

Review

It's Time to Shift the Paradigm: Translation and Clinical Application of Non- $\alpha\nu\beta 3$ Integrin Targeting Radiopharmaceuticals

Susanne Kossatz ^{1,2}, Ambros Johannes Beer ³ and Johannes Notni ^{4,5,*}

¹ Department of Nuclear Medicine, School of Medicine, Technical University of Munich, 81675 Munich, Germany; s.kossatz@tum.de

² Central Institute for Translational Cancer Research (TranslaTUM), School of Medicine, Technical University of Munich, 81675 Munich, Germany

³ Klinik für Nuklearmedizin, Universitätsklinikum Ulm, 89081 Ulm, Germany; ambros.beer@uniklinik-ulm.de

⁴ Department of Pathology, School of Medicine, Technical University of Munich, 81675 Munich, Germany

⁵ TRIMT GmbH, 01454 Radeberg, Germany

* Correspondence: tum@notni.de; Tel.: +49-89-4140-6075; Fax: +49-89-4140-6949

Simple Summary: Cancer cells often present a different set of proteins on their surface than normal cells. This also applies to integrins, a class of 24 cell surface receptors which mainly are responsible for physically anchoring cells in tissues, but also fulfil a plethora of other functions. If a certain integrin is found on tumor cells but not on normal ones, radioactive molecules (named tracers) that specifically bind to this integrin will accumulate in the cancer lesion if injected into the blood stream. The emitted radiation can be detected from outside the body and allows for localization and thus, diagnosis, of cancer. Only one of the 24 integrins, the subtype $\alpha\nu\beta 3$, has hitherto been thoroughly investigated in this context. We herein summarize the most recent, pertinent research on other integrins, and argue that some of these approaches might ultimately improve the clinical management of the most lethal cancers, such as pancreatic carcinoma.



Citation: Kossatz, S.; Beer, A.J.; Notni, J. It's Time to Shift the Paradigm: Translation and Clinical Application of Non- $\alpha\nu\beta 3$ Integrin Targeting Radiopharmaceuticals. *Cancers* **2021**, *13*, 5958. <https://doi.org/10.3390/cancers13235958>

Academic Editor: Melpomeni Fani

Received: 27 October 2021

Accepted: 17 November 2021

Published: 26 November 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: For almost the entire period of the last two decades, translational research in the area of integrin-targeting radiopharmaceuticals was strongly focused on the subtype $\alpha\nu\beta 3$, owing to its expression on endothelial cells and its well-established role as a biomarker for, and promoter of, angiogenesis. Despite a large number of translated tracers and clinical studies, a clinical value of $\alpha\nu\beta 3$ -integrin imaging could not be defined yet. The focus of research has, thus, been moving slowly but steadily towards other integrin subtypes which are involved in a large variety of tumorigenic pathways. Peptidic and non-peptidic radioligands for the integrins $\alpha 5\beta 1$, $\alpha\nu\beta 6$, $\alpha\nu\beta 8$, $\alpha 6\beta 1$, $\alpha 6\beta 4$, $\alpha 3\beta 1$, $\alpha 4\beta 1$, and $\alpha M\beta 2$ were first synthesized and characterized preclinically. Some of these compounds, targeting the subtypes $\alpha\nu\beta 6$, $\alpha\nu\beta 8$, and $\alpha 6\beta 1/\beta 4$, were subsequently translated into humans during the last few years. $\alpha\nu\beta 6$ -Integrin has arguably attracted most attention because it is expressed by some of the cancers with the worst prognosis (above all, pancreatic ductal adenocarcinoma), which substantiates a clinical need for the respective theranostic agents. The receptor furthermore represents a biomarker for malignancy and invasiveness of carcinomas, as well as for fibrotic diseases, such as idiopathic pulmonary fibrosis (IPF), and probably even for Sars-CoV-2 (COVID-19) related syndromes. Accordingly, the largest number of recent first-in-human applications has been reported for radiolabeled compounds targeting $\alpha\nu\beta 6$ -integrin. The results indicate a substantial clinical value, which might lead to a paradigm change and trigger the replacement of $\alpha\nu\beta 3$ by $\alpha\nu\beta 6$ as the most popular integrin in theranostics.

Keywords: $\alpha\nu\beta 6$ -Integrin; radiopharmaceuticals; molecular imaging; translational medicine; pancreatic cancer; theranostics

1. Introduction

Integrins are a family of cell surface receptors which primarily mediate the binding and physical attachment of cells to various insoluble strand proteins, such as collagen, laminin, fibronectin, vitronectin, and others, that constitute the extracellular matrix (ECM) [1]. As such, they fulfil a pivotal role for tissue connectivity and stiffness [2]. Integrins are transmembrane proteins, i.e., they possess an extracellular domain for binding to their ligands, and an intracellular domain which is mostly connected to the actin filaments forming the cytoskeleton (Figure 1). The intracellular domain is furthermore involved in signaling cascades [3]. On the one hand, factors in the cytosol can induce activation and clustering of integrins (inside-out signaling); on the other hand, ligand binding to integrins can initiate intracellular processes (outside-in signaling), thus underscoring the multitude of biological functions attributed to integrins in general [4].

