nanomolar range. Additionally radiolabelling with therapeutic radionuclides of such biomarkers may allow targeted radionuclide therapy of tumours. This review focuses on radiolabelled RGD derivatives used for targeting tumours.

4. ALPHA(V)BETA3 ANTAGONISTS FOR RADIOLABELLING

Integrins are heterodimeric transmembrane glycoproteins consisting of an alpha- and a beta-subunit. It is found that several extracellular matrix proteins like vitronectin, fibrinogen and fibronectin interact via the amino acid sequence arginine-glycine-aspartic acid (RGD, amino acid single letter code) with the integrins (19). Based on these findings linear as well as cyclic peptides including the RGD-sequence have been introduced (for review see (20)). Kessler and co-workers developed the pentapeptide cyclo(-Arg-Gly-Asp-dPhe-Val-) (21) which showed high affinity and selectivity for alpha(v)beta3. This peptide is the most prominent lead structure for the development of radiotracers for the non-invasive determination of the alpha(v)beta3 expression (22). Based on this pentapeptide a great variety of tracers have been synthesized which are discussed later in detail.

Another peptide used as a lead structure is the disulfide-bridged undecapeptide RGD-4C ((Cys^2-Cys^{10},Cys^4-Cys^8)H-Ala-Cys-Asp-Cys-Arg-Gly-Asp-Cys-Phe-Cys-Gly-OH). RGD-4C binds with high affinity (KD ~ 100 nM) to both, the integrin alpha(v)beta3 and alpha(v)beta5 (23). The derivative ((Cys^1-Cys^9,Cys^3-Cys^7)H-Cys-Asp-Cys-Arg-Gly-Asp-Cys-Phe-Cys-OH) was conjugated with HYNIC and labelled using ^{99m}Tc-technetium (24, 25). However, in murine tumour models only marginal tumour uptake was found, which can be explained by the low association constant of this ^{99m}Tc-labelled RGD-4C derivative for alpha(v)beta3 (7 x 10^6 M^{-1}). Thus, either the deletion of the terminal amino acids, the conjugation with HYNIC, and/or the labelling with ^{99m}Tc-technetium impairs the affinity resulting in a peptide that appears unsuitable for in-vivo imaging of alpha(v)beta3 expression.

NC-100717 (26) is a peptide based on the sequence H-Lys-Cys-Arg-Gly-Asp-Cys-Phe-Cys-OH whereby N^{alpha} of Lys^1 is bridged with Cys^8 via a chloroacetyl moiety and Cys^2-Cys^6 via disulfide formation, the N^{ε} is used for derivatisation for radiolabelling with ^{18}F, ^{99m}Tc (NC100692), or other radiometals. Additionally, the C-terminus is derivatised with a PEG linker as biomodifier. These peptides showed *in vitro* affinity and binding to alpha(v)beta3 and alpha(v)beta5 in the nanomolar range (EA-Hy 926 membrane assay).

Another group has derivatised a peptidomimetic vitronectin receptor antagonist SH066 with the bifunctional chelator 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) to give TA138. TA138 and ^{89}Y-TA138 retained antagonist properties and high affinity for integrin alpha(v)beta3 (IC_{50} = 12 and 18 nM, respectively), and good selectivity versus integrin alpha(IIb)beta3 (IC_{50} > 10,000 nM). ^{111}In-TA138 demonstrated high tumour uptake in a mouse mammary adenocarcinoma model (9.39% ID/g at 2 hours p.i.) and low background activity with rapid blood clearance and renal excretion. Further structure activity studies (27) and dimerisation strategies (28) make this class of compounds an interesting alternative to peptides based on the RGD sequence.

Based on the guanidinobenzoyl hydrazino oxopentanoic acid scaffold (GBHO) (29) another class of radioiodine- and ^{18}F-labelled nonpeptidic alpha(v)beta3 antagonists has been synthesized. Compared with [^{125}I]Gluco-RGD, [^{123}I]GBHO-1 (30) showed an approximately 3-fold lower activity accumulation in alpha(v)beta3-expressing tumours. However, due to the

rapid renal and hepatic elimination of [^{123}I]GBHO-1, tumour-to-background ratios are comparable with those obtained with [^{125}I]Gluco-RGD. Gamma-camera images of mice bearing alpha(v)beta3-positive M21 melanomas and corresponding M21-L control tumours demonstrated receptor-specific binding of [^{123}I]GBHO-1. Even higher tumour-to-background ratios can be found for the 4-[^{18}F]fluorobenzoylated derivative [^{18}F]GBHO-2 (5-[N'-(3-guanidinobenzoyl)-hydrazino]-3-(4-[^{18}F]fluorobenzoyl)-amino-5-oxopentanoic acid (31). Initial *in vivo* studies in mice and rats indicate that [^{18}F]GBHO-2 may be an alternative to the [^{18}F]Galacto-RGD for the *in vivo* imaging of alpha(v)beta3-expression.

Dijkgraaf et al. (32) compared ^{111}In-labelled DOTA-E-c(RGDfK) with a DOTA-peptidomimetic and a DOTA-conjugated peptoid-peptide hybrid (DOTA-E-c(nRGDfK)) and found that the peptide and peptidomimetic had higher alpha(v)beta3 affinities and better tumour-targeting characteristics as compared with the hybrid. Anyway, so far radiolabelling approaches have been mainly focused on cyclic RGD peptides and derivatives thereof partly due to the fact that peptidomimetics often are specifically designed to achieve bioavailability after oral administration, which is no requirement for radiopharmaceutical approaches where intravenous application is preferred.

5. RADIOLABELLING STRATEGIES

5.1. Halogens

For the first evaluation and potential use with single photon emission tomography (SPECT) the radioiodinated RGD peptides 3-[*I]Iodo-DTyr^4-cyclo(-Arg-Gly-Asp-DTyr-Val-) and 3-[*I]Iodo-Tyr^5-cyclo(-Arg-Gly-Asp-DPhe-Tyr-) have been synthesized by direct electrophilic radioiodination of the corresponding tyrosine residues in the peptide sequence (33). Both compounds showed *in vitro* affinity and selectivity for the alpha(v)beta3 integrin comparable with the lead structure, and receptor specific accumulation in the tumour *in vivo*. However, the predominantly hepatobiliary elimination resulted in high activity concentration in liver and intestine.

Fluorine-18 is the most frequently used radionuclide in Positron emission tomography due to its favourable half life of 110 minutes. For ^{18}F-labelling using 4-nitrophenyl-2-[^{18}F]fluoropropionate as prosthetic group a carbohydrate derivatised analogue was developed (34). This prosthetic group allowed synthesis of [^{18}F]Galacto-RGD with a maximum decay-corrected radiochemical yield of about 30% and radiochemical purity >98% with a total synthesis time of 200 min. Initial *in vivo* evaluation was carried out using the human melanoma M21 model. The M21 cell line is well characterized concerning alpha(v)beta3 expression. Another advantage is that from M21 cells, stable variants were selected that lack alpha(v) gene expression and thus, fail to express integrin alpha(v)beta3 (M21-L cells) (35). Using this model [^{18}F]Galacto-RGD uptake in the tumour 120 min p.i. was 1.5% ID/g (36). Blocking experiments injecting 6 mg c(RGDfV) per kg mouse 10 min prior to tracer injection reduced tumour accumulation to approx. 35% of control indicating receptor specific accumulation. This is confirmed by biodistribution experiments as well as imaging studies with a small animal PET scanner using nude mice bearing the M21 or the M21-L tumour. Furthermore, imaging studies with mice bearing melanoma tumours with increasing amounts of alpha(v)beta3 positive cells (produced by mixing M21 and M21-L cells) showed that there is a correlation between integrin expression and tracer accumulation (37). Altogether these data demonstrated that non-invasive determination of alpha(v)beta3 expression and quantification with ^{18}F-labelled RGD-peptides is possible.

However, synthesis of ^{18}F-labeled peptides using activated esters is complex and time consuming sometimes requiring complicated protection strategies, therefore chemoselective ^{18}F-labelling strategies based on oxime formation using 4-[^{18}F]fluorobenzaldehyde have been introduced in recent years (38, 39). This prosthetic group has also been used in combination with HYNIC-modified RGD-peptides (40). The resulting 4'-[^{18}F]-fluorobenzylidenehydrazone-6-nicotinamide-c(RGDyK) showed high affinity for alpha(v)beta3 and accumulates specifically in ischemic hindlimb muscle of mice. Most recently, amino-oxy-functionalisation was also used to conjugate [^{18}F]fluorosilyl-benzaldehyde to a RGD-peptide. However, further evaluation is needed to demonstrate the potential of this new prosthetic group (41).

Prante *et al*. (42) used 3,4,6-tri-O-acetyl-2-deoxy-2-[^{18}F]fluoroglucopyranosylphenylthiosulfonate (Ac_3-[^{18}F]FGlc-PTS) as a thiol-reactive glycosyl donor for ^{18}F-glycosylation of peptides. This approach would allow both introduction of the radiolabel and a pharmacokinetic modifier in one synthesis step. Using for first evaluation cyclo(-Arg-Gly-Asp-dPhe-Cys) it was shown that this labelling strategy allows ^{18}F-labelling of the corresponding peptide in high radiochemical yields. Moreover, the *in vitro* assays demonstrated that the alpha(v)beta3 binding affinity remained unaffected.

Another strategy introduced by Cai *et al*. (43) used N-[2-(4-^{18}F-fluorobenzamido)ethyl]maleimide (^{18}F-FBEM) as a thiol-reactive synthon. With this technique ^{18}F-labeling of a monomeric and dimeric thiolated RGD peptide at high specific activities and high radiochemical yields could be carried out. Moreover, an integrin-specific tumour uptake in subcutaneous U87MG glioma and orthotopic MDA-MB-435 breast cancer xenografts with favourable pharmacokinetics was shown. Radiolabelling strategies and RGD peptides used for this purpose are summarized in Table 1.

