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Positron emission tomography tracers for imaging angiogenesis

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Abstract

Position emission tomography imaging of angiogenesis may provide non-invasive insights into the corresponding molecular processes and may be applied for individualized treatment planning of antiangiogenic therapies. At the moment, most strategies are focusing on the development of radiolabelled proteins and antibody formats targeting VEGF and its receptor or the ED-B domain of a fibronectin isoform as well as radiolabelled matrix metalloproteinase inhibitors or $\alpha_v\beta_3$ integrin antagonists. Great efforts are being made to develop suitable tracers for different target structures. All of the major strategies focusing on the development of radiolabelled compounds for use with positron emission tomography are summarized in this review. However, because the most intensive work is concentrated on the development of radiolabelled RGD peptides for imaging $\alpha_v\beta_3$ expression, which has successfully made its way from bench to bedside, these developments are especially emphasized.

Keywords

Angiogenesis; Vascular endothelial growth factor; Fibronectin ED-B domain; Integrin $\alpha_v\beta_3$; Matrix metalloproteinase; Imaging; Positron emission tomography

Angiogenesis and tumour growth

Angiogenesis is a process involving the growth of new blood vessels from pre-existing vessels. It is a normal and vital process in growth and development and is found during embryogenesis, the female reproductive cycle, tissue remodelling and wound healing. However, also numerous disorders are characterized by an imbalance or up-regulation of the angiogenic process. These include psoriasis [1], restenosis [2], rheumatoid arthritis [3], diabetic retinopathy [4] and tumour growth [5]. Angiogenesis research is one of the most rapidly growing biomedical disciplines. Strategies to alter angiogenesis have already been

evaluated in clinical trials. It is estimated that more than 500 million patients potentially benefit from such strategies [6]. These developments are prime examples of targeted therapy that are increasingly changing the face of clinical medicine.

Angiogenesis is a complex multistep process regulated by the balance between pro- and antiangiogenic factors [7, 8]. The angiogenic switch is often triggered by an insufficient oxygen supply resulting in hypoxic cells [9]. Binding of the hypoxia-inducible factor (HIF α) to hypoxia response elements activates expression of vascular endothelial growth factor (VEGF), a key player in the angiogenic process. However, also other stimuli can induce angiogenesis. These include mechanical and metabolic stress, genetic mutations and immune/inflammatory response. Additional regulating factors such as fibroblast growth factors (bFGF, aFGF) and platelet-derived endothelial cell growth factor (PDGF) are secreted by a variety of cells, but can also be found in blood and emanate from the extracellular matrix [8, 10].

After activation of the endothelial cells, proteolytic enzymes such as matrix metalloproteinases (MMPs) are produced to degrade the basement membrane and the extracellular matrix (ECM) [11, 12] providing sufficient space for the sprouting vessels. Moreover, MMPs are involved in controlling the balance between pro- and antiangiogenic factors. On the one hand, they can release matrix-bound proangiogenic factors, and on the other hand, they can play an antiangiogenic role by cleaving matrix components into antiangiogenic factors (for details see [12]).

One receptor class playing an important role during endothelial cell migration are the integrins [13, 14]. But these receptors are not only involved in endothelial cell adhesion but also are important regulators of endothelial cell growth, survival and differentiation. It has been demonstrated that antibodies as well as low molecular weight antagonists, recognizing the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$, block angiogenesis in murine tumour models and in retinal angiogenesis [15–17]. However, based on several knock-out experiments there is evidence that $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are antiangiogenic or negative regulators of angiogenesis rather than proangiogenic [18].

In further steps, extracellular matrix proteins such as tenascin, laminin or collagen type IV are produced to provide new ECM components. Mesenchymal cells release angiopoietin-1, which interacts with Tie-2 receptor tyrosine kinase mediating capillary organization and stabilization [19]. The newly built endothelial cells are reorganized by forming tight junctions with each other leading to tube formation. These new tubes connect with the microcirculation resulting in an operational new vasculature.

Target structures for tracer developments

The angiogenic process offers a variety of target structures for therapeutic interventions [10]. Thus, great efforts are being made to develop antiangiogenic drugs as novel therapeutics in particular for the treatment of tumours. These developments are focused on growth receptor antagonists, metalloproteinase inhibitors, adhesion molecule antagonists and antagonists blocking endothelial cell function (e.g. angiostatin).

In preclinical studies successful inhibition of tumour growth and metastasis formation could be demonstrated for a variety of different compounds. Those encouraging studies have already led to initial clinical trials [20, 21]. However, currently available imaging techniques are limited in monitoring treatment using this class of drugs. Anti-tumour activity is generally assessed by determining the percentage of patients in whom a significant reduction of the tumour size is achieved during a relatively short period of therapy (“response rate”). Thus, this method may not be applicable for a form of therapy that is aimed at disease

stabilization and prevention of metastases. Therefore, new methods are needed for planning and monitoring of treatments targeting the angiogenic process.

There are different approaches including magnetic resonance imaging, Doppler ultrasound and scintigraphic techniques currently being studied. This chapter is focused on positron emission tomography (PET) tracer techniques for angiogenesis imaging.

Several potential targets for the design of radiolabelled compounds for monitoring of angiogenesis are conceivable. At the moment, most of the work on the development of PET tracers for imaging angiogenesis is concentrated on radiolabelled $\alpha_v\beta_3$ antagonists and MMP inhibitors. Some approaches focus on antibodies/proteins targeting the VEGF system and on single-chain Fv antibody fragments selectively binding to a particular fibronectin isoform. All these approaches will be discussed here.

VEGF/VEGFR

VEGF/VEGFR pathway

Vascular endothelial growth factor and its receptors (VEGF/VEGFRs) pathway are considered to be one of the most important regulators of angiogenesis and a key target in anti-cancer treatment [22, 23]. Increased expression of VEGF by tumour cells and increased expression of VEGFR-2(KDR)/VEGFR-1(Flt) by the tumour-associated vasculature are hallmarks of solid tumours, which correlates with tumour growth rate, microvessel density, tumour metastatic potential and poorer patient prognosis in a variety of malignancies [24]. Therefore, non-invasive imaging of VEGF/VEGFR expression in vivo will provide important information about tumour pathology and physiology. In addition, the critical role of VEGF/VEGFRs in cancer progression has been highlighted by the approval of the humanized anti-VEGF monoclonal antibody bevacizumab (Avastin, Genentech) for first-line treatment [25]. However, the evaluation of antiangiogenic treatment efficacy in the early stages in order to assess different dosing regimens is one of the current challenges in VEGFR-targeted therapy. In order to explore the role of VEGF/VEGFRs in cancer, and the potential of VEGFR-targeted therapy, there is a pressing need to develop specific and selective molecular imaging modalities that will enable the measurement of several important parameters such as the concentration and occupancy of VEGFRs in vivo with a non-invasive imaging modality [26].