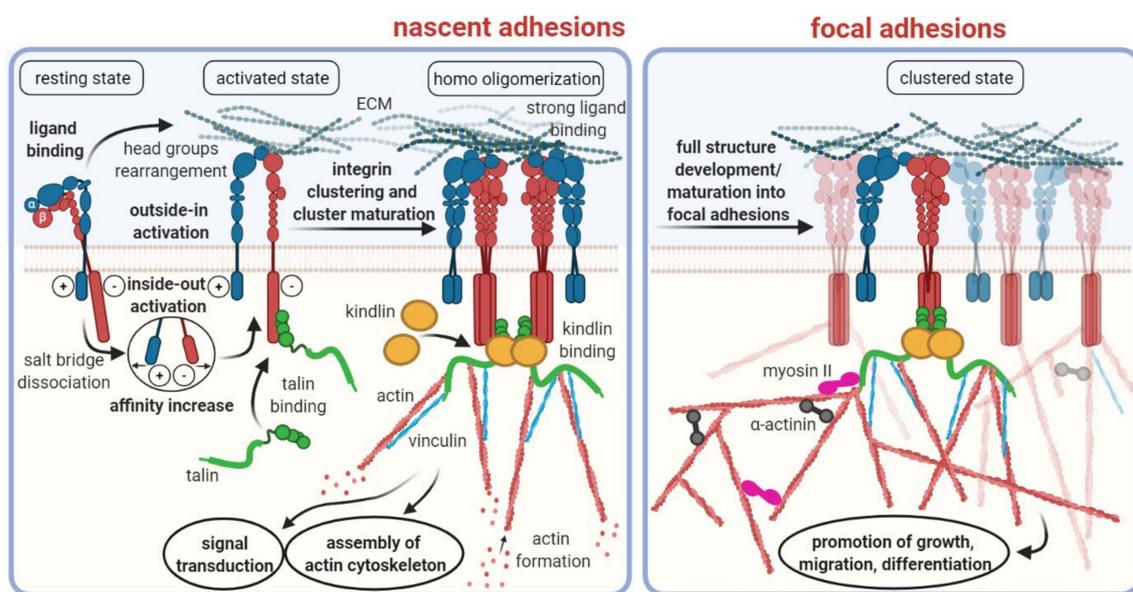


Figure 1. Schematic overview of integrin structures and functions. Note that this scheme illustrates only the processes involved into integrin binding to the extracellular matrix. Other important functionalities of certain integrins, such as TGF- β activation (see below), are not represented herein. Copyright notice: Figure reprinted from Cancers 2021, 13, 1711. Ludwig et al., RGD-Binding Integrins Revisited: How Recently Discovered Functions and Novel Synthetic Ligands (Re-) Shape an Ever-Evolving Field [5]; under Creative Commons CC BY 4.0.

Each integrin is formed by dimerization of two distinct subunits, one α - and one β -chain. 18 different α - and 8 β -proteins have been discovered so far, but not all combinations are capable of pairing—a total of 24 different combinations is known today (Figure 2). Ligand binding occurs on the most distant extracellular contact site of both subunits, where a protein-bound zinc(II) ion and a more or less hydrophobic pocket form a distinct, unique recognition motif stretching across both subunits. Each of the 24 dimers therefore has its own, unique affinity profile and can, for example, specifically recognize a single ECM protein, or bind to a broader range of substrates. It is worth emphasizing that each of the 26 integrin subunits is genetically encoded, transcribed, and translated independently. Studies addressing the expression and functions of a particular integrin therefore require, for example, the determination of mRNA and/or cytosolic concentration of both comprising subunits, or the direct detection of the actual dimer. This implicates that the abundance of a particular subunit, e.g., $\beta 1$, does not determine any functionality or property of one of its respective dimers, e.g., $\alpha v \beta 1$, $\alpha 5 \beta 1$, et cetera. On the other hand, the lack of a particular subunit may have specific and well-defined consequences due to a loss of all respective dimers, which is why many researchers actually investigated the

impact of knockout of a certain ITG gene to conclude on the respective dimers' functions from the resulting malfunctions observed in mice.