5.2. Technetium

Technetium-99m is the radionuclide of choice for SPECT applications due to its wide availability based on generator production and its favourable decay characteristics with a gamma energy of 140 keV and 6 hr half life. A variety of bifunctional chelators are available for radiolabelling of small peptides. Recently, cyclo(-Arg-Gly-Asp-dTyr-Lys-) was derivatised with a variety of

Table 1. Characteristics of radiohalogen labelled RGD peptides

Name	Sequence	Pharmacokinetic modifier / Spacer	Labelling strategy	Radionuclide	Reference
(*I)-RGD	c (RGDyK)	-	Iodogen	^{123}I, ^{125}I	(33)
(*I)-RGD	c (RGDfY)		Iodogen	^{123}I, ^{125}I	(33)
(*I)Gluco-RGD	c (RGDyK)	Glucose-based SAA[1]	Iodogen	^{123}I, ^{125}I	(36)
(^{18}F)Galcto-RGD	c (RGDfK)	Galactose-based SAA[1]	FP[3]	^{18}F	(34)
(^{18}F)dAsp$_3$-RGD	c (RGDfK)	gAbu- (Asp)$_3$ [2]	FP[3]	^{18}F	(63)
^{125}I-RGD-PEG	c (RGDyK)	PEG	-	^{125}I	(66)
(^{18}F)FB-RGD	c (RGDyK)	-	FB[4]	^{18}F	(96)
(^{18}F)PEG-FB-RGD	c (RGDyK)	PEG	FB[4]	^{18}F	(67)
(^{18}F)FGlc-RGD	c (RGDfC)	FDG	Ac$_3$- (^{18}F)FGlc-PTS[5]	^{18}F	(42)
(^{18}F)-FBEM-SRGD	c (RGDyK)	acetylthioacetate (SATA)	^{18}F-FBEM [6]	^{18}F	(43)

[1] SAA: sugar amino acid, [2] gAbu: gamma amino butyric acid, [3] FP: 4-nitrophenyl-2- (18F)fluoropropionate, [4] FB: N-succinimidyl-4- ((18)F)fluorobenzoate, [5] PTS: 1-phenylthiosulfonate[6] ^{18}F-FBEM: N- (2- (4-18F-fluorobenzamido)ethyl)maleimide

ligands including hydrazino nicotinic acid (HYNIC), a pyrazolyl-derivative, an isonitril-conjugate and a Cys-moiety allowing radiolabelling with different Technetium-cores including Tc(CO)$_3$, Tc-nitrido, Tc-hydrazino and the so called Tc-3+1 approach (44). The compounds could be labelled with high specific activities in high radiochemical yields and showed high *in vitro* stability for all Tc-cores studied. However, plasma protein binding and lipophilicity varied considerably between different radiolabelled conjugates, resulting in significant differences concerning pharmacokinetic behaviour as well as tumour uptake (0.2-2.7%ID/g). Highest specific tumour uptake was found for HYNIC-cyclo(-Arg-Gly-Asp-dTyr-Lys-) with ethylenediamnin N,N`diacetic acid (EDDA) as coligand. Other coligands based on tricine showed higher values of protein binding or lower *in vitro* binding affinity (45). Tumour/background ratios of ^{99m}Tc-EDDA/HYNIC-RGD were comparable to [^{18}F]Galacto-RGD indicating that this compound could be a promising candidate for imaging alpha(v)beta3 expression using SPECT. Other groups have used tricine /TPPTS as coligands for radiolabelling HYNIC-derivatised monomers, dimers and tetramers (46, 47).

A promising compound based on a tridentate pyrazolyl derivative using $[Tc(CO)_3(H_2O)_3]^+$ for radiolabelling was recently reported (48). The resulting ^{99m}Tc-tricarbonyl labelled peptide showed high and specific tumour uptake and high *in vivo* stability, the higher lipophilicity compared to HYNIC derivatised peptides resulted in somewhat higher hepatobiliary excretion and liver uptake. Other developments of ^{99m}Tc-tricarbonyl labelling of RGD peptides are based on a 5-carboxylate-2,2'-bipyridine derivatisation (49) or on a His-derivatised peptide resulting in a bidentate chelating system (50, 51). Jung *et al*. (52) have used a glucoseamin derivatised RGD peptide (glucosamino-Asp-cyclic(Arg-Gly-Asp-dPhe-Lys)) functionalized with an aminodiacetic acid for Tc-tricarbonyl labelling. This derivative had a high binding affinity to purified alpha(v)beta3 integrin (IC_{50} = 1.5 nM) and showed rapid blood clearance, with substantially lower liver uptake and higher tumour uptake compared with ^{125}I-c(Arg-Gly-Asp-dTyr-Val) in mice. Therefore it may be an attractive alternative to radiohalogenated RGD peptides for angiogenesis-imaging research. Another ^{99m}Tc-labelling approach was based on the tetrapeptide sequence H-Asp-Lys-Cys-Lys-OH resulting in a triamidomonothiol chelating sequence (53). For [^{99m}Tc]DKCK-RGD gamma-camera images 4 h p.i. showed a clearly contrasting tumour, but also high activity concentration in the kidneys, which may be due to the lysine containing tetrapeptide used as chelating sequence.

NC100692 is a peptide containing a cyclic RGD sequence coupled to an ethylene glycol biomodifier at the C-terminal end of the peptide and a diamine dioxime chelate for ^{99m}Tc-labelling linked to the N-terminal end (54). It binds with high affinity in particular to the alpha(v)beta3 integrin as shown by *in vitro* binding assays and competitive radiolabelled ligand assays. The use of NC100692 in ischemic models shows high uptake in areas of neovascularisation with alpha(v)beta3 integrin overexpression (55), and binding of NC100692 has been confirmed to be localized on endothelial cells in the regions of angiogenesis (56). The approaches towards ^{99m}Tc labelled RGD peptides including labelling moieties and Tc-cores applied are summarized in Table 2.

5.3. Other radiometals

Within recent years also other radiometals have gained increasing interest as radiolabels for small biomolecules such as peptides. These include ^{111}In for SPECT, ^{90}Y and ^{177}Lu for therapeutic applications and ^{68}Cu and ^{68}Ga for PET. Van Hagen *et al*. (57) synthesized a diethylentriamine-pentaacetic acid-(DTPA) conjugated RGD-peptide and demonstrated alpha(v)beta3 selective binding on blood vessels of human tumour tissue sections using receptor autoradiography and immunohistochemistry. DTPA-RGD-peptides have also been synthesized using a solid-phase system (58). The *in vitro* and *in vivo* assays of the ^{111}In-labelled derivatives showed selective binding and accumulation using the alpha(v)beta3 positive Melanoma M21 model. The authors suggest that this approach may allow construction of DTPA-containing peptide libraries for high throughput screening. DTPA, however, is not the ligand of choice for radiolabelling trivalent metals, especially for PET and therapy applications.

A DOTA conjugated RGD-peptide (DOTA-RGDyK) was labelled with ^{64}Cu-copper (59). This tracer showed lower tumour uptake and retention in a murine orthotopic human breast cancer model as compared with the radioiodinated cyclo(-Arg-Gly-Asp-dTyr-Lys-) and unfavourable activity retention in liver and kidneys. The

Table 2. Characteristics of Technetium-99m labelled RGD peptides

Name	Sequence	Labelling moiety	Tc-core	Reference
^{99m}Tc-EDDA/HYNIC-RGD	c (RGDyK)	HYNIC[1]/ EDDA[2]	^{99m}Tc-HYNIC	(45)
^{99m}Tc-Tricine-TPPTS/HYNIC-RGD	c (RGDfK)	HYNIC[1]/ Tricine	^{99m}Tc-HYNIC	(47)
(^{99m}Tc (CO)$_3$)-PZ1-RGD	c (RGDyK)	pyrazolyl	^{99m}Tc (CO)$_3$	(48)
(^{99m}Tc (CO)$_3$)- BPy-RGD	c (RGDyK)	5-carboxylate-2,2'-bipyridine	^{99m}Tc (CO)$_3$	(49)
(^{99m}Tc (CO)$_3$)- His-RGD	C (RGDfK)	His	^{99m}Tc (CO)$_3$	(50)
(^{99m}Tc (CO)$_3$)- Glucosamino-Asp-RGD	C (RGDfK)	aminodiacetic acid	^{99m}Tc (CO)$_3$	(52)
(^{99m}Tc-NS$_3$) L2- RGD	c (RGDyK)	isonitrile	^{99m}Tc-NS$_3$ (3+1)	(44)
(^{99m}Tc-PNP) Cys- RGD	c (RGDyK)	Cys	^{99m}Tc-Nitrido	(44)
^{99m}Tc-DKCK-RGD	c (RGDfK)	H-Asp-Lys-Cys-Lys-OH	^{99m}Tc-oxo	(53)
^{99m}Tc-NC100692	YCRGDCFC	aminoxime	^{99m}Tc-oxo	(26)
^{99m}Tc-RGD-4C	CDCRGDCFC	HYNIC / Tricine	^{99m}Tc-HYNIC	(24)

[1] HYNIC: 6-Hydrazinonicotinic acid, [2] EDDA: Ethylenediamine N,N´diacetic acid

activity concentration in the liver could be due to transfer of ^{64}Cu from DOTA to superoxide dismutase and/or the persistent localization of the final metabolite ^{64}Cu-DOTA-Lys-OH in this tissue. Tumour/blood and tumour/muscle ratios of approximately 7 and 8, respectively, allowed acquisition of clear tumour/background contrast images 1 h p.i. using a small animal scanner. However, highest activity concentration was found in liver, intestine and bladder indicating that further optimization of the tracer is needed.

Another DOTA-derivatised RGD peptide (cyclo(-Arg-Gly-Asp-dPhe-Lys(DOTA)), DOTA-RGDfK) was used by Decristoforo *et al*. for radiolabelling with ^{111}In and ^{68}Ga (60). Both radiolabelled peptides showed specific binding to alpha(v)beta3 integrin with comparable internalisation and tumour uptake values to [^{18}F]Galacto-RGD in an alpha(v)beta3 positive melanoma M21 model. However, protein binding was considerably higher for [^{68}Ga]DOTA-RGDfK resulting in higher background activity *in vivo* and lower tumour/background values as compared to [^{18}F]Galacto-RGD.

Radiolabelling with ^{177}Lu and ^{90}Y was reported for studying the therapeutic potential of radiolabelled RGD peptides. Jansen *et al*. (47) used an ^{90}Y-labelled RGD dimer (DOTA-E-[c(RGDfK)]$_2$) to study the effect of dose fractionation in OVCAR-3 ovarian carcinoma mouse xenografts. However, they found that the therapeutic efficacy of the radiolabelled peptide was not significantly improved by dose fractionation.

Using ^{111}In and ^{177}Lu labelled DOTA-E-c(RGDfK) the same group studied the effect on intraperitoneally (i.p.) growing OVCAR-3 human ovarian carcinomas (61) and found that intraperitoneal growth of the tumour could be significantly delayed by injecting 37 MBq ^{177}Lu-labelled peptide i.p. indicating the therapeutic potential of radiolabelled RGD peptides. Radiometal labelled RGD peptides are summarized in Table 3.

6. OPTIMIZING PHARMAKOKINETICS

Optimal tumour targeting of radiolabelled RGD peptides is achieved with tracers having a rapid uptake in the tumour and at the same time a rapid washout and excretion from non target tissues. In this respect hydrophilic properties are warranted, and a variation in charge may be applied to achieve rapid renal excretion and low retention in organs such as kidney or liver. Therefore pharmacokinetic properties have been varied using chemical modifications ("pharmacokinetic modifiers") whereby different approaches have been reported.