Probes used for VEGF/VEGFR imaging

The probes currently used for imaging VEGF/VEGFRs pathway fall into two categories. The first category includes antibodies against VEGF. Over-expression of VEGF occurs in many human tumour types [27]. Tumour cells produce VEGF, which can lead to paracrine effects in the microenvironment. VEGF₁₂₁ is freely soluble, VEGF₁₆₅ is secreted, whereas a significant fraction remains localized to the extracellular matrix, such as VEGF₁₈₉ and VEGF₂₀₆ [28]. This will most likely lead to locally high VEGF levels. Non-invasive measurement of VEGF in the tumour might give insight to the available target for VEGF-dependent antiangiogenic therapy and thus assist in tumour response prediction. The humanized monoclonal antibody bevacizumab blocks VEGF-induced endothelial cell proliferation, permeability and survival, and it inhibits human tumour cell line growth in preclinical models [29, 30]. Small animal PET imaging with ⁸⁹Zr-bevacizumab showed higher tumour uptake compared with human ⁸⁹Zr-IgG in a human SKOV-3 ovarian tumour xenograft. Non-invasive quantitative small animal PET was correlated with invasive ex vivo biodistribution. Similar results were observed with ¹²⁴I-labelled monoclonal antibody VG67e [31] that binds to human VEGF. HuMV833, the humanized version of a mouse monoclonal anti-VEGF antibody MV833, was also labelled with ¹²⁴I and the distribution and biological effects of HuMV833 in patients in a phase I clinical trial were investigated

[32]. These results demonstrated that radiolabelled antibody is a new class of tracer for non-invasive in vivo imaging of VEGF in the tumour microenvironment. However, antibody distribution and clearance were quite heterogeneous not only between and within patients but also between and within individual tumours [32]. In addition, the radiolabelled antibody tumour accumulation is not always correlated with the level of VEGF expression in the tissue as determined by in situ hybridization and enzyme-linked immunosorbent assay (ELISA) [33]. Furthermore, due to the large size of the antibody, it is hard to penetrate into the centre of the tumour and it usually takes several hours or even days before high-contrast images can be obtained for antibody-based tracers. Using engineered antibody fragments with compromised binding affinity can partially overcome this problem.

The second category includes radiolabelled VEGF-A and its derivatives for imaging VEGFRs. Several studies have been reported on the use of appropriately labelled VEGF proteins for PET [34]. However, most of the reported wild-type VEGF-based imaging agents are unsuitable for clinical translation because of the unacceptably high major organ, such as liver and kidney, uptake [35] or uncertain binding activity of the protein, owing to damage caused by random radiolabelling or bioconjugation [36–39]. Therefore, optimization of VEGF protein-based probes without changing the conformation of the protein and compromising its functional activity is required.

The VEGF family is composed of seven members with a common VEGF homology domain [25]. VEGF-A is a dimeric, disulphide-bound glycoprotein existing in at least seven homodimeric isoforms. Besides the difference in molecular weight, these isoforms also differ in their biological properties such as the ability to bind to cell surface heparin sulphate proteoglycans [25]. Both VEGF₁₆₅ and VEGF₁₂₁ have been used for VEGFR imaging [38, 40]. The advantages of this class of tracers is that they are natural ligands for VEGFRs and have high binding affinity to the receptors. However, VEGF₁₆₅ and VEGF₁₂₁ bind to both VEGFR-1 and VEGFR-2, and their binding affinity to VEGFR-1 was even higher than that to VEGFR-2, which resulted in high kidney retention, an organ that expresses a high level of VEGFR-1 [41]. Compared with VEGF₁₂₁, VEGF₁₆₅ is less soluble and contains an extra domain for heparin binding, resulting in increased non-specific binding and low tumour to background ratio. Therefore, optimization of VEGF protein probes is mainly based on VEGF₁₂₁ protein instead of VEGF₁₆₅ protein.

For labelling VEGF with ^{99m}Tc a variety of single-chain as well as “tagged” VEGF derivatives have been introduced which will be discussed in another review of this issue. The development of PET tracers is, at the moment, mainly based on mutants of VEGF₁₂₁.

MicroPET imaging with ⁶⁴Cu-labelled wild-type VEGF₁₂₁ reveals rapid, specific and prominent uptake in highly vascularized tumour xenografts over-expressing VEGFR-2. However, the highest uptake was in the kidneys [40]. Many other VEGF-based tracers have been reported for SPECT and optical imaging of tumour angiogenesis, most of which have had high kidney uptake [35, 38, 42, 43]. The kidney is usually the dose-limiting organ because of its high VEGFR-1 expression, which can lead to significant uptake of VEGF protein-based tracers. The uptake of VEGF-based tracers in the kidneys was mainly due to VEGFR-1 binding (and some renal clearance), while the tumour uptake was mainly related to VEGFR-2 expression. Several separate domains of VEGF interact with VEGFR-2 and VEGFR-1. Alanine-scanning mutagenesis has revealed that Arg(82), Lys(84) and His(86) are critical for the binding of VEGF to VEGFR-2, while Asp(63), Glu(64) and Glu(67) are required for the binding of VEGF to VEGFR-1 [44]. Based on this knowledge, the D63AE64AE67A mutant of VEGF₁₂₁ (VEGF_{DEE}), in which Asp(63), Glu(64) and Glu(67) of VEGF₁₂₁ protein critical for VEGFR-1 binding were mutated to Ala [45], was conjugated with DOTA and labelled with ⁶⁴Cu (⁶⁴Cu-DOTA-VEGF_{DEE}) for PET imaging. As

compared with ^{64}Cu -DOTA-VEGF₁₂₁, ^{64}Cu -DOTA-VEGF_{DEE} had comparable tumour targeting efficacy but much reduced renal accumulation (Fig. 1). Further improvement in VEGFR-2 binding affinity/specificity, pharmacokinetics and tumour-targeting efficacy by generating other VEGF₁₂₁ mutants with higher VEGFR-2 specificity is considered a good direction for clinical translation of VEGF protein-based imaging probes.

Perspective of VEGF/VEGFR PET imaging

PET imaging of VEGFR expression was first fulfilled by using radiolabelled anti-VEGFR antibodies. Although VEGFR specificity in vivo was demonstrated in these studies, the poor immunoreactivity (<35%) of the radiolabelled antibody limits the potential use of this type of tracer. Recombinant VEGF₁₂₁ has been labelled with ^{64}Cu for PET imaging of tumour angiogenesis and VEGFR expression [45]. PEGylated VEGF₁₂₁ site-specifically labelled with ^{64}Cu showed considerably prolonged blood clearance, higher tumour uptake and lower kidney uptake [46]. Overall, PET imaging using VEGF protein-based radiotracers is a feasible option to non-invasively detect VEGFR expression in vivo.