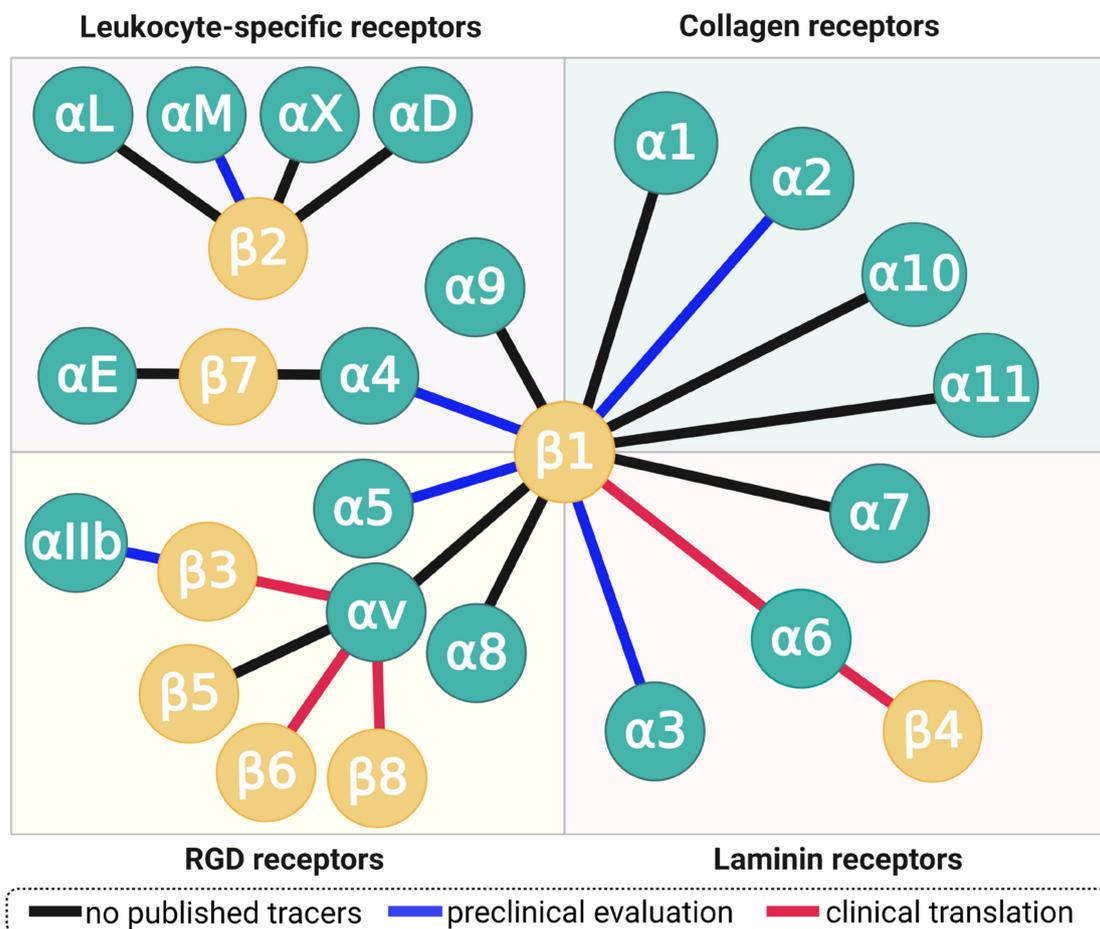


Figure 2. The family integrin receptors. Each connecting line represents an integrin, formed by dimerization of one α - and one β -subunit. Blue lines indicate integrins for which radiolabeled small-molecule ligands, peptides, or peptidomimetics have been characterized in small animals; red lines denote clinical translation, regardless of cohort size (i.e., including single-case applications). Copyright notice: Figure reprinted from *EJNMMI Res.* 2021, 11, doi:10.1186/s13550-021-00842-2. Steiger et al., There is a world beyond $\alpha v \beta 3$ -integrin: Multimeric ligands for imaging of the integrin subtypes $\alpha v \beta 6$, $\alpha v \beta 8$, $\alpha v \beta 3$, and $\alpha 5 \beta 1$ by positron emission tomography [6]; under Creative Commons CC BY 4.0.

Certain integrin subunits are capable of forming a multitude of dimers, most notably, $\beta 1$, which dimerizes with 12 different α -chains, and αv which pairs with $\beta 1$, $\beta 3$, $\beta 5$, $\beta 6$, and $\beta 8$ (Figure 2). Although all αv integrins (plus $\alpha 5 \beta 1$, $\alpha 8 \beta 1$, and $\alpha IIb \beta 3$) recognize the short tripeptide sequence arginine–glycine–aspartate (RGD), they nevertheless are expressed by different cell types and, in part, bind to proteins with different structures and completely different biological functions. For example, $\alpha 5 \beta 1$ -integrin is expressed by activated endothelial cells, binds to the ECM protein fibronectin, and regulates angiogenesis [7]—whereas the dimer $\alpha v \beta 6$ is exclusively expressed by epithelial cells and releases transforming growth factor beta (TGF- β) [8] by snatching and physically deforming latency associated peptide (LAP), the “protective envelope” that wraps up TGF- β while lounging in the interstitium (vide infra) [9]. Both integrins exert their functions by binding to an RGD motif in their respective substrates, which clearly demonstrates that RGD-recognizing integrins neither necessarily share the same class of molecular targets or biochemical purpose, nor does presence of the RGD motif in recognized proteins implicate a similar biological function or mechanism [5].

That being said, it appears worthwhile to have a closer look at the common perception of integrins and their ligands in the context of molecular imaging and radiopharmacy [6]. An in-depth look at the literature of the last 1–2 decades reveals that the overwhelming majority of studies has been conducted using (radio)labeled ‘RGD peptides’ [10–12]. This term mostly refers to cyclic pentapeptides of the cyclo(RDGxX) type [13] which are often abbreviated as ‘cRGD’ and possess a high affinity and selectivity for $\alpha v\beta 3$ -integrin [14] ($x = \text{D-Phe, D-Tyr}$; $X = \text{Val, Lys, Glu}$; conjugation and/or labeling is usually done on the Lys or Glu side chains). Since $\alpha v\beta 3$ was the first integrin shown to be closely associated with angiogenesis [15,16], most of these investigations aimed at targeting angiogenesis in various settings and diseases—above all, in cancer [17–19] but also in arthritis, wound healing, and other instances of medical interest.

The narrative of targeting $\alpha v\beta 3$ -integrin for imaging of angiogenesis using (radio) labeled ‘RGD peptides’ became so popular that the terms ‘ $\alpha v\beta 3$ ’, ‘RGD’, and ‘angiogenesis’ were (and still are) frequently used in an almost synonymous manner, such as in the phrase “angiogenesis imaging using Ga-68-RGD PET/CT” [20]. A strict causal relationship, however, does not exist for any combination of these terms. For example, neither the αv - [21] nor the $\beta 3$ -subunit [22] (and, therefore, $\alpha v\beta 3$) is strictly required for angiogenesis. A lack of $\beta 3$ can, for example, be compensated by up-regulation of integrin-independent angiogenic pathways, such as vascular endothelial growth factor receptor 2 (VEGFR2) signaling [23]. $\alpha v\beta 3$ is not only expressed on activated endothelial cells but also on many tumor cells and on macrophages [24]. It was shown recently that homing of cRGD-decorated nanocarriers to tumor vasculature may also occur by action of phagocytes, substantiating that targeting angiogenic sites with cRGD-containing constructs may involve other mechanisms than straightforward binding to endothelial $\alpha v\beta 3$ -integrin [25]. Moreover, there are selective ligands for $\alpha v\beta 3$ -integrin which do not rely on the RGD motif [26,27]. Vice versa, many (cyclic) peptides have been described which contain the RGD sequence but do not preferably bind to $\alpha v\beta 3$ but another integrin, such as $\alpha v\beta 5$, $\alpha v\beta 6$, $\alpha v\beta 8$, or $\alpha 5\beta 1$ [5]. The “classical” cRGD pentapeptides [13], i.e., c(RGDxK) and its congeners (see above), are nonetheless selective for $\alpha v\beta 3$ [28]; a fact which is sometimes disregarded and leads to confusion about the integrins and biological structures targeted by the respective conjugates [29].