The glycosylation approach was first described by Haubner and co-worker (36, 62) and is based on the introduction of sugar amino acids (sugar derivatives with an amino and a carboxylate function) which allows straight forward integration into peptide chemistry. The sugar amino acids (SAA) were conjugated via the ε-amino function of the corresponding lysine in the pentapeptide sequence. In a murine tumour model the resulting [*I]Gluco-RGD (62, see Figure 1) and [^{18}F]Galacto-RGD (34, 36, 37) showed an initially increased activity concentration in blood, very similar kinetics in kidneys and more important, a clearly reduced activity concentration in liver and an increased activity uptake and retention in the tumour compared to the first generation, unmodified peptides.

Another strategy is to introduce hydrophilic D-amino acids to improve pharmacokinetics of peptide-based tracer (63). Therefore, peptides containing three D-serine or D-aspartic acids and a γ-amino butyric acid for ^{18}F-labelling via prosthetic groups were coupled with the corresponding cyclic RGD-peptide. D-amino acids were used to improve the metabolic stability of the compounds. The peptides showed high alpha(v)beta3 selectivity *in vitro* and receptor specific accumulation *in vivo*. The tumour uptake in a murine melanoma model was lower as found with the glycosylated RGD-peptides. However, due to the rapid predominantly renal elimination of [^{18}F]dAsp^3-RGD tumour/background ratios calculated from small animal PET images were comparable with [^{18}F]Galacto-RGD.

PEGylation, the technology of poly(ethlyene glycol) (PEG) conjugation, is known to improve many properties of peptides and proteins including plasma stability, immunogenicity and pharmacokinetics (64, 65). In many cases it is used to prolong median circulation times and half lives of proteins and polypeptides by shifting the elimination pathway from renal to hepatobiliary excretion. Since renal filtration is dependant on both the molecular mass and the volume occupied, this effect strongly depends on the molecular weight of the PEG moiety. In a first study, Chen *et al*. (66) attached a 2 kDa PEG moiety to the ε-amino function of cyclo(-Arg-Gly-Asp-dTyr-Lys-) and compared the ^{125}I-labelled PEGylated derivative (^{125}I-RGD-PEG) with the radioiodinated cyclo(-Arg-Gly-Asp-

Table 3. Characteristics of radiometal labelled RGD peptides

Name	Sequence	Chelator	Radionuclide	Reference
^{111}In-DTPA-RGD	c (RGDyK)	DTPA[1]	^{111}In	(57)
^{111}In-DTPA-Bz-NH-c (KRGDf)	c (RGDfK)	DTPA[1]	^{111}In	(58)
^{111}In-DTPA-Bz-NH-c (ERGDf)	c (RGDfE)	DTPA[1]	^{111}In	(58)
^{64}Cu-DOTA-RGD	c (RGDyK)	DOTA[2]	^{64}Cu	(69)
^{64}Cu-DOTA-PEG-RGD	c (RGDyK)	DOTA[2]	^{64}Cu	(69)
^{111}In-DOTA-RGD	c (RGDfK)	DOTA[2]	^{111}In	(60)
^{68}Ga-DOTA-RGD	c (RGDfK)	DOTA[2]	^{68}Ga	(60)
^{111}In-DOTA-E-c (RGDfK)	c (RGDfK)	DOTA[2]	^{111}In	(61)
^{177}Lu-DOTA-E-c (RGDfK)	c (RGDfK)	DOTA[2]	^{177}Lu	(61)

[1] DTPA: Diethylendiaminetetraacetic acid , [2] DOTA: ,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid

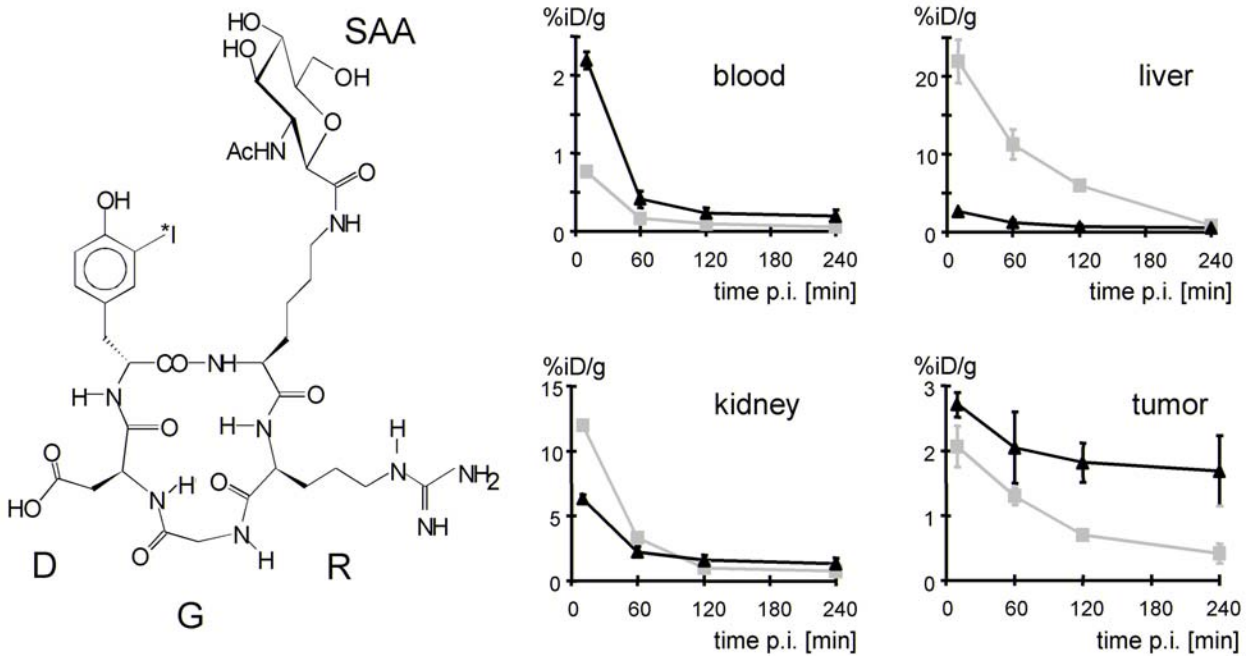

Figure 1. Structure of the glycosylated [*I]Gluco-RGD (left) and comparison of the kinetics of the first generation peptide [*I]c(RGDyV) (grey) and [*I]Gluco-RGD (black) obtained from biodistribution studies (right). The data demonstrate that glycosylation resulted in an initially increased activity concentration in blood, very similar kinetics in kidneys and clearly reduced activity concentration in liver and increased tracer accumulation and retention in the receptor positive tumor.

Tyr-Lys-) (^{125}I-RGD). The PEGylated derivative showed a more rapid blood clearance, a decreased activity concentration in the kidneys and slightly increased activity retention in the tumour. However, tumour uptake for ^{125}I-RGD was higher as found for ^{125}I-RGD-PEG for all time points. Moreover, as explained above, increased activity retention in liver and intestine was found. In another study [^{18}F]FB-RGD, a [^{18}F]fluorobenzoyl labelled RGD peptide, and the PEGylated analogue [^{18}F]FB-PEG-RGD (PEG, MW = 3.4 kDa) have been compared (67). Again activity retention of the PEGylated peptide in the tumour was improved compared with the lead structure. However, initial elimination from blood was slower and activity concentration in liver and kidneys was higher as for [^{18}F]FB-RGD, which already is considered to be rather lipophilic. Anyway, [^{18}F]FB-RGD was used to image brain tumour growth in a murine tumour model (68). In this model longitudinal microPET imaging allowed visualization and quantification of anatomical variations during brain tumour growth and angiogenesis. In addition, the effect of PEGylation was studied by comparing ^{64}Cu-DOTA-RGD and ^{64}Cu-DOTA-PEG-RGD (PEG, MW = 3.4 kDa) (69). As discussed above ^{64}Cu-DOTA-RGD showed significant liver uptake. Thus, in this case, PEGylation reduced activity concentration in liver and small intestine and resulted in a faster blood clearance, while the tumour uptake as well as retention was not affected.

Dijkgraaf *et al*. (70) studied the influence of linker variation on the targeting properties of an ^{111}In-labelled DOTA-derivatised RGD-dimer. Linker variation did not affect affinity for alpha(v)beta3 as well as tumour uptake. Insertion of lysine or of glutamic acid resulted in enhanced retention in the kidneys. PEG4-insertion appeared to be the most suitable linker as compared with glutamic acid and lysine because it has the highest tumour/blood ratio and the lowest uptake in the kidney and liver.

Altogether, these studies revealed very different effects of PEGylation on the pharmacokinetics and tumour uptake of RGD-peptides which seems to strongly depend

on the nature of the lead structure. However pharmacokinetic modifyers may be necessary in general to adjust especially lipophilicity and charge towards the desired pharmacokinetic properties. In some cases, however, radiolabelling strategies themselves can serve to modulate pharmacokinetics. Especially HYNIC-derivatisation for radiolabelling with ^{99m}Tc using hydrophilic coligands (45) or DOTA-conjugation (60) for other radiometals have shown to serve this purpose.

7. THE "MULTIMERISATION" APPROACH

Besides monomeric RGD-peptides also multimeric compounds presenting more than one RGD-site have been introduced. This "multimerisation" approach may result in an improved target affinity and prolonged target retention mainly due to an increased apparent ligand concentration and/or, especially by lager molecules, due to strong cooperative binding. Janssen *et al*. (47, 71) synthesized a dimeric RGD-peptide by coupling two cyclo(-RGDfK) via a glutamic acid linker. For radiolabelling DOTA or HYNIC were conjugated to the free amino function of the linker moiety. The dimeric ^{99m}Tc-HYNIC-E-[c(RGDfK)]$_2$ revealed a 10-fold higher affinity for the alpha(v)beta3 integrin as the monomeric ^{99m}Tc-HYNIC-c(RGDfK). Moreover, also activity retention in the tumour was improved compared with the monomeric compound. However, activity retention was also high in kidneys.