Nuclear imaging of VEGF/VEGFR expression could be a valuable tool for evaluation of patients with a variety of diseases [45, 47, 48], and particularly for monitoring those undergoing antiangiogenic therapies that block VEGF/VEGFR-2 function [49]. While SPECT imaging can be used for simultaneous imaging of multiple radionuclides since the gamma ray emitted from different radioisotopes can be differentiated based on the energy, PET is the most widely studied modality used for VEGFR imaging. However, before widespread use in patient trials, considerable improvements in the pharmacokinetic properties and better targeting efficacy of these compounds need to be achieved and validated.

ED-B domain targeting mAbs

Biological background

Fibronectin exists in several isoforms (e.g. III CS, ED-A, ED-B) [50]. These isoforms are involved in a variety of processes including cell migration, wound healing and oncogenic transformation. It has been shown that the isoform containing the ED-B domain is important in vascular proliferation and is widely expressed in fetal and neoplastic tissues, whereas its distribution is highly restricted in normal adult tissue [51]. It was demonstrated that fluorescently labelled anti-ED-B single-chain Fv antibody fragments selectively accumulates around blood vessels in tumour tissue in a murine tumour model [52].

Development of PET tracer targeting the ED-B domain

Based on these findings a radioiodinated anti-ED-B antibody fragment with picomolar antigen binding affinity has been synthesized. The radiolabelled single-chain fragment scFv(L19) showed high accumulation in different tumour models [53]. In addition, microautoradiography and immunohistochemical staining demonstrated selective accumulation in tumour vessels. In contrast, no activity accumulation was found in vessels of other organs. In an initial clinical study [54], including patients with lung, colorectal or brain cancer, in 16 of 20 patients injected with the ^{123}I -labelled dimeric single-chain fragment L19(scFv)₂, different levels of tracer accumulation were found either in the primary tumour or metastases 6 h post-injection (p.i.). These data indicate that radiolabelled antibody fragments against the ED-B domain of fibronectin are potential new tracers for non-invasive angiogenesis monitoring. Meanwhile, a variety of different formats have been tested to identify the best-suited radioimmunoconjugate [55, 56]. These studies include the complete human IgG1 L19-IgG1 (~150 kDa), the “small immunoprotein” L19-SIP (~80 kDa) and the single-chain fragment scFv(L19) (~50 kDa). The fastest clearance but also the

lowest stability was found for scFv(L19). But even the elimination kinetics of the single-chain fragment is not compatible with the short half-life of standard PET isotopes for labelling peptides such as ^{18}F and ^{68}Ga . Thus, PET isotopes with longer half-lives like ^{76}Br (half-life 16.2 h) and ^{124}I (half-life 4.18 days) have been introduced to develop radiolabelled antibody fragments which could be used for imaging ED-B domain expression with PET. In one approach L-19-SIP was labelled with ^{76}Br via enzymatic radiobromination. The stability of the compound in serum was comparable with that found for the ^{125}I -labelled derivative. However, the tumour to blood ratio was not higher than 1.2 even after 24 h p.i. resulting in high background activity in the body of F9 tumour-bearing mice [57]. Recently, ^{124}I -L19-SIP was introduced for immuno-PET imaging of tumour vasculature and guidance of ^{131}I -L19-SIP radio-immunotherapy [58]. ^{124}I -L19-SIP showed comparable tumour to background ratios as found for ^{131}I -L19-SIP and improved ratios compared with ^{76}Br -L19-SIP. This led to good tumour contrast prominent in the stomach and to a lesser extent in the bladder of FaDu-bearing mice at 24 h p.i. (Fig. 2). Background activity, which may mainly be due to some deiodination, had almost disappeared 48 h after tracer injection. However, further studies have to be carried out demonstrating that this tracer might be suitable for imaging ED-B domain expression in patients using PET.

Radiolabelled MMP inhibitors

MMP activity—MMPs are capable of degrading proteins of the extracellular matrix [59]. The MMP family is divided into five classes. The different classes include collagenases, gelatinases, stromelysins, membrane type (MT)-MMPs and non-classified MMPs [60]. MMP activity is controlled by a balance between the expression of endogenous MMP inhibitors and proenzyme synthesis [61]. An increased proenzyme production results in degradation of the basement membrane and the extracellular matrix [62]. The gelatinases MMP-2 and MMP-9 are most consistently detected in malignant tissue [63, 64]. Their over-expression correlates with tumour aggressiveness and meta-static potential. Due to their important role in tumour-induced angiogenesis and metastasis, MMPs are potential targets for therapeutic interventions [11, 65]. Thus, great efforts are being made to develop MMP inhibitors. Many of them are in preclinical or already in clinical studies.

Peptide-based inhibitors—By using the phage display library approach the disulphide bridged decapeptide CTTHWGFTLC was found to selectively inhibit MMP-2 and MMP-9 [66]. This peptide suppressed the migration of endothelial and tumour cells in vitro and mediated the homing of phages in the tumour vasculature in vivo. Based on these results the peptide with the sequence yCTTHWGFTLC including a D-tyrosine at the N-terminal end was synthesized, radioiodinated and evaluated [67]. In vitro data showed that the modified peptide has similar inhibitory capacities for MMP-2 as found for the lead structure and that the tracer was stable towards degradation by activated MMP-2 and MMP-9. However, in vivo studies revealed low metabolic stability and high lipophilicity resulting in moderate tumour uptake. Moreover, high activity concentration in the liver and kidneys was found, indicating that further improvements concerning metabolic stability as well as pharmacokinetic behaviour have to be carried out to make this compound suitable for imaging MMP expression.

Small molecular mass inhibitors—Due to the importance of MMPs during tumour-induced angiogenesis and tumour metastasis numerous peptidomimetic and non-peptidic inhibitors are currently being investigated. Detailed structure activity investigations [68–71] showed that some of the most important characteristics for high affinity binding are: (a) a coordination site binding to the catalytic zinc ion, (b) a lipophilic site interacting with a hydrophobic cleft and (c) hydrophobic interactions between the sulfonyl amide substituent of the corresponding ligand and the binding pocket.