During the last two decades, translational and clinical studies involving integrin-targeted radiopharmaceuticals were nonetheless focused almost exclusively on $\alpha v\beta 3$ -integrin ligands based on cyclic RGD peptides [12,30]. Their overwhelming popularity may have shaped and defined the general opinion about integrin tracers and their clinical potential. In spite of a large number of clinical studies and an even larger number of synthesized tracers, a well-defined clinical application of $\alpha v\beta 3$ -integrin targeted compounds could not be established throughout this time [31]. A broad clinical application or even a breakthrough similar to prostate-specific membrane antigen (PSMA) targeted agents [32] never occurred, and this is also quite improbable to happen in the future, in view of the substantial efforts already made [30]. A certain degree of resignation and a comparably low level of interest in integrin-targeted radiopharmaceuticals within the radiopharmaceutical and nuclear medicine communities thus comes as no surprise. We nonetheless believe that integrins could turn out to be valuable theranostic targets after all—on the condition that a broader view is established and pertinent research will continue off the beaten tracks. This review will thus summarize the latest reports on clinical translation of radiolabeled ligands for integrins other than $\alpha v\beta 3$, in order to shed light on the untapped potential and the hidden treasures offered by this multifaceted class of receptors. A brief glance on $\alpha v\beta 3$ -integrin driven theranostics and the associated challenges is nonetheless included as well.

2. $\alpha v\beta 3$ -Integrin—A Cul-De-Sac?

The above-mentioned availability of cRGD peptides as versatile ligands for $\alpha v\beta 3$ -integrin [33] and the knowledge about the importance of this receptor for a highly relevant biological mechanism—angiogenesis—triggered the development of the respective radiola-

beled derivatives [34,35] and the first clinical application of an $\alpha v\beta 3$ -directed PET tracer, [^{18}F]Galacto-RGD [36–38]. Other, similar tracers followed shortly thereafter, and clinical studies were conducted to establish the novel class of radiopharmaceuticals for cancer imaging [39–41], more or less explicitly seeking to augment or even replace [^{18}F]2-fluoro-2-deoxyglucose (FDG). Many of these investigations also aimed to exploit the fact that $\alpha v\beta 3$ -integrin is frequently expressed by tumor cells, not only endothelial cells involved in angiogenesis.

Triggered by the early success of ‘theran(g)ostics’, above all, the application of imaging agents in tandem with particle-emitting nuclides for diagnosis and endoradiotherapy of somatostatin-receptor 2 (SSTR2) positive neuroendocrine tumors (NET) [42], it appeared logical and obvious to adapt the same scheme to radiolabeled RGD peptides. In practice, this turned out to be more complicated than expected, because compared to SSTR2-expression in NET, $\alpha v\beta 3$ -integrin expression showed a higher degree of interindividual variation for most tumor entities, and $\alpha v\beta 3$ -integrin dependent uptake in target-positive lesions is usually substantially lower as compared to SSTR2-mediated accumulation of radiopharmaceuticals [43,44]. Target-to-organ ratios are furthermore lower because $\alpha v\beta 3$ -integrin is physiologically expressed to a certain extent in various organs and tissues (Figure 3) [30,36].