A more systematic study on the influence of multimerisation on receptor affinity and tumour uptake was carried out by the groups of Wester and Kessler (38, 39, 72). They synthesized a series of monomeric, dimeric, tetrameric and octameric RGD-peptides. These compounds contain different numbers of c(RGDfE) peptides which are connected via PEG linker and lysine moieties, which are used as branching units. Labelling was based on a chemoselective oxime formation between an aminooxo function at the peptide site and a ^{18}F-labelled aldehyde. They found an increasing binding affinity in the series monomer, dimer, tetramer and octamers in an *in vitro* binding assay (Figure 2). Initial PET images resulting from a clinical PET scanner confirmed these findings. The image of mice with both a receptor positive and a receptor negative melanoma showed an increasing activity accumulation only in the receptor positive tumour in the series monomer, dimer and tetramer. Moreover, PET studies comparing a tetrameric structure containing four c(RGDfE) peptides with a tetrameric compound containing only one c(RGDfE) and three c(RaDFE) peptides (which do not bind to the alpha(v)beta3 integrin) showed for the pseudo monomeric tetramer a 3-fold lower activity accumulation in the tumour as for the "real" tetramer, indicating that the higher uptake in the tumour is due to multimerisation and not based on other structural effects.

In another study, a dimeric cyclic RGD peptide E[c(RGDyK)]$_2$ was labelled by conjugating a 4-[^{18}F]fluorobenzoyl moiety to the amino function of the glutamate (73, 74). The dimeric RGD peptide demonstrated significantly higher tumour uptake and prolonged tumour retention in comparison with a monomeric RGD peptide analogue [^{18}F]FB-c(RGDyK). Moreover, the dimeric RGD peptide had predominant renal excretion, whereas the monomeric analogue was excreted primarily via the biliary route. Thus, the authors conclude that the synergistic effect of polyvalence and improved pharmacokinetics may be responsible for the superior imaging characteristics of [^{18}F]FB-E[c(RGDyK)]$_2$. To improve labelling yields in a further study a mini-PEG spacer (11-amino-3,6,7-trioxaundecanoic acid) was introduced (75). This resulted in an improved amino function reactivity and thus, in an increased overall radiolabelling yield. Moreover, [^{18}F]FB-mini-PEG-E[c(RGDyK)]$_2$ showed reduced renal and comparable tumour uptake as found for [^{18}F]FB-E[c(RGDyK)]$_2$. The same approach was used to synthesize a tetrameric RGD-peptide (76). In this case the tetrameric [^{18}F]FB-mini-PEG-E{E[c(RGDyK)]$_2$}$_2$ showed no increased *in vitro* binding affinity as compared to the monomer and dimer. However, dynamic animalPET studies demonstrated an increased tumour uptake up to 120 min p.i. compared with [^{18}F]FB-mini-PEG-E[c(RGDyK)]$_2$. Unfortunately, similar increase in tracer concentration is also found in kidneys, liver and muscle resulting in comparable tumour/background ratios for both compounds.

Similar effects as for the ^{18}F-labelled dimer [^{18}F]FB-E[c(RGDyK)]$_2$ have been found for dimeric ^{64}Cu-labelled analogues (77). In contrast to [^{18}F]FB-mini-PEG-E{E[c(RGDyK)]$_2$}$_2$, the comparison with the tetrameric [^{64}Cu]DOTA-E[E-c(RGDyK)$_2$]$_2$ (78) showed significantly higher integrin binding affinity for the tetramer than the corresponding monomeric and dimeric RGD analogues, most likely due to a polyvalence effect. Again tumour uptake was rapid and high, and the tumour washout was slow. The positive effect of "multimerisation" on tumour uptake is further confirmed by introduction of a ^{64}Cu-labelled octrameric RGD-peptide (79) as well as ^{111}In-labelled monomeric, dimeric, and tetrameric analogues (80). However, also in this case uptake in different organs including kidneys and muscle was increased indicating that a favourable balance between binding epitope density and tracer size is important for the design of the optimal tracer.

As described the scaffolds of the multimers are based on glutamate or lysine linker moieties. However, most recent approaches uses a regioselectivity addressable functionalised template (RAFT) (81) or dendrimers (82) as scaffold for the synthesis of multimeric RGD-peptides. For the [^{99m}Tc]RAFT-RGD four cyclic RGD sequences are tethered on a cyclodecapeptide platform and labelled with ^{99m}T using the tricarbonyl strategy. The *in vivo* biodistribution studies using two different murine tumour models showed that the tumour uptake of the tetramere is significantly higher as the tumour uptake of the corresponding monomer. The other approach uses the 1,3-dipolar cycloaddition for conjugating the cyclic RGD-peptides to the branching unit which are coupled with DOTA for ^{111}In-labelling. Monomeric, dimeric and tetrameric peptides have been synthesized. *In vitro* binding studies showed for the tetrameric compound higher alpha(v)beta3 affinity compared with the monomer and dimer. Moreover, tracer uptake in the tumour increases in

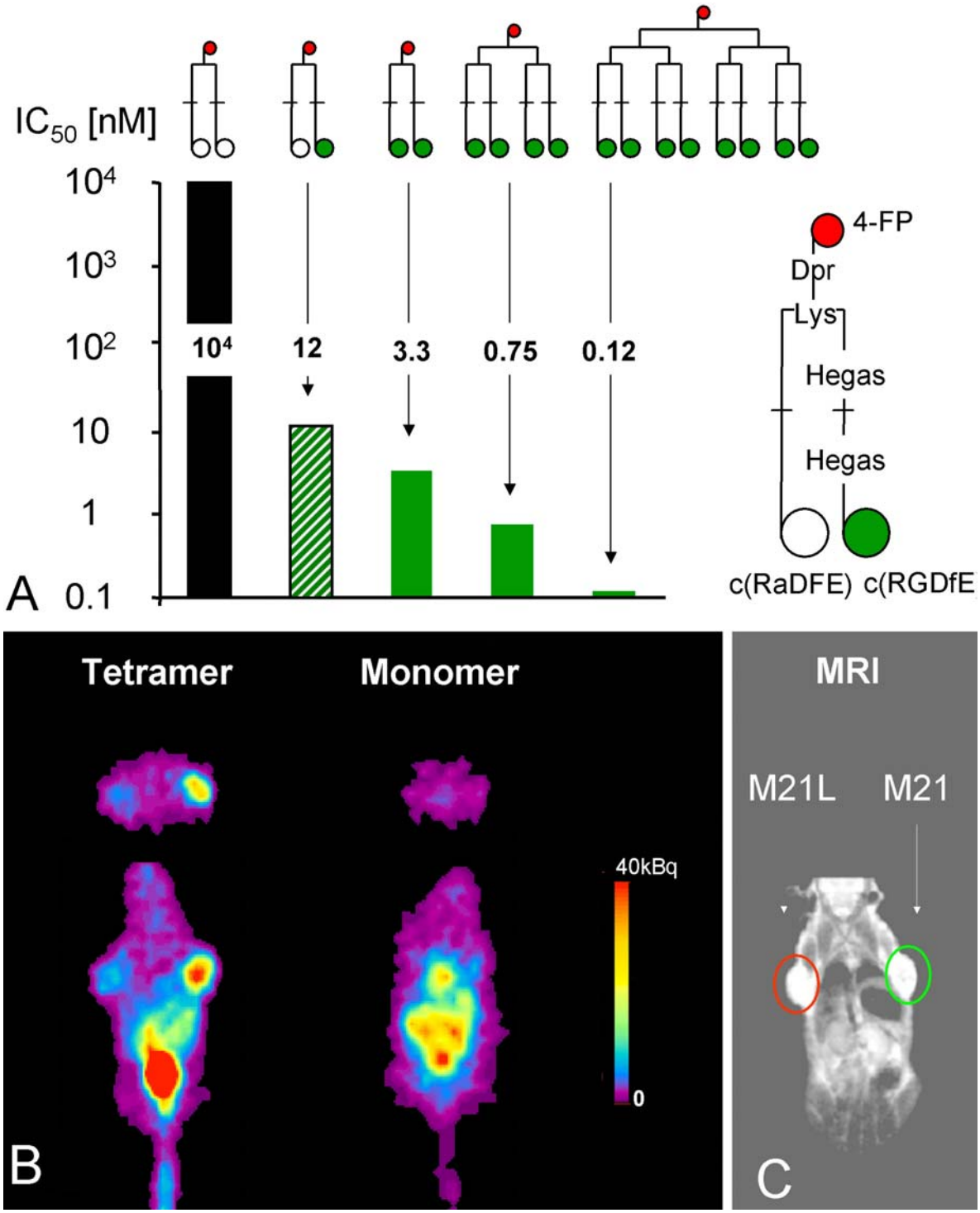

Figure 2. In vitro binding studies of different multimeric RGD-peptides using immoblised integrins alpha(v)beta3 and vitronectin as endogenous ligand (A) showed an increasing affinity for the alpha(v)beta3 integrin with increasing numbers of binding epitopes. The multimers contain one to eight RGD-peptides (green circle) linked via an ethylenglycol linker and lysine moieties. For labelling via ^{18}F-fluorobenzaldehyde multimers were functionalised using an oxoamino group. For some derivatives also a negative control peptide c(RADfE) was introduced. For imaging a nude mouse model bearing the alpha(v)beta3 positive melanoma M21 as well as the receptor negative M21-L tumor was used. The images obtained from a clinical scanner (B) demonstrate the improved targeting properties of the tetramer compared with the monomeric peptide, (C) shows the corresponding MRI images (unpublished data from Wester and co-worker).

Table 4. Characteristics of multimeric RGD peptides

monomer sequence	number[1]	linker / branching unit	spacer	chelator / prosthetic group	isotope	Reference
c (RGDfK)	2	Glu	-	HYNIC	^{99m}Tc	(47; 71)
c (RGDfK)	2	Glu	-	DOTA	^{111}In / ^{90}Y	(71)
c (RGDfE)	2 / 4	Lys	HEGAS[2]	FBA[3]	^{18}F	(39; 40; 72)
c (RGDyK)	2	Glu	-	SFB[4]	^{18}F	(73; 74)
c (RGDyK)	2 / 4	Glu	-	DOTA	^{64}Cu	(77; 78))
c (RGDfK)	2 / 4	Glu	-	DOTA	^{111}In	(80)
c (RGDfK)	2	Glu / Lys / PEG	-	DOTA	^{111}In	(70)
c (RGDfK)	4	dendrimer	-	DOTA	^{111}In	(82)
c (RGDyK)	2 / 4	Glu	PEG[5]	SFB	^{18}F	(75; 76)
c (RGDyK)	4 / 8	Glu	-	DOTA	^{64}Cu	(79)
c (RGDfK)	4	RAFT[6]	-	His	^{99m}Tc (CO)$_3$	(81)

[1] number of monomeric units used in the multimer, [2] 20-amino-3,6,9,12,15,18-hexaoxaeicosanoic acid, [3] 4-(^{18}F)fluorobenzaldehyde, [4] succinimidyl-4- (^{18}F)fluorobenzoate, [5] polyethylenglycol, [6] regioselectivity addressable functionalised template

the series monomer, dimer and tetramer. However, increasing activity concentration is also found in a variety of organs including kidneys, liver and intestine.