Starting from different D-amino acid scaffolds, ^{18}F -labelled MMP-2 inhibitors have been developed [72]. In vitro studies demonstrated micromolar inhibitory activities. Based on another N-sulfonylamino acid derivative a ^{11}C -labelled analogue was synthesized, which showed strong inhibitory effectiveness for the gelatinases MMP-2 and MMP-9 [73]. A broad range of MMP inhibitors belonging again to the N-sulfonylamino acid family were used as additional lead structures for the synthesis of ^{11}C - and ^{18}F -labelled derivatives [74–77]. They were either labelled at the phenyl group or at the hydroxamic acid function. Some of the described radiolabelled compounds have comparable inhibitory effectiveness on MMP-1 as found for the parent structure CGS 27023A. Recently radiolabelled biphenylsulphonamide-based MMP inhibitors have been developed [78], which showed high inhibitory effectiveness on MMP-13. Oltenfreiter et al. [79, 80] labelled hydroxamic and carboxylic acid containing MMP inhibitors also based on amino acid scaffolds. Labelling was carried out via an electrophilic aromatic substitution using ^{123}I -iodine. Again these compounds showed high inhibitory capacities on gelatinases.

However, in contrast to the promising in vitro data, the data resulting from the in vivo evaluations were not so promising (for example see Fig. 3), although the normal mice biodistribution data of some tracers indicated favourable pharmacokinetics [73, 79, 80] and metabolic stability [73]. However, the biodistribution studies as well as microPET images using murine tumour models demonstrated low tracer accumulation in the corresponding tumours [76, 81, 82].

Radiolabelled integrin antagonists (RGD peptides)

Targeting integrin expression

Integrins are cell adhesion receptors which are not only involved in mediating migration of endothelial cells but are also important regulators of endothelial cell growth, survival and differentiation [13, 14]. The integrin $\alpha_v\beta_3$ is one member of this class of receptors. It has been shown that it plays an essential role in the regulation of tumour growth, local invasiveness and metastatic potential [83, 84], but is also highly expressed on activated endothelial cells during angiogenesis [15]. However, several knock-out experiments gave evidence that $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are antiangiogenic or negative regulators of angiogenesis rather than proangiogenic [18]. In any event, inhibition of $\alpha_v\beta_3$ -mediated interactions has been found to induce apoptosis not only of activated endothelial cells but also of $\alpha_v\beta_3$ -positive tumour cells, resulting in a direct cytotoxic effect on these cells [85]. Thus, the use of $\alpha_v\beta_3$ antagonists is currently being evaluated as a strategy for anticancer therapy [86], which is focused on the prevention of metastasis and disease stabilization rather than reduction of tumour mass during a relatively short therapy period. Thus, such therapy regimens would benefit from new biomarkers allowing monitoring of $\alpha_v\beta_3$ expression before and during therapy.

Integrin $\alpha_v\beta_3$ is a heterodimeric transmembrane glycoprotein consisting of two subunits. It has been found that several extracellular matrix proteins like vitronectin, laminin and fibronectin interact via the amino acid sequence Arg-Gly-Asp (RGD) with this integrin [87]. Based on these findings Kessler and co-workers developed the $\alpha_v\beta_3$ targeting pentapeptide cyclo(-Arg-Gly-Asp-DPhe-Val-) [88] which is the most prominent lead structure for the development of radiotracers for the non-invasive determination of this receptor [89]. Another lead structure is based on the sequence H-Lys-Cys-Arg-Gly-Asp-Cys-Phe-Cys-OH (NC-100717) including to bridging systems (N^α of Lys¹ is bridged with Cys⁸ via a chloroacetyl moiety and Cys²-Cys⁶ via disulphide formation) [90]. The side chain amino function of the lysine is used for derivatization allowing radiolabelling with ^{18}F , $^{99\text{m}}\text{Tc}$ or other radiometals. C-terminal modifications include the introduction of a PEG linker as biomodifier.

Some other approaches focused on peptidomimetics as targeting structures. These include antagonists which are conjugated with 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (DOTA) to give the high affinity antagonist TA138 [91] or use a guanidinobenzoyl hydra-zino oxopentanoic acid scaffold (GBHO) [92] which allows radioiodine and ^{18}F labelling. Despite initial in vivo studies in mice and rats indicating that [^{18}F]GBHO-2 is a potential alternative to the ^{18}F -Galacto-RGD for the in vivo imaging of $\alpha_v\beta_3$ no further studies were published. Thus far, radiolabelling approaches have been mainly focused on cyclic RGD peptides and derivatives partly due to the fact that peptidomimetics often are specifically designed to achieve bioavailability after oral administration, which is not required for radiopharmaceutical approaches where intravenous application is preferred.

PET tracers for imaging $\alpha_v\beta_3$

Monomeric tracer—labelling strategies—Due to its favourable beta energy and half-life, ^{18}F is the most frequently used radionuclide in PET. The main approach to label peptides with ^{18}F involves prosthetic groups. The most prominent ^{18}F -labelled tracer for imaging $\alpha_v\beta_3$ expression is [^{18}F]Galacto-RGD, which is labelled via conjugation of 4-nitrophenyl-2-[[^{18}F]fluoropropionate [93]. This compound resulted from an optimization strategy introducing sugar moieties to improve pharmacokinetics (see below). In murine tumour models as well as in patients (for details see also below) this tracer showed receptor-specific tumour accumulation and good elimination kinetics resulting in high contrast images demonstrating that non-invasive determination of $\alpha_v\beta_3$ expression and quantification with ^{18}F -labelled RGD peptides is feasible (Fig. 4).

However, synthesis of ^{18}F -labelled 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 [94, 95] and more recently also with [[^{18}F]fluorosilyl benzaldehyde [96] have been introduced. The 4-[[^{18}F]fluorobenzaldehyde has also been used in combination with HYNIC-modified RGD peptides [97] resulting in 4'-[[^{18}F]fluorobenzylidenehydrazide-6-nicotinamide-c(RGDyK). [^{18}F]-AH111585 [98] is an aminooxy-functionalized double-bridged RGD peptide which already entered clinical trials (see below). Other strategies use thiol-reactive groups. For example 3,4,6-tri-*O*-acetyl-2-deoxy-2-[[^{18}F]fluoroglucopyranosyl phenylthiosulfonate (Ac_3 -[[^{18}F]FGlc-PTS) [99] was used 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 (see below). Another group introduced *N*-[2-(4- ^{18}F -fluorobenzamido)ethyl]maleimide (^{18}F -FBEM) [100] as a thiol-reactive synthon. With this technique ^{18}F -labelling of a monomeric and dimeric thiolated RGD peptide at high specific activities and high radiochemical yields could be carried out.

Recently, the Huisgen [3+2] azide-alkyne cycloaddition (more prominent as “click chemistry”) has found its way into radiopharmaceutical chemistry. The main advantages of this reaction, which can be carried out under mild Cu-promoted reaction conditions, are selectivity, reliability and short reaction time. A comparison of different strategies of chemoselective labelling of functionalized double-bridged RGD peptides confirmed that “click labelling” of peptides may be an attractive alternative to aminooxy aldehyde condensation [101]. [^{18}F]RGD-K5, which is another click chemistry-derived RGD-based peptidomimetic PET tracer with high $\alpha_v\beta_3$ binding affinity, has already entered initial clinical trials [102] (see also below).