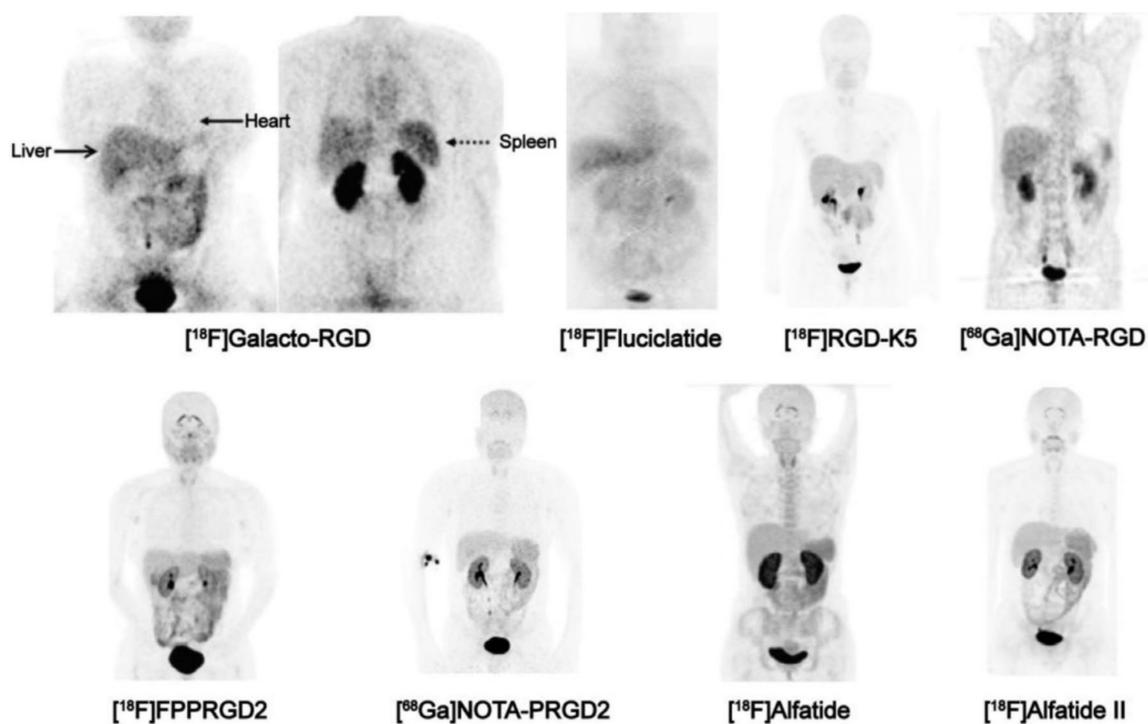


Figure 3. Biodistribution of PET imaging agents based on RGD peptides, 1 h p.i., in healthy human subjects (except [^{18}F]Galacto-RGD, which shows a patient with osteomyelitis). Images in the upper row were obtained with monomeric agents (i.e., comprising only one RGD per molecule), the bottom row with dimers (two RGD per molecule). Note the typical pattern of physiological uptake in liver, spleen, intestines, thyroid, and plexus choroideus, arising from low-level $\alpha v\beta 3$ -integrin expression in these organs. The pattern is more clearly discernible for the dimers (bottom row), because their generally higher affinity [45] causes a higher sensitivity and thus, a higher uptake in tissues with low $\alpha v\beta 3$ expression density. Strong signals in kidneys and urinary bladder are caused by renal excretion of all tracers. Copyright notice: Figure reprinted from *Theranostics* 2016;6:78–92. Chen et al., *Clinical Application of Radiolabeled RGD Peptides for PET Imaging of Integrin $\alpha v\beta 3$* [30]; under Creative Commons CC BY 4.0.

Targeted radionuclide therapy using popular radiometals like ^{177}Lu , ^{90}Y , or ^{225}Ac [46] therefore bears a considerable risk of critical off-target doses and, therefore, radiotoxicity. These circumstances might eventually have impeded a notable clinical impact; at least,

we are not aware of a broad clinical application of $\alpha v\beta 3$ -integrin targeted radionuclide therapeutics, regardless of compound structure or radionuclide. The same applies for tumor imaging with RGD-peptide based PET or SPECT imaging agents. In view of the fact that many attempts have been made but apparently none resulted in a broad clinical success or the approval of a pertinent radiopharmaceutical by the FDA or the EMA, we conclude that $\alpha v\beta 3$ -integrin is almost certainly not an optimal target for radionuclide theranostics.

However, we believe that there is still a considerable potential of $\alpha v\beta 3$ -integrin imaging, provided the study design of future clinical trials goes beyond the popular “if you can see it, you can treat it” theranostic approach. $\alpha v\beta 3$ -Integrin has not only been suggested as a predictive marker for many cancers [5,19,47], but also for non-oncological conditions, such as evaluation of cardiac remodeling [48–50] and plaque vulnerability [51,52], or rheumatoid arthritis [53,54]. Future studies seeking to establish clinical applications for $\alpha v\beta 3$ -integrin PET might therefore be successful if they are based on a robust biochemical rationale that truly reflects the complexity of $\alpha v\beta 3$ -dependent processes, and address a real clinical need, i.e., enable an improved, image-based clinical decision making for a relevant medical question [47].

3. $\alpha v\beta 6$ -Integrin—A Rising Star?

3.1. Relevance of $\alpha v\beta 6$ -Integrin for TGF- β Activation, Fibrosis, and Carcinoma Invasiveness

Other than $\alpha v\beta 3$, $\alpha v\beta 6$ -integrin is exclusively expressed by epithelial cells [55]. The search for ligands targeting this integrin has been pursued already for a long time because of its key function as an activator of transforming growth factor β (TGF- β) [56]. TGF- β , in turn, is a pleiotropic cytokine which is highly conserved across species and produced by virtually all mammalian cells. TGF- β is involved in a large number of human pathologies [57], rendering it a highly relevant pharmacological target and, as such, a subject of intense research [58]. Most importantly, TGF- β is a universal growth suppressor which regulates gene transcription, DNA duplication and, thus, cell proliferation (mitosis) via smad-dependent signaling—not only for resident cells but also immune cells, such as leukocytes. As such, it is essential for tissue homeostasis, suggesting that pathologically low systemic TGF- β levels may be associated with autoimmune diseases and a higher probability of developing cancer. TGF- β overabundance or a loss of essential components in the respective signaling cascade may therefore trigger a broad variety of disease morphologies, such as fibrosis, atherosclerosis, and tumorigenesis, as well as tumor invasion and metastasis [57].

However, TGF- β is not secreted by cells into the interstitium in its active form (i.e., freely diffusible and able to bind to its receptors). Cells rather produce and sequester a complex with another protein called latency-associated peptide (LAP) [59]. Briefly, this aggregate named small latent complex (SLC) is linked to the ECM by another protein referred to as latent TGF- β binding protein (LTBP), and TGF- β must be released from the entire prodomain, called large latent complex (LLC), to take action. This activation is a key functionality of $\alpha v\beta 6$ -integrin [60]. Figure 4 illustrates that on a molecular level, this process occurs by binding of the integrin to an RGD motif in LAP; then, a pulling force is exerted via the $\beta 6$ -subunit which is intracellularly bound to the actin filaments forming the cytoskeleton [61]. The force changes the structure of the ‘envelope’ LAP, the inter-protein binding forces are weakened, and TGF- β is released [9].