Altogether, in many cases the "multimerisation" approach lead to increased binding affinity and tumour uptake as well as retention of the tracer and can, by using appropriate linker moieties and molecular size, improve the pharmacokinetics of peptide based tracer. An overview of multmerisation approaches is shown in Table 4.

8. HETEROMULTIMERS

It is obvious that tumours over-express a variety of different receptors. By designing compounds which combine the binding epitopes of these receptors a new class of tracers for multireceptor tumour targeting may become available. Such heteromultimeric systems may enhance tracer accumulation by taking advantage of the heterogeneity of receptor expression on target cells (targeting of the cumulative receptor density). E.g. Reubi and co-worker have studied over-expression of peptide receptors on breast cancer (83) and neuroendocrine tumours (84) and postulated that by combination of binding epitopes for the gastrin-releasing peptide receptor and the neuropeptide Y receptor approx. 93% of breast carcinoma and their lymph node metastases should be detectable. Furthermore, heterodimers offer the possibility to addressing successive biochemical processes within the cell, e.g. using the first bioactive structure as a vehicle and addressing unit and the second one as effector. Some of these strategies are currently evaluated using RGD-sequences as ligands.

De Jong *et al*. (85) conjugated cyclo(-Arg-Gly-Asp-dPhe-Lys-) via a DTPA moiety with octreotate and labelled it with ^{111}In. This heterodimer showed comparable high affinity and selectivity for the somatostatin receptor subtype 2 (sst2) and the integrin alpha(v)beta3 as the monomers. However, tracer accumulation in the tumour was mainly determined by binding of the compound to sst2. Although the authors explained this by a higher affinity of octreotate for sst2 than of the RGD-peptide for alpha(v)beta3, a higher sst2 density and fast internalization of sst2 could also significantly contribute to this finding. Anyway, based on these data a new approach was studied where the octreotate part of the radiolabelled heterodimeric peptide was used as a selective shuttle transporting the RGD-peptide, which is known not only to bind to integrins but also to induce apoptosis via caspase-3 activation (86), into the tumour cell. This combination approach is expected to increase the therapeutic efficacy of somatostatin-based receptor-targeted radionuclide therapy (87). Initial *in vitro* studies showed an increased caspase-3 activation and cell death after incubation with the hybrid peptide (88). However, in further studies high renal uptake of RGD-^{111}In-DTPA-octreotate was found which let the authors conclude that this compound is not suitable for radionuclide therapy (89).

9. CLINICAL EVALUATION

The promising preclinical data of [^{18}F]Galacto-RGD were the basis for initial clinical evaluation of this tracer in a small number of tumour patients. Nine patients with malignant melanoma, sarcoma, osseous metastasis from renal cell carcinoma and a villonodular synovitis were imaged with PET (37). In these tumours a very variable uptake was observed. Standard uptake values (SUVs) ranged from 1.2 to 10.0. While for one melanoma patient multiple lesions were detected by a [^{18}F]FDG scan, indicating viable tumour cells, no [^{18}F]Galacto-RGD uptake was found. For other patients similar uptake patterns were observed for both tracers. These high inter- as well as intraindividual variances in tracer accumulation in the different lesions indicate great diversity in alpha(v)beta3 expression and demonstrate the importance of non-invasive tools for planning and controlling of corresponding alpha(v)beta3-targeted antiangiogenic therapies.

Further biodistriubtion (90) and dosimetry studies (91) confirmed the favourable pharmacokinetics of [^{18}F]Galacto-RGD with rapid renal excretion and elimination from the blood pool. Background activity in lung and muscle tissue was low and the calculated effective dose found was approx. 19 µSv/MBq which is very similar to a [^{18}F]FDG scan. The metabolic stability analysed from blood samples was high up to 120 min after tracer injection. Tumour kinetics were consistent with a two-tissue compartment model with reversible specific binding. Distribution volume values were in average four times higher for tumour tissue than for muscle tissue. The data suggest that there was only minimal free and bound (specific or unspecific) tracer in muscle tissue. Thus,

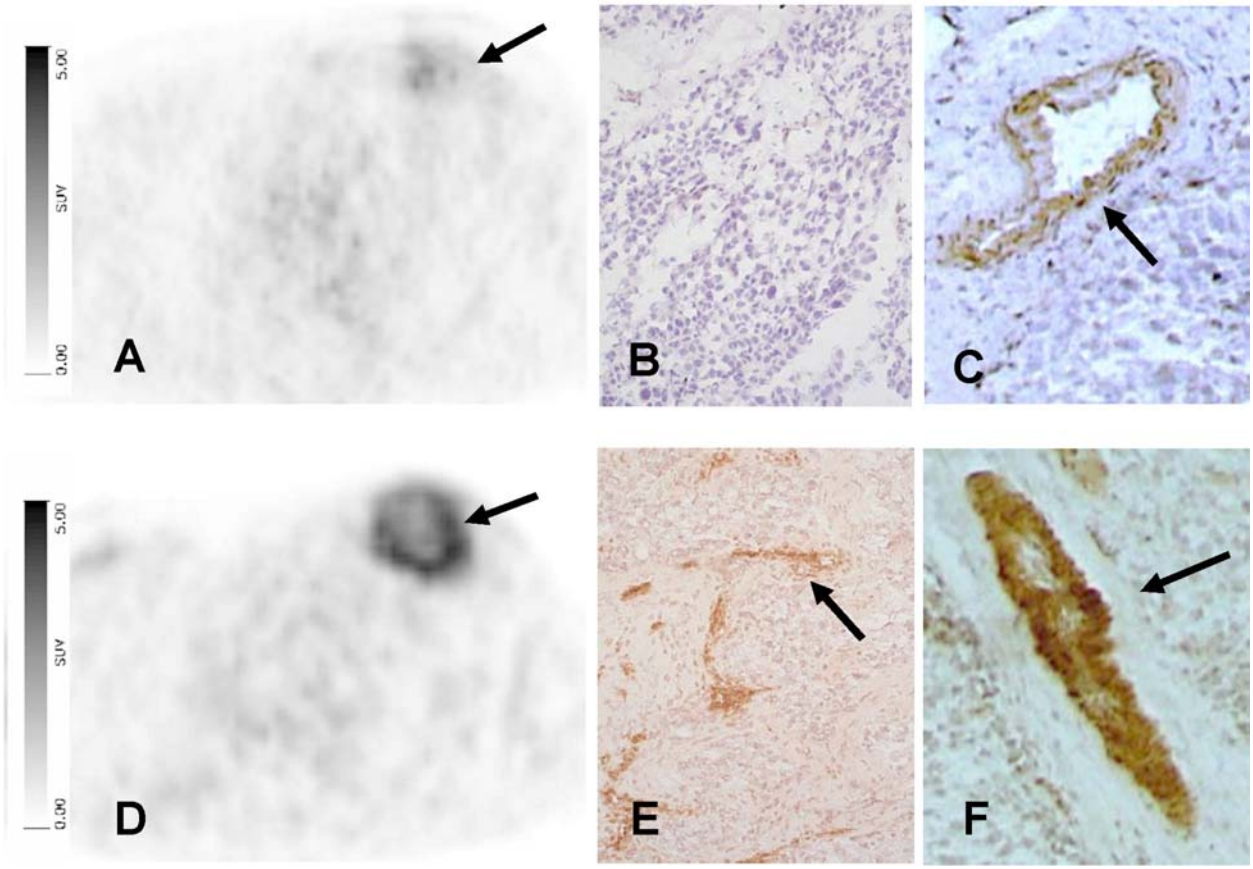

Figure 3. Comparison of [^{18}F]Galacto-RGD PET (A, D) and immunohistochemistry of alpha(v)beta3 expression (B, C, E, F). In the upper row a patient with a large invasive ductal carcinoma of the left breast and low [^{18}F]Galacto-RGD uptake (A, arrow). The corresponding immunohistochemistry shows negative staining of most parts of the tumour (B low magnification) and only faint positive staining of single vessels (C high magnification, arrow). In the lower row a patient with an invasive ductal carcinoma of the left breast and intense [^{18}F]Galacto-RGD uptake (D, arrow). The corresponding immunohistochemistry (E low magnification, F high magnification) shows intense staining of tumour vessels (arrow). These data demonstrate that of [^{18}F]Galacto-RGD and PET allows non-invasive monitoring of alpha(v)beta3 integrin expression and indicate the benefits for corresponding therapy planning and controlling (with kind permission from ref. 94).

[^{18}F]Galacto-RGD demonstrates a highly favourable biodistribution in humans with specific receptor binding.

In a recent study 19 patients with solid tumours (musculoskeletal system n=10, melanoma n=4, head and neck cancer n=2, glioblastoma n=2, breast cancer n=1) were examined with PET using [^{18}F]Galacto-RGD before surgical removal of the lesions (92). Tumour tissue sections from representative areas with low as well as intense SUVs were collected and imunohistochemistry was performed. Intensity of staining and the microvessel density of alpha(v)beta3-positive vessels were determined and correlated with SUV and tumour/blood-ratios. For 17 of the 19 lesions varying tracer accumulation with SUVs ranging from 1.2–10.0 were found. But more important, the correlation of SUV and tumour/blood-ratios with the intensity of immunohistochemical staining as well as with the microvessel density were significant. Moreover, immunohistochemistry confirmed lack of alpha(v)beta3 expression in normal tissue and in the two tumours without tracer uptake.

Two recent studies investigated the feasibility of using [^{18}F]Galacto-RGD in specific patient groups. In eleven patients with squamous Cell Carcinoma (93) 10 out of 12 tumours were identified, which confirmed alpha(v)beta3 expression mainly on tumour vessels by ex vivo Immunhistochemistry. Comparable results were obtained in 19 patients with breast cancer with predominant expression of alpha(v)beta3 integrin on microvessels (94, two examples of images and corresponding immunhistochemistry are shown in Figure 3). In an additional study Beer *et al* compared [^{18}F]Galacto-RGD and [^{18}F]FDG uptake in 18 patients with different primary or metastatic cancers, whereby no correlation between glucose metabolism ([^{18}F]FDG uptake) and alpha(v)beta3 expression could be found (95).

These studies demonstrate that molecular imaging of alpha(v)beta3 expression with [^{18}F]Galacto-RGD in humans correlates with alpha(v)beta3 expression as determined by immunohistochemistry. The variable tracer uptake which is in correlation with variable

alpha(v)beta3 expression shows the value of non-invasive techniques for appropriate selection of patients entering clinical trials of alpha(v)beta3-targeting therapies. PET with [^{18}F]Galacto-RGD might therefore be used as a new marker of activated endothelial and tumour cells and for individualized planning of therapeutic strategies with alpha(v)beta3-targeted drugs.