Due to increasing availability, in the last few years, metal-based PET isotopes such as ^{64}Cu and ^{68}Ga have become more and more interesting for labelling of peptides. Thus, a variety

of tracers allowing labelling with these isotopes have been introduced. In one study a DOTA-conjugated RGD peptide (DOTA-RGDyK) was labelled with ^{64}Cu [103]. Tumour to blood and tumour to muscle ratios of approximately 7 and 8, respectively, allowed acquisition of clear tumour to background contrast images 1 h p.i. using a small animal scanner. However, the highest activity concentration was found in liver, intestine and bladder indicating that further optimization of the tracer is needed.

Another DOTA-derivatized RGD peptide (DOTA-RGDfK) was used for radiolabelling with ^{111}In and ^{68}Ga [104]. Both radiolabelled peptides showed specific binding to $\alpha_v\beta_3$ with comparable internalization and tumour uptake values to [^{18}F]Galacto-RGD in an $\alpha_v\beta_3$ -positive melanoma M21 model. However, the tendency to interact with proteins in the blood was considerably higher for [^{68}Ga] DOTA-RGDfK resulting in higher blood pool activity in vivo and thus in lower tumour to background ratios compared to [^{18}F]Galacto-RGD.

An alternative chelator fitting well with the binding properties of Ga^{3+} is the 1,4,7-triazacyclononane-1,4,7-triacetic acid system. This chelator shows a high complex binding constant forming a very stable gallium complex even at room temperature. Jeong et al. [105] used isothiocyanatobenzyl-1,4,7-triazacyclononane-1,4,7-triacetic acid (SCN-Bz-NOTA) and conjugated it with c(RGDyK). Small animal PET imaging of mice bearing SNU-4C xenografts showed a good contrasting tumour but also high activity concentration in kidneys and bladder. Based on these results first studies in patients have been initiated and data can be found in the corresponding paragraph below. Another NOTA-based chelating system has already been used in combination with a somatostatin derivative [106]. An advantage of 1,4,7-triazacyclononane-1-glutaric acid-4,7-diacetic acid (NODAGA) might be that the linker moiety does not contain an aromatic system, which may have a negative effect on the hydrophilicity of the corresponding tracer (especially for small peptides; for protein labelling this would have no effect). Most recently, this chelating system was conjugated to c(RGDfK). The resulting [^{68}Ga] NODAGA-RGD showed clearly reduced protein binding properties in vitro and decreased activity concentration in blood in a murine tumour model compared with [^{68}Ga] DOTA-RGD [107]. The improved imaging properties and the straightforward accessibility make it an interesting alternative to ^{18}F -labelled RGD peptides for imaging $\alpha_v\beta_3$ expression.

Optimization approaches

Optimization of pharmacokinetics: Desired features for optimal tumour targeting include rapid uptake and long retention 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 kidneys or liver. Therefore, pharmacokinetic properties have been varied using chemical modifications (“pharmacokinetic modifiers”).

One approach introduced glycosylation to improve the pharmacokinetic properties [108, 109]. Thus, sugar amino acids (SAA) were conjugated via the ϵ -amino function of the corresponding lysine with the pentapeptide sequence. In a murine tumour model the resulting [^{125}I]Gluc-RGD [109] and [^{18}F]Galacto-RGD [93, 108, 110] showed an initially increased activity concentration in blood, very similar kinetics in kidneys and more importantly, a clearly reduced activity concentration in the liver and an increased activity uptake and retention in the tumour compared to the first-generation, unmodified peptides.

More recently, it was suggested that a glycoside precursor be used for combining ^{18}F -labelling and glycosylation. One approach used the chemoselective oxime formation strategy to conjugate [^{18}F]FDG to the aminooxy-functionalized RGD peptide [111]. This would be an elegant way to use the most widely applied PET tracer directly to label

peptides. However, labelling cannot be carried out without removing unlabelled glucose which is a competitor for the conjugation reaction. If no-carrier-added (n.c.a.) [^{18}F] FDG is used the [^{18}F]-labelled peptide can be produced in high yield resulting in a tracer with suitable pharmacokinetics. Thus, if the necessary n.c.a. preparation of [^{18}F] FDG prior to reaction with the aminoxy-functionalized peptide can be implemented in a fully automated [^{18}F] FDG synthesis, this [^{18}F]fluoroglucosylation strategy may represent a promising way to produce [^{18}F]-labelled peptides. Another approach involved click chemistry techniques to synthesize a glycosylated RGD peptide [112]. Therefore, 2-deoxy-2-[^{18}F]fluoroglucosyl azide was used as labelling precursor and a propargylglycine modified RGD peptide as targeting structure. The Cu-catalysed [3+2] cycloaddition results in the desired product in good chemical yields in a total synthesis time of approximately 70 min, which is a clear improvement compared with [^{18}F]-Galacto-RGD. However, further preclinical and clinical evaluations have to be carried out before final assessment.

PEGylation is known to improve many properties of peptides and proteins including plasma stability, immune reactivity and pharmacokinetics [113, 114]. 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 hepatic excretion. Since renal filtration is dependent on both the molecular mass and the volume occupied, this effect is strongly related to the molecular weight of the PEG moiety. In a first study, a 2-kDa PEG moiety was attached to the ϵ -amino function of cyclo(-Arg-Gly-Asp-DTyr-Lys-) and the [^{125}I]-labelled PEGylated derivative (^{125}I -RGD-PEG) was compared with the non-PEGylated [^{125}I -c(RGDyK)] [115]. The PEGylated RGD showed more rapid blood clearance, decreased activity concentration in the kidneys and slightly increased activity retention in the tumour but also lower tumour uptake and increased activity retention in liver and intestine as found for [^{125}I -RGD]. 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) were compared [116]. 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 than for [^{18}F]FB-RGD. Anyway, in a brain tumour model longitudinal microPET imaging allowed visualization and quantification of anatomical variations during tumour growth and angiogenesis. In an additional study, [^{64}Cu]-DOTA-RGD and [^{64}Cu]-DOTA-PEG-RGD (PEG, MW=3.4 kDa) [117] were compared. [^{64}Cu]-DOTA-RGD showed significant liver uptake, which could be reduced by PEGylation. Moreover, for [^{64}Cu]-DOTA-PEG-RGD faster blood clearance was found, while the tumour uptake as well as retention were not affected.