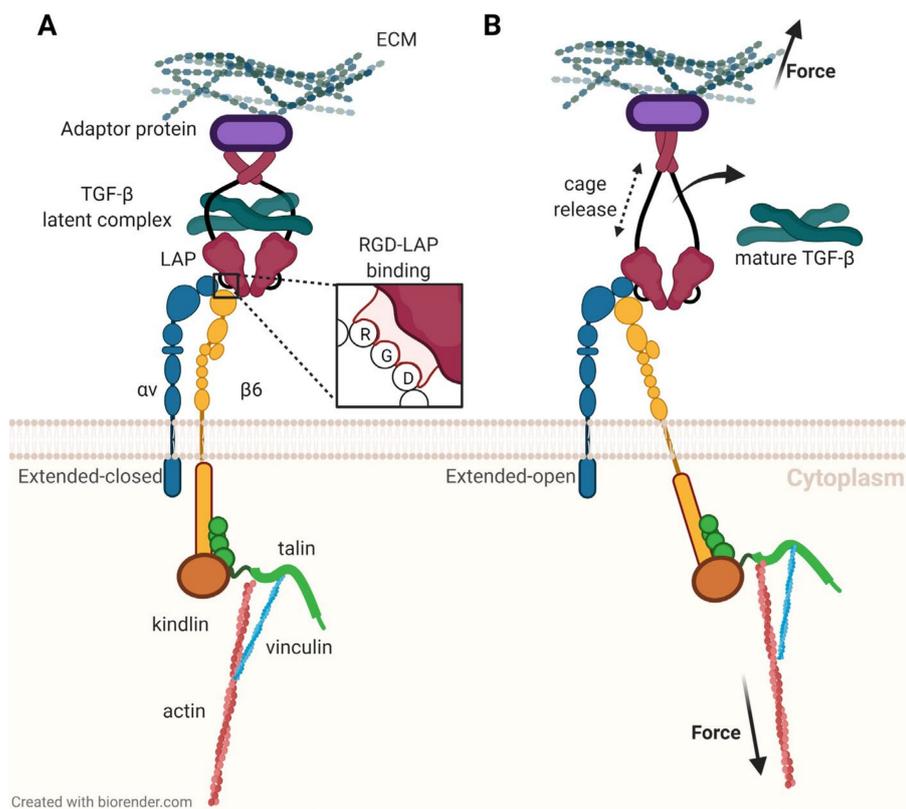


Figure 4. Activation of latent transforming growth factor β (TGF- β) by interaction of $\alpha v\beta 6$ -integrin with latency-associated peptide (LAP), according to a study by Dong et al. [61]. (A): $\alpha v\beta 6$ -integrin binds to an RGD motif in LAP. (B): A pulling force transmitted by the $\beta 6$ -subunit deforms LAP and releases TGF- β .

Overexpression of $\alpha v\beta 6$ -integrin therefore endows cells with the capability to enhance TGF- β levels in their vicinity, which is why its expression is tightly linked to diseases associated with TGF- β dysregulation. Assuming that, e.g., tumor cells have become insensitive to the antiproliferative signaling of TGF- β due to loss of smad4 [62] or p53 [63], a high TGF- β concentration actually promotes their proliferation and invasion of the surrounding tissue [64], because it suppresses the growth of normal cells, suppresses the immune system's antitumor activity [65], triggers epithelial–mesenchymal transition (EMT) [66], and promotes angiogenesis. In accordance with this notion, several immunohistochemical studies have confirmed a high level of $\alpha v\beta 6$ -integrin at the infiltrative margins of epithelial cancers, particularly on carcinoma cells invading the stroma [6,67,68].

Since a therapeutic intervention by systemic inhibition of TGF- β may cause the aforementioned complications associated with low systemic TGF- β [69,70], the targeting of its activators, e.g., $\alpha v\beta 6$ -integrin [71,72], has been suggested as a more site-specific approach [47,67,73]. This strategy is currently pursued for treatment of fibrosis, a class of diseases associated with elevated TGF- β signaling [58] and $\alpha v\beta 6$ -integrin expression [74]. Rather than a systemic TGF- β blockade with, e.g., antibodies, an anti-fibrotic therapy using a small-molecule $\alpha v\beta 6$ -integrin inhibitor has been adapted successfully for control of pulmonary fibrosis [75]. $\alpha v\beta 6$ -Inhibitors have furthermore been suggested as cancer therapeutics [71,76], above all, for pancreatic cancer [72,77], which is characterized by a particularly high $\alpha v\beta 6$ -integrin expression rate. An investigation of nearly 400 patient specimens of pancreatic ductal adenocarcinoma (PDAC) revealed $\beta 6$ positivity in 88% of primaries, in virtually lymph node and distant metastases—and also in many of its immediate precursor lesions, grade 3 pancreatic intraepithelial neoplasia (PanIN3) [78]. The integrin has furthermore been found in high density in many other carcinomas [55], like head-and-neck squamous cell (HNSCC), lung adeno (NSCLC), ovarian, and others,

and furthermore has a prognostic value for some heterogeneous entities, for example, colorectal carcinoma (CRC) [79]. Finally, $\alpha v\beta 6$ -integrin might be a target of interest in the context of COVID-19 [80].

3.2. Towards Clinical Application of $\alpha v\beta 6$ -Integrin Imaging

The described biochemical and clinical background points at an urgent need for $\alpha v\beta 6$ -integrin imaging agents, specifically PET tracers, because the high intrinsic sensitivity and resolution of PET could potentially deliver highly valuable information to advance the clinical management of the aforementioned diseases. This encompasses not only initial diagnostics of cancers and fibrosis based on PET imaging. A personalized management of $\alpha v\beta 6$ -integrin targeted therapies, e.g., the image-based patient stratification, the early assessment of the efficacy of anti-fibrotic or anti-cancer drugs in order to adapt and improve dosage schemes, or the early identification of non-responders to save patients from a therapy's side effects by discontinuation, might eventually generate an even higher clinical impact [47]. The search for $\alpha v\beta 6$ -integrin targeted tracers has therefore been initiated quite some time ago [19].