The second RGD based tracer that has been evaluated in humans is ^{99m}Tc-NC100692. In a proof of concept study 16 patients with equiviocal mammographic findings and 4 patients with benign lesions were included. Nineteen of 22 histopathological confirmed malignant lesions were detected using ^{99m}Tc-NC100692 scintigraphy. The use of ^{99m}Tc-NC100692 in subjects with breast cancer was well tolerated and indicates the potential of this tracer for tumour imaging, however immunhistoligical correlation with alpha(v)beta3 expression was not reported (96).

10. PERSPECTIVE

In conclusion, great efforts have been made to develop radiolabelled RGD peptides for the non-invasive determination of the alpha(v)beta3 expression for monitoring angiogenetic processes. The clinical data available so far show that by using [^{18}F]Galacto-RGD alpha(v)beta3 expression in tumours can be monitored and further clinical studies will have to provide data on the potential of this imaging technique in various clinical situations. Recent approaches towards multimers and alternative radiolabelling strategies show great potential *in vitro* and in preclinical models with significant improvement of targeting and even the potential of therapeutic application. Whether this can be translated into a clinical setting still has to be shown.

11. ACKNOWLEDGEMENT

Hans-Jürgen Wester, is kindly acknowledged for supplying some of his groups unpublished data.

12. REFERENCES

1. Storgard C M, D. G. Stupack, A. Jonczyk, S. L. Goodman, R. I. Fox, D. A. Cheresh: Decreased angiogenesis and arthritic disease in rabbits treated with an alphavbeta3 antagonist. *J Clin Invest* 103, 47-54 (1999)

2. Creamer D, D. Sullivan, R. Bicknell, J. Barker: Angiogenesis in psoriasis. *Angiogenesis* 5, 231-236 (2002)

3. Bishop G G, J. A. McPherson, J. M. Sanders, S. E. Hesselbacher, M. J. Feldman, C. A. McNamara, L. W. Gimple, E. R. Powers, S. A. Mousa, I. J. Sarembock: Selective alpha(v)beta(3)-receptor blockade reduces macrophage infiltration and restenosis after balloon angioplasty in the atherosclerotic rabbit. *Circulation* 103, 1906-1911. (2001)

4. Chavakis E, B. Riecke, J. Lin, T. Linn, R. G. Bretzel, K. T. Preissner, M. Brownlee, H. P. Hammes: Kinetics of integrin expression in the mouse model of proliferative retinopathy and success of secondary intervention with cyclic RGD peptides. *Diabetologia* 45, 262-267 (2002)

5. Folkman J: Role of angiogenesis in tumor growth and metastasis. *Semin Oncol* 29, 15-18 (2002)

6. Ellis L M, W. Liu, F. Fan, Y. D. Jung, N. Reinmuth, O. Stoeltzing, A. Takeda, M. Akagi, A. A. Parikh, S. Ahmad: Synopsis of angiogenesis inhibitors in oncology. *Oncology* 16, 14-22 (2002)

7. Kuwano M, J. Fukushi, M. Okamoto, A. Nishie, H. Goto, T. Ishibashi, M. Ono: Angiogenesis factors. *Intern Med* 40, 565-572 (2001)

8. Eliceiri B P, D. A. Cheresh: Adhesion events in angiogenesis. *Curr Opin Cell Biol* 13, 563-568 (2001)

9. Chang C, Z. Werb: The many faces of metalloproteases: cell growth, invasion, angiogenesis and metastasis. *Trends Cell Biol* 11, S37-43 (2001)

10. Jin H, J. Varner: Integrins: roles in cancer development and as treatment targets. *Br J Cancer* 90, 561-565 (2004)

11. Eliceiri B P, D. A. Cheresh: Role of alpha v integrins during angiogenesis. *Cancer J Sci Am* 6, S245-249 (2000)

12. Hynes R O, B. L. Bader, K. Hodivala-Dilke: Integrins in vascular development. *Braz J Med Biol Res* 32, 501-510 (1999)

13. Hood JD, D. A. Cheresh: Role of integrins in cell invasion and migration. *Nat Rev Cancer* 2, 91-100 (2002)

14. Ruoslahti E: Specialization of tumour vasculature. *Nat Rev Cancer* 2, 83-90 (2002)

15. Brooks P C, A. M. Montgomery, M. Rosenfeld, R. A. Reisfeld, T. Hu, G. Klier, D. A. Cheresh: Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell* 79, 1157-1164 (1994)

16. Hynes R O: A reevaluation of integrins as regulators of angiogenesis. *Nat Med* 8, 918-921 (2002)

17. Taga T, A. Suzuki, I. Gonzalez-Gomez, F. H. Gilles, M. Stins, H. Shimada, L. Barsky, K. I. Weinberg, W. E. Laug: alpha v-Integrin antagonist EMD 121974 induces apoptosis in brain tumor cells growing on vitronectin and tenascin. *Int J Cancer* 98, 690-697 (2002)

18. Dredge K, A. G. Dalgleish and J. B. Marriott: Recent developments in antiangiogenic therapy. *Expert Opin Biol Ther* 2, 953-966 (2002)

19. Ruoslahti E, M. D. Pierschbacher: New perspectives in cell adhesion: RGD and integrins. *Science* 238, 491-497 (1987)

20. Haubner R, D. Finsinger, H. Kessler: Stereoisomeric Peptide Libraries and Peptidomimetics for Designing Selective Inhibitors of the alpha(v)beta3 Integrin for a New

Cancer Therapy. *Angew Chem Int Ed Engl* 36, 1374-1389 (1997)

21. Aumailley M, M. Gurrath, G. Muller, J. Calvete, R. Timpl, H. Kessler: Arg-Gly-Asp constrained within cyclic pentapeptides. Strong and selective inhibitors of cell adhesion to vitronectin and laminin fragment P1. *FEBS Lett* 291, 50-54
(1991)

22. Haubner R H, H. J. Wester, W. A. Weber, M. Schwaiger: Radiotracer-based strategies to image angiogenesis. *Q J Nucl Med* 47, 189-199 (2003)

23. Assa-Munt N, X. Jia, P. Laakkonen, E. Ruoslahti: Solution structures and integrin binding activities of an RGD peptide with two isomers. *Biochemistry* 40, 2373-2378 (2001)

24. Su Z F, G. Liu, S. Gupta, Z. Zhu, M. Rusckowski, D. J. Hnatowich: In vitro and in vivo evaluation of a Technetium-99m-labeled cyclic RGD peptide as a specific marker of alpha(V)beta(3) integrin for tumor imaging. *Bioconjug Chem* 13, 561-570 (2002)

25. Su Z F, J. He, M. Rusckowski, D. J. Hnatowich: In vitro cell studies of technetium-99m labeled RGD-HYNIC peptide, a comparison of tricine and EDDA as co-ligands. *Nucl Med Biol* 30, 141-149 (2003)

26. Indrevoll B, G. M. Kindberg, M. Solbakken, E. Bjurgert, J. H. Johansen, H. Karlsen, M. Mendizabal, A. Cuthbertson: NC-100717: a versatile RGD peptide scaffold for angiogenesis imaging. *Bioorg Med Chem Lett* 16, 6190-6193 (2006)

27. Harris T D, S. Kalogeropoulos, T. Nguyen, G. Dwyer, D. S. Edwards, S. Liu, J. Bartis, C. Ellars, D. Onthank, P. Yalamanchili, S. Heminway, S. Robinson, J. Lazewatsky, J. Barrett: Structure-activity relationships of 111In- and 99mTc-labeled quinolin-4-one peptidomimetics as ligands for the vitronectin receptor: potential tumor imaging agents. *Bioconjug Chem* 17, 1294-1313 (2006)

28. Harris T D, E. Cheesman, A. R. Harris, R. Sachleben, D. S. Edwards, S. Liu, J. Bartis, C. Ellars, D. Onthank, P. Yalamanchili, S. Heminway, P. Silva, S. Robinson, J. Lazewatsky, M. Rajopadhye, J. Barrett: Radiolabeled divalent peptidomimetic vitronectin receptor antagonists as potential tumor radiotherapeutic and imaging agents. *Bioconjug Chem* 18, 1266-1279 (2007)

29. Sulyok G A, C. Gibson, S. L. Goodman, G. Holzemann, M. Wiesner, H. Kessler: Solid-phase synthesis of a nonpeptide RGD mimetic library: new selective alphavbeta3 integrin antagonists. *J Med Chem* 44, 1938-1950 (2001)

30. Haubner R, G. Sulyok, W. Weber, W. Linke, C. Bodenstein, H. J. Wester, H. Kessler, M. Schwaiger: Synthesis and first evaluation of [I-123]Aza-RGD-1: a radiolabeled RGD-Mimetic for the noninvasive determination of alpha(v)beta3 integrin expression. *Eur J Nucl Med* 28, 1175 (2001)

31. Haubner R, G. Thumshirn, W. A. Weber, M. Herz, H. J. Wester, H. Kessler, M. Schwaiger: [F-18]GBHO-2: a RGD-mimetic for the non-invasive determination of the alpha(v)beta3 expression (german). *Nuclear Medicine* 42, 45P (2003)

32. Dijkgraaf I, J. A. Kruijtzer, C. Frielink, A. C. Soede, H. W. Hilbers, W. J. Oyen, F. H. Corstens, R. M. Liskamp, O. C. Boerman: Synthesis and biological evaluation of potent alphavbeta3-integrin receptor antagonists. *Nucl Med Biol* 33, 953-961 (2006)

33. Haubner R, H. J. Wester, U. Reuning, R. Senekowitsch-Schmidtke, B. Diefenbach, H. Kessler, G. Stocklin, M. Schwaiger: Radiolabeled alpha(v)beta3 integrin antagonists: a new class of tracers for tumor targeting. *J Nucl Med* 40, 1061-1071 (1999)

34. Haubner R, B. Kuhnast, C. Mang, W. A. Weber, H. Kessler, H. J. Wester, M. Schwaiger: [18F]Galacto-RGD: synthesis, radiolabeling, metabolic stability, and radiation dose estimates. *Bioconjug Chem* 15, 61-69 (2004)

35. Felding-Habermann B, B. M. Mueller, C. A. Romerdahl, D. A. Cheresh: Involvement of integrin alpha V gene expression in human melanoma tumorigenicity. *J Clin Invest* 89, 2018-2022 (1992)

36. Haubner R, H. J. Wester, W. A. Weber, C. Mang, S. I. Ziegler, S. L. Goodman, R. Senekowitsch-Schmidtke, H. Kessler, M. Schwaiger: Noninvasive imaging of alpha(v)beta3 integrin expression using 18F-labeled RGD-containing glycopeptide and positron emission tomography. *Cancer Res* 61, 1781-1785 (2001)