Most recently, Liu et al. [118] compared glycosylated and PEGylated RGD monomers and dimers for PET imaging of $\alpha_v\beta_3$ expression and found in a murine tumour model that for [^{18}F]Galacto-RGD and the [^{18}F]fluoropropionic acid-labelled dimeric PEGylated ([^{18}F]FP-PRGD2) as well as glycosylated RGD peptide ([^{18}F]FP-SRGD2) tumour to background ratios 2 h p.i. are comparable. However, tumour uptake at this time point is 2–3 times higher for the dimers resulting in better tumour uptake in the corresponding microPET images for the latter compounds (Fig. 5). Due to easier production procedures an investigative new drug application for the PEGylated dimer was applied for and recently approved by the US Food and Drug Administration (FDA). At the moment, a side-by-side comparison of [^{18}F] FP-PRGD2 and [^{18}F]Galacto-RGD in humans is in progress.

Optimizing binding affinity—the “multimerization” approach: As already indicated in the last paragraph one approach to improve target affinity and retention is the so-called multimerization approach, which means that more than one binding epitope is included in the targeting molecule. The improvement is argued to be mainly due to an increased apparent ligand concentration and/or, especially by larger molecules, due to strong

cooperative binding. In one study a dimeric RGD peptide coupling two c(RGDfK) via a glutamic acid linker [119, 120] has been synthesized. For radiolabelling DOTA or HYNIC were conjugated. The resulting dimeric ^{99m}Tc -HYNIC-E-[c(RGDfK)]₂ revealed a tenfold higher affinity for $\alpha_v\beta_3$ and an improved tumour retention but also a higher uptake in kidneys compared with the monomeric ^{99m}Tc -HYNIC-c(RGDfK).

In another approach a series of monomeric, dimeric, tetrameric and octameric RGD peptides linked via PEG moieties and labelled via oxime formation using ^{18}F -fluorobenzaldehyde [94, 95, 121] have been studied. Increasing binding affinities in the series of monomer, dimer, tetramer and octamers have been found. Initial PET images resulting from a clinical PET scanner confirmed these findings. The images of melanoma-bearing mice showed increasing activity accumulation in the series monomer, dimer and tetramer. Another group studied a glutamic acid bridged dimeric RGD peptide, which was labelled by conjugating a 4- ^{18}F fluorobenzoyl moiety [122, 123]. The dimeric RGD peptide demonstrated higher tumour uptake and prolonged tumour retention compared with the monomeric analogue [^{18}F]FB-c(RGDyK). Moreover, the dimeric RGD peptide had predominantly renal excretion, whereas the monomeric analogue was excreted primarily through the biliary route. It was concluded 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. Labelling yields could be improved by introducing [^{18}F]FB-mini-PEG-E[c(RGDyK)]₂ [124].

Similar effects have been found for multimeric ^{64}Cu -labelled analogues [125]. The tetrameric [^{64}Cu]DOTA-E[E-c(RGDyK)]₂ [126] showed significantly higher integrin binding affinity than the corresponding monomeric and dimeric RGD analogues. Again tumour uptake was rapid and high, and the tumour washout was slow. The positive effect of multimerization on tumour uptake is further confirmed by introduction of a ^{64}Cu -labelled octameric RGD peptide [127]. However, again also uptake in different organs including kidneys and muscle is increased indicating that a favourable balance between binding epitope density and tracer size is important for the design of the optimal tracer.

Recently approaches were described which used the regioselectivity addressable functionalized template (RAFT) [128] or dendrimers [129] 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. The biodistribution studies using murine tumour models showed that the tumour uptake of the tetramer is higher than that of the corresponding monomer. The other approach used the 1,3-dipolar cycloaddition for conjugating the cyclic RGD peptides to the scaffold. Monomeric, dimeric and tetrameric peptides have been synthesized. In vitro binding studies and biodistribution studies showed higher binding affinity and tumour uptake for the tetrameric compound as compared to the monomer and dimer. However, increasing activity concentration is also found in a variety of organs including kidneys, liver and intestine.

Altogether, in many cases the multimerization approach led 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.

Clinical evaluation

Molecular imaging of angiogenesis with PET might be extremely valuable in the clinical setting, e.g. for response assessment to antiangiogenic or combined cytotoxic/anti-angiogenic therapy. However, up to date no data are available to elucidate the clinical value of PET imaging of angiogenesis or whether it is superior or complementary to functional imaging of angiogenesis, like with dynamic contrast-enhanced (DCE) MRI. However, some

recent preclinical studies indicate the potential value of PET imaging of $\alpha_v\beta_3$ expression for response assessment. One study suggested that [^{64}Cu]RGD has the potential to monitor the physiological changes in the bone metastatic microenvironment in an animal model of osteolytic bone metastases after osteoclast-inhibiting bisphosphonate therapy [130]. Another study in an animal model of Lewis lung cell cancer (LLC) showed that PET imaging with [^{18}F]AH111585 ([^{18}F]fluciclatide) was able to visualize reduction of microvessel density during low-dose paclitaxel therapy, while uptake of [^{14}C]FDG did not decrease [131]. Thus, [^{18}F]AH111585 might be of potential value for assessment of response to antiangiogenic therapy. Clinical trials with this tracer are currently ongoing. Most clinical data, however, are currently still found for [^{18}F]Galacto-RGD. [^{18}F]Galacto-RGD was the first PET tracer applied in patients and could successfully image $\alpha_v\beta_3$ with good tumour to background ratios [110]. In all patients, rapid, predominantly renal tracer elimination was observed, resulting in low background activity in most regions of the body. High inter- and intra-individual variance in tracer accumulation in tumour lesions was noted, suggesting substantial heterogeneity of $\alpha_v\beta_3$ expression. Further biodistribution studies have confirmed rapid clearance of [^{18}F]Galacto-RGD from the blood pool and primarily renal excretion. Background activity in lung and muscle tissue was low and the calculated effective dose was found to be similar to a [^{18}F]FDG scan [132]. Distribution volume (D_v) values, which are supposed to reflect the receptor concentration in tissue, were on average four times higher for tumour tissue than for muscle tissue, suggesting specific tracer binding. [^{18}F]Galacto-RGD uptake was correlated with $\alpha_v\beta_3$ expression as determined by immunohistochemistry. Nineteen patients with solid tumours were examined with PET using [^{18}F]Galacto-RGD before surgical removal of the lesions [133]. Standardized uptake values (SUVs) and tumour to blood ratios correlated significantly with the intensity of immunohistochemical staining as well as with the microvessel density. Moreover, immunohistochemistry confirmed lack of $\alpha_v\beta_3$ expression in normal tissue and in the two tumours without tracer uptake. Different tumour entities were systematically examined with respect to their $\alpha_v\beta_3$ expression patterns with [^{18}F]Galacto-RGD PET. In squamous cell carcinoma of the head and neck (SCCHN) good tumour to background ratios were found. Immunohistochemistry demonstrated predominantly vascular $\alpha_v\beta_3$ expression, suggesting that in SCCHN [^{18}F]Galacto-RGD PET might be used as a surrogate parameter of angiogenesis [134]. In patients with glioblastoma, normal brain tissue did not show significant tracer accumulation. In glioblastoma multiforme moderate and heterogeneous tracer uptake was noticed, with a maximum in the highly proliferating and infiltrating areas of tumours. Immunohistochemical staining was prominent in tumour microvessels as well as glial tumour cells. In areas of highly proliferating glial tumour cells, tracer uptake in the PET images correlated with immunohistochemical $\alpha_v\beta_3$ expression of corresponding tumour samples [135]. However, tracer uptake was substantially lower as compared to results in tumours outside the CNS. As [^{18}F]Galacto-RGD does not cross the intact blood-brain barrier, this might be an important factor influencing tracer uptake in CNS lesions. Therefore, results obtained in the CNS and outside the CNS probably have to be interpreted differently.