Hausner and colleagues from UC Davis pioneered this field with a report on an ^{18}F labeled $\alpha v\beta 6$ -integrin binding peptide named A20FMDV (sequence: NAVPNLRGDLQVLAQK-VART) and its use for PET imaging of tumor xenografts in mice [81]. The same group continued with preclinical optimization of the radiolabeled A20FMDV derivatives, e.g., by examining the effect of PEG28-linkers on both termini of the peptide [82,83], and investigated other labeling approaches, e.g., fluorination by means of strain-promoted click chemistry (SPAAC) [84] or AIF chemistry [85]. They ultimately selected the best-performing candidate for translation, which was [^{18}F]fluorobenzoyl-PEG28-A20FMDV-PEG28, renamed it to [^{18}F] $\alpha v\beta 6$ -BP (an abbreviation of [^{18}F] $\alpha v\beta 6$ binding peptide), and tested it for PET imaging of lung adenocarcinoma and breast carcinoma in humans [86]. The same compound was used for imaging of lung fibrosis in humans [87] and subsequently for monitoring the therapeutic effect of an inhaled small-molecule $\alpha v\beta 6$ -integrin inhibitor named GSK3008348 [88], and recently tested for PET/CT of SARS-CoV-2 infection related lesions in the lung [89].

Another approach was pursued by Kimura and colleagues from Stanford, who developed a series of selective $\alpha v\beta 6$ -integrin ligands based on an engineered peptide knot scaffold with three cysteine bridges, referred to as cysteine knot peptides or 'knottins' [90–92]. Eventually, ^{68}Ga and ^{18}F -labeled PET tracers based on one of the peptides, referred to as R₀1-MG, were characterized in tumor-xenografted mice, and utilized for imaging of pancreatic, cervical and lung cancer, as well as of idiopathic pulmonary fibrosis (IPF) [93].

Researchers from Heidelberg University (Germany) exploited a phage-display engineering approach on an established cysteine-bridged scaffold, the sunflower trypsin inhibitor. They developed two $\alpha v\beta 6$ -integrin targeted peptides named SFITGv6 (sequence: GRCRFRGDLMQLCYPD) [94] and SFLAP3 (sequence: GRCTGRGDLGRLCYPD) [95]. Both peptides were conjugated to DOTA and labeled with ^{68}Ga , and used for PET-imaging of patients with HNSCC [95], hypopharynx tumor, [94], NSCLC [94,96], and pancreatic cancer [97].

Researchers from Beijing recently introduced ^{68}Ga -cycratide, a ^{68}Ga -labeled DOTA conjugate of a cyclic peptide with the sequence c(RGDLATLK). They reported in-vitro as well as preclinical data in tumor-bearing mice, and, furthermore, performed PET imaging in five healthy volunteers and two pancreatic cancer patients [98].

At TU Munich (Germany), Kessler and coworkers developed a class of cyclic nonapeptides with a high $\alpha v\beta 6$ -integrin selectivity and metabolic stability [99]. The most selective structure with the sequence c(FRGDLAFp(NMe)K) was conjugated to the ^{68}Ga chelator TRAP [100] by means of Cu^{II} -catalyzed azide-alkyne cycloaddition (CuAAC) [101,102], giving rise to monomeric, dimeric, and trimeric conjugates [103]. A preclinical characterization in tumor-xenografted mice revealed that the multimers exhibited extraordinarily high affinities (IC_{50} of 260 and 23 pM for the monomer and the trimer, respectively). The trimer

unfortunately showed a high degree of nonspecific uptake in the bowel organs (intestines and liver), whereas various monomeric conjugates showed insufficient tumor accumulation [104]. A revision of the peptide sequence finally led to the development of Trivehexin, a TRAP-based trimer of c(YRGDLAYp(NMe)K) [105]. ^{68}Ga -Trivehexin was subsequently tested for imaging of patients with PDAC [106] as well as HNSCC and adenocarcinoma of the parotid duct [105].

3.3. Clinical $\alpha\nu\beta6$ -Integrin PET for Cancer Imaging

To the best of our knowledge, no more than the above-mentioned, radiolabeled $\alpha\nu\beta6$ -integrin specific ligands have been investigated in humans so far, although other highly promising radiotracers have been synthesized and characterized preclinically [107–110]. The already published clinical data nonetheless allow for a glance into the future and illustrate the potential of this class of PET imaging agents.

Overall, the hitherto translated PET tracers show predominantly renal clearance; hence, a strong signal is invariably observed in the kidneys and the urinary bladder (Figures 5 and 6). In addition, some of the agents show more or less prominent non-specific uptake in several organs, most notably, in stomach and intestines. Figure 5 demonstrates that $\alpha\nu\beta6$ -integrin imaging might nevertheless be suitable for visualization of a variety of cancers. This is in accordance with a solid body of evidence that this integrin is expressed in many carcinomas [55]. A particularly high-expression density and, therefore, a high diagnostic relevance of pertinent imaging agents, has been suggested for HNSCC [55]. In line with this notion, a good tumor delineation could be observed for a large sublingual carcinoma using ^{68}Ga -Trivehexin (Figure 5C), and for a hypopharynx tumor using ^{68}Ga -DOTA-SFITGv6 (Figure 5A).