37. Haubner R, W. A. Weber, A. J. Beer, E. Vabuliene, D. Reim, M. Sarbia, K. F. Becker, M. Goebel, R. Hein, H. J. Wester, H. Kessler, M. Schwaiger: Noninvasive visualization of the activated alphavbeta3 integrin in cancer patients by positron emission tomography and [18F]Galacto-RGD. *PLoS Med* 2, e70 (2005)

38. Poethko T, M. Schottelius, G. Thumshirn, M. Herz, R. Haubner, G. Henriksen, H. Kessler, M. Schwaiger, H. J. Wester: Chemoselective pre-conjugate radiohalogenation of unprotected mono- and multimeric peptides via oxime formation. *Radiochimica Acta* 92, 317-327 (2004)

39. Poethko T, M. Schottelius, G. Thumshirn, U. Hersel, M. Herz, G. Henriksen, H. Kessler, M. Schwaiger, H. J. Wester: Two-step methodology for high-yield routine radiohalogenation of peptides: 18F-labeled RGD and octreotide analogs. *J Nucl Med* 45, 892-902 (2004)

40. Lee Y S, J. M. Jeong, H. W. Kim, Y. S. Chang, Y. J. Kim, M. K. Hong, G. B. Rai, D. Y. Chi, W. J. Kang, J. H. Kang, D. S. Lee, J. K. Chung, M. C. Lee, Y. G. Suh: An improved method of 18F peptide labeling: hydrazone formation with HYNIC-conjugated c(RGDyK). *Nucl Med Biol* 33, 677-683 (2006)

41. Schirrmacher E, B. Wängler, M. Cypryk, G. Bradtmöller, M. Schäfer, M. Eisenhut, K. Jurkschat, R. Schirrmacher. Synthesis of p-(Di-tert-butyl[18F]Fluorosilyl)benzaldehyde ([18F]SiFA-A) with high specific activity by isotopic exchange: a convienient labeling synthon for the 18F-labeling of N-amino-oxy derivatized peptides. *Bioconjug Chem* 18, 2085-2089 (2007)

42. Prante O, J. Einsiedel, R. Haubner, P. Gmeiner, H. J. Wester, T. Kuwert, S. Maschauer: 3,4,6-Tri-O-acetyl-2-deoxy-2-[18F]fluoroglucopyranosyl phenylthiosulfonate: a thiol-reactive agent for the chemoselective 18F-glycosylation of peptides. *Bioconjug Chem* 18, 254-262 (2007)

43. Cai W, X. Zhang, Y. Wu, X. Chen: A thiol-reactive 18F-labeling agent, N-[2-(4-18F-fluorobenzamido)ethyl]maleimide, and synthesis of RGD peptide-based tracer for PET imaging of alpha v beta 3 integrin expression. *J Nucl Med* 47, 1172-1180 (2006)

44. Decristoforo C, I. Santos, H. J. Pietzsch, J. U. Kuenstler, A. Duatti, C. J. Smith, A. Rey, R. Alberto, E. Von Guggenberg, R. Haubner: Comparison of in vitro and in vivo properties of [99mTc]cRGD peptides labeled using different novel Tc-cores. *Q J Nucl Med Mol Imaging* 51, 33-41 (2007)

45. Decristoforo C, B. Faintuch-Linkowski, A. Rey, E. von Guggenberg, M. Rupprich, I. Hernandez-Gonzales, T. Rodrigo, R. Haubner: [99mTc]HYNIC-RGD for imaging integrin alphavbeta3 expression. *Nucl Med Biol* 33, 945-952 (2006)

46. Liu S, W. Y. Hsieh, Y. Jiang, Y. S. Kim, S. G. Sreerama, X. Chen, B. Jia, F. Wang: Evaluation of a (99m)Tc-labeled cyclic RGD tetramer for noninvasive imaging integrin alpha(v)beta3-positive breast cancer. *Bioconjug Chem* 18, 438-446 (2007)

47. Janssen M, W. J. Oyen, L. F. Massuger, C. Frielink, I. Dijkgraaf, D. S. Edwards, M. Radjopadhye, F. H. Corstens, O. C. Boerman: Comparison of a monomeric and dimeric radiolabeled RGD-peptide for tumor targeting. *Cancer Biother Radiopharm* 17, 641-646 (2002)

48. Alves S, J. D. Correia, L. Gano, T. L. Rold, A. Prasanphanich, R. Haubner, M. Rupprich, R. Alberto, C. Decristoforo, I. Santos, C. J. Smith: In vitro and in vivo evaluation of a novel 99mTc(CO)3-pyrazolyl conjugate of cyclo-(Arg-Gly-Asp-d-Tyr-Lys). *Bioconjug Chem* 18, 530-537 (2007)

49. Zhang X, X. Chen: Preparation and characterization of 99mTc(CO)3-BPy-RGD complex as alphav beta3 integrin receptor-targeted imaging agent. *Appl Radiat Isot* 65, 70-78 (2007)

50. Fani M, D. Psimadas, C. Zikos, S. Xanthopoulos, G. K. Loudos, P. Bouziotis, A. D. Varvarigou: Comparative evaluation of linear and cyclic 99mTc-RGD peptides for targeting of integrins in tumor angiogenesis. *Anticancer Res* 26, 431-434 (2006)

51. Psimadas D, M. Fani, C. Zikos, S. Xanthopoulos, S. C. Archimandritis, A. D. Varvarigou: Study of the labeling of two novel RGD-peptidic derivatives with the precursor [99mTc(H2O)3(CO)3]+ and evaluation for early angiogenesis detection in cancer. *Appl Radiat Isot* 64, 151-159 (2006)

52. Jung K H, K. H. Lee, J. Y. Paik, B. H. Ko, J. S. Bae, B. C. Lee, H. J. Sung, D. H. Kim, Y. S. Choe, D. Y. Chi: Favorable biokinetic and tumor-targeting properties of 99mTc-labeled glucosamino RGD and effect of paclitaxel therapy. *J Nucl Med* 47, 2000-2007 (2006)

53. Haubner R, F. Bruchertseifer, M. Bock, H. Kessler, M. Schwaiger, H. J. Wester: Synthesis and biological evaluation of a (99m)Tc-labelled cyclic RGD peptide for imaging the alphavbeta3 expression. *Nuklearmedizin* 43, 26-32 (2004)

54. Oulie I, L. Roed, K. G. Toft, T. Skotland: Quantification of NC100692, a new tracer for 99mTc-imaging of angiogenesis, in human plasma using reversed-phase liquid chromatography coupled with electrospray ionization ion-trap mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 852, 605-610 (2007)

55. Lindsey M L, G. P. Escobar, L. W. Dobrucki, D. K. Goshorn, S. Bouges, J. T. Mingoia, D. M. McClister, Jr., H. Su, J. Gannon, C. MacGillivray, R. T. Lee, A. J. Sinusas, F. G. Spinale: Matrix metalloproteinase-9 gene deletion facilitates angiogenesis after myocardial infarction. *Am J Physiol Heart Circ Physiol* 290, 26 (2006)

56. Hua J, L. W. Dobrucki, M. M. Sadeghi, J. Zhang, B. N. Bourke, P. Cavaliere, J. Song, C. Chow, N. Jahanshad, N. van Royen, I. Buschmann, J. A. Madri, M. Mendizabal, A. J. Sinusas: Noninvasive imaging of angiogenesis with a 99mTc-labeled peptide targeted at alphavbeta3 integrin after murine hindlimb ischemia. *Circulation* 111, 3255-3260 (2005)

57. van Hagen P M, W. A. Breeman, H. F. Bernard, M. Schaar, C. M. Mooij, A. Srinivasan, M. A. Schmidt, E. P. Krenning, M. de Jong: Evaluation of a radiolabelled cyclic DTPA-RGD analogue for tumour imaging and radionuclide therapy. *Int J Cancer* 90, 186-198 (2000)

58. Wang W, J. S. McMurray, Q. Wu, M. L. Campbell, C. Li: Convenient solid-phase synthesis of diethylenetriaminepenta-acetic acid (DTPA)- conjugated cyclic RGD peptide analogues. *Cancer Biother Radiopharm* 20, 547-556 (2005)

59. Chen X, R. Park, M. Tohme, A. H. Shahinian, J. R. Bading, P. S. Conti: MicroPET and autoradiographic imaging of breast cancer alpha v-integrin expression using 18F- and 64Cu-labeled RGD peptide. *Bioconjug Chem* 15, 41-49 (2004)

60. Decristoforo C, I. Hernandez-Gonzales, J. Carlsen, M. Rupprich, M. Husiman, I. Virgolini, H. J. Wester, R. Haubner: Ga-68- and In-111-labelled DOTA-RGD peptides for imaging of alpha(v)beta3 integrin expression. *Eur J Nucl Med Mol Imaging*, (2008 in press)

61. Dijkgraaf I, J. A. Kruijtzer, C. Frielink, F. H. Corstens, W. J. Oyen, R. M. Liskamp, O. C. Boerman: Alpha v beta 3 integrin-targeting of intraperitoneally growing tumors with a radiolabeled RGD peptide. *Int J Cancer* 120, 605-610 (2007)

62. Haubner R, H. J. Wester, F. Burkhart, R. Senekowitsch-Schmidtke, W. Weber, S. L. Goodman, H. Kessler, M. Schwaiger: Glycosylated RGD-containing peptides: tracer for tumor targeting and angiogenesis imaging with improved biokinetics. *J Nucl Med* 42, 326-336 (2001)

63. Haubner R, B. Kuhnast, H. J. Wester, W. A. Weber, R. Huber, R. Senekowitsch-Schmidtke, S. I. Ziegler, S. L. Goodman, H. Kessler, M. Schwaiger: [F-18]-RGD-Peptides Conjugated with Hydrophilic Tetrapeptides for the Noninvasive Determination of the Alpha(v)beta3 Integrin. *J Nucl Med* 43 (Suppl), 89P (2002)

64. Harris J M, N. E. Martin, M. Modi: Pegylation: a novel process for modifying pharmacokinetics. *Clin Pharmacokinet* 40, 539-551 (2001)

65. Harris J M, R. B. Chess: Effect of pegylation on pharmaceuticals. *Nat Rev Drug Discov* 2, 214-221 (2003)

66. Chen X, R. Park, A. H. Shahinian, J. R. Bading, P. S. Conti: Pharmacokinetics and tumor retention of 125I-labeled RGD peptide are improved by PEGylation. *Nucl Med Biol* 31, 11-19 (2004)

67. Chen X, R. Park, Y. Hou, V. Khankaldyyan, I. Gonzales-Gomez, M. Tohme, J. R. Bading, W. E. Laug, P. S. Conti: MicroPET imaging of brain tumor angiogenesis with 18F-labeled PEGylated RGD peptide. *Eur J Nucl Med Mol Imaging* 31, 1081-1089 (2004)