Recently, the SPECT tracer [$^{99\text{m}}\text{Tc}$]NC100692 was introduced by GE Healthcare for imaging $\alpha_v\beta_3$ expression in humans and was first evaluated in breast cancer by Bach-Gansmo et al. [136]; 19 of 22 tumours could be detected with this agent, which was safe and well tolerated by the patients. Moreover, also a PET imaging agent was introduced. First studies with [^{18}F]AH111585 (now also called [^{18}F]fluciclatide) in humans have demonstrated favourable biodistribution of this tracer with predominantly renal excretion [137]. In 7 patients with metastasized breast cancer all 18 tumours detected by CT were visible on the [^{18}F]AH111585 PET images (Fig. 6) [138]. However, in the case of liver metastases, lesions were identified only indirectly as photopenic regions because of the high background activity in normal liver tissue. Increased uptake compared with background was demonstrated in metastases in lung, pleura, bone, lymph node and primary tumour. The

authors concluded that [^{18}F]AH111585 is safe, metabolically stable and able to detect breast cancer lesions by PET in most anatomical sites. Currently, a proof of concept study in up to 30 patients is being performed in patients with brain tumours, lung cancers, SCCHN, differentiated thyroid carcinoma, sarcoma and melanoma to correlate dynamic and static [^{18}F]AH111585 PET imaging with histological parameters of angiogenesis (including $\alpha_v\beta_3$ expression) and DCE CT [139].

In a first clinical study with [^{18}F]RGD-K5 the tracer was administered to 12 patients with breast cancer, who also received a separate [^{18}F]FDG PET [140]. No adverse reactions were observed; 157 lesions were identified by [^{18}F]FDG PET, of which 122 also showed elevated [^{18}F]RGD-K5 uptake. Similar as was found for other integrin targeting PET tracers, most lesions (80%) showed higher [^{18}F]FDG uptake. However, in a few cases (4%) [^{18}F]RGD-K5 uptake was higher than [^{18}F]FDG. Analogous to [^{18}F] Galacto-RGD, [^{18}F]RGD-K5 accumulation in lesions showed no correlation with [^{18}F]FDG uptake. Biodistribution and radiation dosimetry was performed in four patients [141], resulting in an estimated effective dose of 0.015 mSv/MBq when using a 1-h bladder voiding interval, which is comparable to other integrin targeting PET tracers. Organs with the highest doses were the bladder wall, gall bladder and the kidneys. Recently, a non-randomized uncontrolled pilot phase II clinical study was started to assess the usefulness of [^{18}F]RGD-K5 PET imaging in predicting efficacy and early response monitoring in a combination therapy of the anti-VEGF antibody bevacizumab plus chemotherapy [142].

Another clinically used tracer is [^{68}Ga]NOTA-RGD. First studies were performed in patients with [^{18}F]FDG and [^{68}Ga]NOTA-RGD PET with hepatic metastases of colorectal cancer before a combination therapy of FOLFOX and bevacizumab [143]. Static PET images with [^{68}Ga]NOTA-RGD were acquired 30 min after injection. In all patients, hypermetabolic liver lesions were visualized on [^{18}F]FDG PET. Three patients also had mild [^{68}Ga]NOTA-RGD accumulation in liver lesions, and the other half showed no [^{68}Ga]NOTA-RGD uptake. Interestingly, the patients with [^{68}Ga]NOTA-RGD uptake in the hepatic metastases showed partial response to the antiangiogenic combination therapy, whereas the other patients had stable or progressive disease. These encouraging findings corroborate the hypothesis that PET imaging with integrin $\alpha_v\beta_3$ targeting tracers might help in patient selection for antiangiogenic therapies; however, validation in larger studies is needed.

Summary and perspective

There is a keen interest in techniques allowing non-invasive imaging of angiogenesis. Thus, a variety of approaches are currently being studied, which include MRI, optical imaging, ultrasound imaging and nuclear medicine tracer techniques such as SPECT and PET. Radiolabelled probes have mainly been developed for four different targets involved in the angiogenic process, namely VEGF and its receptors, the ED-B domain of a fibronectin isoform, matrix metalloproteinases and integrin $\alpha_v\beta_3$.

Despite the fact that there are a variety of approaches for the development of radiolabelled VEGF derivatives and anti ED-B domain antibody formats for use with SPECT, relatively less work was done to develop tracers for use with PET. This may be mainly due to the fact that the biological half-lives of these proteins and antibody formats are not compatible with the short physical half-lives of the most common isotopes used for PET. To overcome such obstacles, ^{64}Cu , ^{76}Br and ^{124}I with a half-life of 12.7 h, 16 h and 4.2 days, respectively, have been introduced. In one approach using ^{64}Cu -labelled VEGF mutants activity accumulation in the dose-limiting kidneys could be reduced compared to the native VEGF₁₂₁. However, further data including studies in patients are needed to demonstrate the potential usefulness of this class of tracers in clinical settings. A very similar situation concerning the

development of PET tracers for imaging the ED-B domain of the fibronectin isoform is that although promising data were found for the ^{124}I -L19-SIP, much more data are needed before a final decision can be made. Moreover, due to the long half-life of ^{124}I radiation burden for the patients might be a problem in clinical settings.

A great variety of different ^{11}C - and ^{18}F -labelled small molecular MMP inhibitors have been developed in the past few years. Many of them demonstrated comparable binding affinity and subtype selectivity as found for the lead structures. But, at the moment, corresponding data from murine tumour models could not confirm that this class of tracers allows monitoring of tumour-induced angiogenesis. Thus, before further compounds are introduced a detailed verification of the corresponding animal models might be of great importance.

Great efforts have been made to develop radiolabelled RGD peptides for the non-invasive determination of $\alpha_v\beta_3$ expression for monitoring angiogenic processes. The most detailed studied PET tracer yet is ^{18}F Galacto-RGD. The initial clinical studies demonstrated that molecular imaging of $\alpha_v\beta_3$ expression with ^{18}F Galacto-RGD correlates with $\alpha_v\beta_3$ expression as determined by immunohistochemistry after surgical removal of the tumour. Moreover, the variable tracer uptake which is in correlation with variable $\alpha_v\beta_3$ expression shows the value of non-invasive techniques for appropriate selection of patients entering clinical trials of $\alpha_v\beta_3$ -targeting therapies. Meanwhile at least three other compounds, either labelled with ^{18}F or ^{68}Ga , entered initial clinical studies. With all these $\alpha_v\beta_3$ -targeting tracers tumours could be monitored and further clinical studies will provide data on the potential of this imaging technique in various clinical situations and will provide the tracer with the best performance for imaging $\alpha_v\beta_3$ expression using PET. Recent approaches towards multimers showed great potential in vitro and in preclinical animal models with significant improvement of targeting. For one of these dimeric compounds a clinical study has been approved most recently, which will reveal if this strategy will also have a positive impact on the imaging properties in patients. Cumulatively, the available data indicate great potential of radiolabelled RGD peptides and PET to be used as a new marker of activated endothelial and tumour cells and for individualized planning of therapeutic strategies with $\alpha_v\beta_3$ -targeted drugs.

Overall, it is likely that angiogenesis will eventually be assessed not by using a single parameter, target structure or imaging technique but rather by a combination of parameters that allow for a multimodal/multiparametric imaging evaluation of the intricacies of the angiogenic cascade. Combined MR/PET scanners [144] might help in this respect, as they could provide functional imaging by DCE MRI and molecular imaging with PET in a one-stop-shop examination (Fig. 7). With PET/MR inserts for brain imaging already being in use and whole-body hybrid MR/PET scanners being developed, assessing the different aspects of angiogenesis at the structural, functional and molecular levels before, during and after antiangiogenic therapy within one examination will likely become a reality and help further steps toward personalized medicine.

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Fig. 1. Serial microPET imaging 1, 4 and 20 h p.i. of tumour-bearing mice injected intravenously with $^{64}\text{Cu-DOTA-VEGF}_{121}$ or $^{64}\text{Cu-DOTA-VEGF}_{\text{DEE}}$, respectively. Coronal slices containing the tumours (*arrowheads*) are shown. The study showed that $^{64}\text{Cu-DOTA-VEGF}_{\text{DEE}}$ had significantly lower kidney uptake than $^{64}\text{Cu-DOTA-VEGF}_{121}$ while the tumour and other major organ uptake of both tracers was comparable (reproduced with permission [30])

Fig. 2.

Small animal PET images of a tumour-bearing nude mouse injected with the anti ED-B domain antibody format [^{124}I]L19-SIP. Coronal images 24 h (**a**) and 48 h (**b**) after tracer injection showed clearly visible tumours on both flanks of the mouse. At 24 h p.i. high uptake was also found in the stomach (may be due to some deiodination) and to a lesser extent in the bladder which disappeared at 48 h p.i. (reproduced with permission [53])

Fig. 3. Planar scintigraphy imaging of tumour-bearing mice injected with 2-(4-[^{123}I]Iodo-biphenyl-4-sulfonylamino)-3-(1H-indol-3-yl)-propionic acid at 6, 24 and 48 h p.i. demonstrated low tracer uptake in the tumour (indicated by *arrows*) (reproduced with permission [77])

Fig. 4.

Correlation of tracer accumulation and $\alpha_v\beta_3$ expression. **a–c** Patient with a soft tissue sarcoma dorsal of the right knee joint. **a** The sagittal section of a [^{18}F]Galacto-RGD PET acquired 170 min p.i. showed circular peripheral tracer uptake in the tumour with variable intensity and a maximum SUV at the apical-dorsal aspect of the tumour (arrow). **b** The image fusion of the [^{18}F] Galacto-RGD PET and the corresponding CT scan showed that the regions of intense tracer uptake correspond with the enhancing tumour wall, whereas the non-enhancing hypodense centre of the tumour showed no tracer uptake. **c** Immunohistochemistry of a peripheral tumour section using an anti- $\alpha_v\beta_3$ monoclonal antibody demonstrated intense staining predominantly of tumour vasculature. **d–f** Patient with malignant melanoma and a lymph node metastasis in the right axilla. **d** The axial section of a [^{18}F] Galacto-RGD PET acquired 140 min p.i. shows intense focal uptake in the lymph node (arrow). **e** Image fusion of the [^{18}F]Galacto-RGD PET and the corresponding CT scan. **f** Immunohistochemistry of the lymph node using the anti- $\alpha_v\beta_3$ monoclonal antibody demonstrated intense staining predominantly of tumour cells and also blood vessels (reproduced with permission [105])

Fig. 5. Comparison of small animal PET images of tumour-bearing mice. *First row* Coronal images 20, 60 and 120 min after injection of ^{18}F -Galacto-RGD. *Second and third rows* Coronal images 20, 60 and 120 min after injection of ^{18}F -FP-PRGD2 and ^{18}F -FP-SRGD2, respectively. For all tracers best tumour to background ratios are found after 120 min p.i. However, due to higher absolute uptake the dimeric RGD peptides showed superior imaging quality in this animal model (reproduced with permission [113])

Fig. 6.

^{18}F -AH111585 PET and corresponding CT showed increased signal intensity in the periphery of lesions in patients with lung and pleural metastases (a). Intralesion heterogeneity of tracer uptake within the pleural metastasis is found in the PET image, which was identified as non-necrotic by the CT section (b). Liver metastasis was imaged as hypointense lesion because of high background signal (c). High tracer uptake in spleen is possibly due to blood pooling (reproduced with permission [131])

Fig. 7.

Example of multiparametric multimodality MRI and PET imaging in a patient with lung cancer in the left upper lobe (*arrows*). In the *upper row* in the images before start of chemotherapy, FDG PET/CT shows the intense glucose metabolism of the tumour, and [^{18}F]Galacto-RGD PET/MRI image fusion shows intense $\alpha_v\beta_3$ expression of the tumour. Functional imaging by MRI shows restricted water diffusion in the ADC map of the diffusion-weighted MRI (“apparent diffusion coefficient”) as an indicator of high cellularity and elevated tissue perfusion in the parametric map of K^{trans} from DCE MRI. Two weeks after start of chemotherapy, glucose metabolism shows no substantial change, ADC has slightly increased (about 10%) and [^{18}F]Galacto-RGD uptake has decreased by 20%. K^{trans} has decreased especially in the central parts of the tumour. This demonstrates that changes of functional and molecular parameters of angiogenesis and tumour biology can be measured early after onset of chemotherapy by combined MRI and PET, which might be helpful for response assessment in the future (unpublished data from A. Beer)