68. Chen X, R. Park, V. Khankaldyyan, I. Gonzales-Gomez, M. Tohme, R. A. Moats, J. R. Bading, W. E. Laug, P. S. Conti: Longitudinal MicroPET Imaging of Brain Tumor Growth with F-18-labeled RGD Peptide. *Mol Imaging Biol* 29, 1-7 (2005)

69. Chen X, Y. Hou, M. Tohme, R. Park, V. Khankaldyyan, I. Gonzales-Gomez, J. R. Bading, W. E. Laug, P. S. Conti: Pegylated Arg-Gly-Asp peptide: 64Cu labeling and PET imaging of brain tumor alphavbeta3-integrin expression. *J Nucl Med* 45, 1776-1783 (2004)

70. Dijkgraaf I, S. Liu, J. A. Kruijtzer, A. C. Soede, W. J. Oyen, R. M. Liskamp, F. H. Corstens, O. C. Boerman: Effects of linker variation on the in vitro and in vivo characteristics of an 111In-labeled RGD peptide. *Nucl Med Biol* 34, 29-35 (2007)

71. Janssen M L, W. J. Oyen, I. Dijkgraaf, L. F. Massuger, C. Frielink, D. S. Edwards, M. Rajopadhye, H. Boonstra, F. H. Corstens, O. C. Boerman: Tumor targeting with radiolabeled alpha(v)beta(3) integrin binding peptides in a nude mouse model. *Cancer Res* 62, 6146-6151 (2002)

72. Thumshirn G, U. Hersel, S. L. Goodman, H. Kessler: Multimeric cyclic RGD peptides as potential tools for tumor targeting: solid-phase peptide synthesis and chemoselective oxime ligation. *Chemistry* 9, 2717-2725 (2003)

73. Chen X, M. Tohme, R. Park, Y. Hou, J. R. Bading, P. S. Conti: Micro-PET imaging of alphavbeta3-integrin expression with 18F-labeled dimeric RGD peptide. *Mol Imaging* 3, 96-104 (2004)

74. Zhang X, Z. Xiong, Y. Wu, W. Cai, J. R. Tseng, S. S. Gambhir, X. Chen: Quantitative PET Imaging of Tumor Integrin {alpha}v{beta}3 Expression with 18F-FRGD2. *J Nucl Med* 47, 113-121 (2006)

75. Wu Z, Z. B. Li, W. Cai, L. He, F. T. Chin, F. Li, X. Chen: (18)F-labeled mini-PEG spacered RGD dimer ((18)F-FPRGD2): synthesis and microPET imaging of alpha (v)beta (3) integrin expression. *Eur J Nucl Med Mol Imaging* 34, 1823-1831 (2007)

76. Wu Z, Z. B. Li, K. Chen, W. Cai, L. He, F. T. Chin, F. Li, X. Chen: microPET of tumor integrin alphavbeta3 expression using 18F-labeled PEGylated tetrameric RGD peptide (18F-FPRGD4). *J Nucl Med* 48, 1536-1544 (2007)

77. Chen X, S. Liu, Y. Hou, M. Tohme, R. Park, J. R. Bading, P. S. Conti: MicroPET imaging of breast cancer alphav-integrin expression with 64Cu-labeled dimeric RGD peptides. *Mol Imaging Biol* 6, 350-359 (2004)

78. Wu Y, X. Zhang, Z. Xiong, Z. Cheng, D. R. Fisher, S. Liu, S. S. Gambhir, X. Chen: microPET imaging of glioma integrin {alpha}v{beta}3 expression using (64)Cu-labeled tetrameric RGD peptide. *J Nucl Med* 46, 1707-1718 (2005)

79. Li Z B, W. Cai, Q. Cao, K. Chen, Z. Wu, L. He, X. Chen: (64)Cu-labeled tetrameric and octameric RGD peptides for small-animal PET of tumor alpha(v)beta(3) integrin expression. *J Nucl Med* 48, 1162-1171 (2007)

80. Dijkgraaf I, J. A. Kruijtzer, S. Liu, A. C. Soede, W. J. Oyen, F. H. Corstens, R. M. Liskamp, O. C. Boerman: Improved targeting of the alpha(v)beta (3) integrin by multimerisation of RGD peptides. *Eur J Nucl Med Mol Imaging* 34, 267-273 (2007)

81. Sancey L, V. Ardisson, L. M. Riou, M. Ahmadi, D. Marti-Batlle, D. Boturyn, P. Dumy, D. Fagret, C. Ghezzi, J. P. Vuillez: In vivo imaging of tumour angiogenesis in mice with the alpha(v)beta (3) integrin-targeted tracer (99m)Tc-RAFT-RGD. *Eur J Nucl Med Mol Imaging* 4, 4 (2007)

82. Dijkgraaf I, A. Y. Rijnders, A. Soede, A. C. Dechesne, G. W. van Esse, A. J. Brouwer, F. H. Corstens, O. C. Boerman, D. T. Rijkers and R. M. Liskamp: Synthesis of DOTA-conjugated multivalent cyclic-RGD peptide

dendrimers via 1,3-dipolar cycloaddition and their biological evaluation: implications for tumor targeting and tumor imaging purposes. *Org Biomol Chem* 5, 935-944 (2007)

83. Reubi C, M. Gugger and B. Waser: Co-expressed peptide receptors in breast cancer as a molecular basis for in vivo multireceptor tumour targeting. *Eur J Nucl Med Mol Imaging* 29, 855-862 (2002)

84. Reubi J C, B. Waser: Concomitant expression of several peptide receptors in neuroendocrine tumours: molecular basis for in vivo multireceptor tumour targeting. *Eur J Nucl Med Mol Imaging* 30, 781-793 (2003)

85. De Jong M, H. F. Bernard, W. A. Breeman, A. Srinivasan, M. A. Schmidt, E. P. Krenning, M. Van Hagen: A Radiolabeled Cyclic RGD-DTPA-Octreotate Analog for Tumor Imaging and Radionuclide Therapy. *J Nucl Med* 42 Suppl, 111P (2001)

86. Buckley C D, D. Pilling, N. V. Henriquez, G. Parsonage, K. Threlfall, D. Scheel-Toellner, D. L. Simmons, A. N. Akbar, J. M. Lord, M. Salmon: RGD peptides induce apoptosis by direct caspase-3 activation. *Nature* 397, 534-539 (1999)

87. Bernard B, A. Capello, M. van Hagen, W. Breeman, A. Srinivasan, M. Schmidt, J. Erion, A. van Gameren, E. Krenning, M. de Jong: Radiolabeled RGD-DTPA-Tyr3-octreotate for receptor-targeted radionuclide therapy. *Cancer Biother Radiopharm* 19, 173-180 (2004)

88. Capello A, E. P. Krenning, B. F. Bernard, W. A. Breeman, M. P. van Hagen, M. de Jong: Increased cell death after therapy with an Arg-Gly-Asp-linked somatostatin analog. *J Nucl Med* 45, 1716-1720 (2004)

89. Capello A, E. P. Krenning, B. F. Bernard, W. A. Breeman, J. L. Erion, M. de Jong: Anticancer activity of targeted proapoptotic peptides. *J Nucl Med* 47, 122-129 (2006)

90. Beer A J, R. Haubner, M. Goebel, S. Luderschmidt, M. E. Spilker, H. J. Wester, W. A. Weber, M. Schwaiger: Biodistribution and pharmacokinetics of the alphavbeta3-selective tracer 18F-galacto-RGD in cancer patients. *J Nucl Med* 46, 1333-1341 (2005)

91. Beer A J, R. Haubner, I. Wolf, M. Goebel, S. Luderschmidt, M. Niemeyer, A. L. Grosu, M. J. Martinez, H. J. Wester, W. A. Weber, M. Schwaiger: PET-based human dosimetry of 18F-galacto-RGD, a new radiotracer for imaging alpha v beta3 expression. *J Nucl Med* 47, 763-769 (2006)

92. Beer A J, R. Haubner, M. Sarbia, M. Goebel, S. Luderschmidt, A. L. Grosu, O. Schnell, M. Niemeyer, H. Kessler, H. J. Wester, W. A. Weber, M. Schwaiger: Positron emission tomography using [18F]Galacto-RGD identifies the level of integrin alpha(v)beta3 expression in man. *Clin Cancer Res* 12, 3942-3949 (2006)

93. Beer A J, A. L. Grosu, J. Carlsen, A. Kolk, I. Stangler, P. Watzlowik, H. J. Wester, R. Haubner, M. Schwaiger: [18F]Galacto-RGD Positron Emission Tomography for Imaging of avß3 expression on the Neovasculature in Patients with Squamous Cell Carcinoma of the Head and Neck. *Clin Cancer Res* 13, 6610-6 (2007)

94. Beer A J, M. Niemeyer, J. Carlsen, M. Sarbia, J. Nährig, P. Watzlowik, H. J. Wester, N. Harbeck, M. Schwaiger: Patterns of avß3 Expression in Primary and Metastatic Human Breast Cancer as shown by [18F]Galacto-RGD PET. *J Nucl Med* 49, 255-259 (2008)

95. Beer A J, S. Lorenzen, S. Metz, K. Herrmann, P. Watzlowik, H. J. Wester, C. Peschel, P. Lordick, M. Schwaiger: Comparison of Integrin avß3 Expression and Glucose Metabolism in Primary and Metastatic Lesions in Cancer Patients: a PET Study Using [18F]Galacto-RGD and [18F]FDG. *J Nucl Med* 49:22-9 (2008)

96. Bach-Gansmo T, R. Danielsson, A. Saracco, B. Wilczek, T. V. Bogsrud, A. Fangberget, A. Tangerud, D. Tobin: Integrin receptor imaging of breast cancer: a proof-of-concept study to evaluate 99mTc-NC100692. *J Nucl Med* 47, 1434-1439 (2006)

97. Chen X, R. Park, A. H. Shahinian, M. Tohme, V. Khankaldyyan, M. H. Bozorgzadeh, J. R. Bading, R. Moats, W. E. Laug, P. S. Conti: 18F-labeled RGD peptide: initial evaluation for imaging brain tumor angiogenesis. *Nucl Med Biol* 31, 179-189 (2004)

Key Words: Angiogenesis, Radiolabelled Rgd-Peptides, Integrin alpha (v)beta3, Tracer Techniques, Molecular Imaging, Review

Send correspondence to: Clemens Decristoforo, Clinical Department of Nuclear Medicine, Medical University Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria, Tel: 43512504 80951, Fax: 43512504 6780951, E-mail: Clemens.Decristoforo@uki.at

http://www.bioscience.org/current/vol14.htm