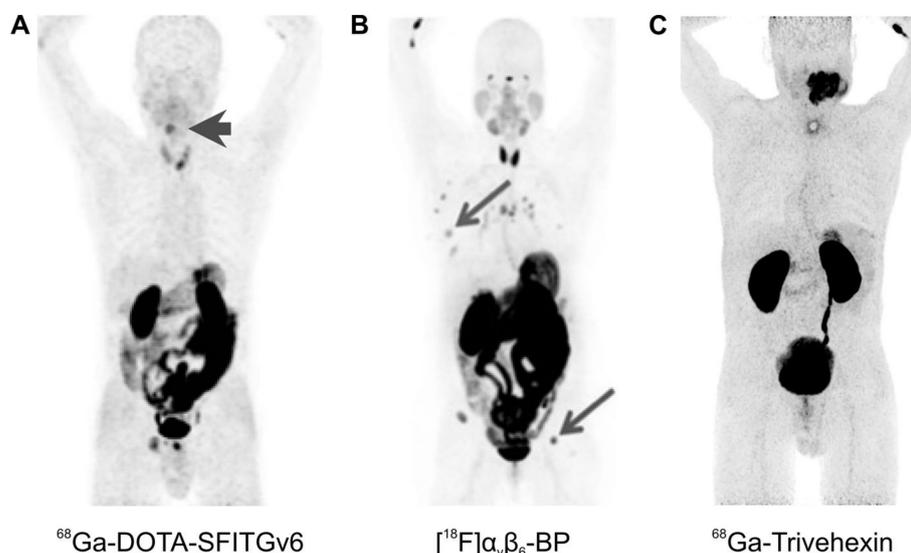


Figure 5. $\alpha\nu\beta6$ -integrin PET imaging in cancer patients (maximum intensity projections, 1 h p.i.) with different radiopharmaceuticals; arrows (wherever shown) indicate tumor lesions. (A): ^{68}Ga -DOTA-SFITGv6 PET of a recurrent hypopharynx tumor. (B): A stage IV invasive mammary carcinoma, imaged using ^{18}F $\alpha\nu\beta6$ -BP. (C): ^{68}Ga -Trivehexin PET of a large oral squamous cell carcinoma. Copyright notice: Images were adapted and reprinted from: (A) Clin. Cancer Res. 2017, 23, 4170–4180. Altmann et al., Identification of a Novel ITG $\alpha\nu\beta6$ -Binding Peptide Using Protein Separation and Phage Display [94]; and (B) Clin. Cancer Res. 2019, 25, 1206–1215; Hausner et al., Preclinical Development and First-in-Human Imaging of the Integrin $\alpha\nu\beta6$ with ^{18}F $\alpha\nu\beta6$ -Binding Peptide in Metastatic Carcinoma [86]; with permission from AACR and not included in CC-BY 4.0 of this article. (C) Eur. J. Nucl. Med. Mol. Imaging 2021, doi:10.1007/s00259-021-05559-x. Quigley et al., PET/CT imaging of head-and-neck and pancreatic cancer in humans by targeting the “Cancer Integrin” $\alpha\nu\beta6$ with Ga-68-Trivehexin [105]; under Creative Commons CC BY 4.0.

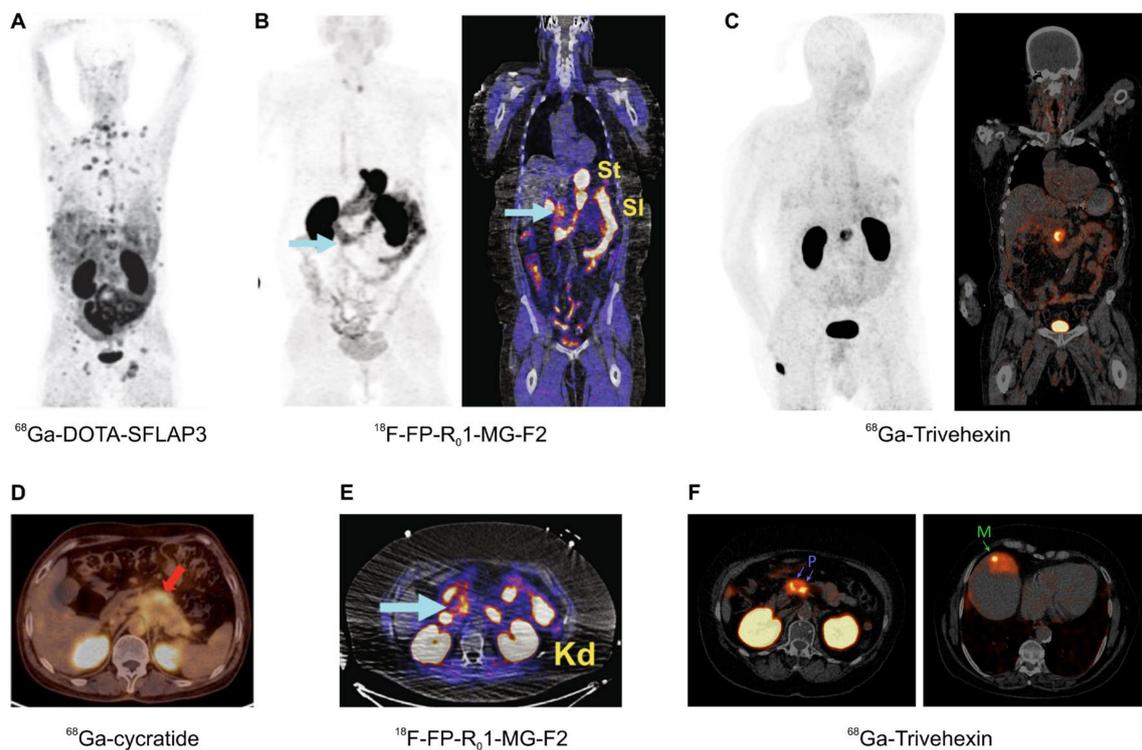


Figure 6. $\alpha\beta6$ -integrin PET/CT imaging in pancreatic cancer (MIPs (A–C), coronal slices (B,C), and axial slices (D–F); (A–E), 1 h p.i.; F, 2 h p.i.); arrows (wherever shown) indicate tumor lesions. (A): Highly metastatic pancreatic carcinoma, imaged using ⁶⁸Ga-DOTA-SFLAP3. (B,E): MIP and fusion images of a non-metastatic pancreatic carcinoma, obtained using the knottin peptide based tracer ¹⁸F-FP-R₀1-MG-F2. (C,F): ⁶⁸Ga-Trivehexin PET/CT of a non-metastatic (C) and metastatic (F) PDAC (“P” indicates the primaries, “M” a liver metastasis). (D): Axial fusion PET/CT of a pancreatic cancer obtained with ⁶⁸Ga-cycratide. Copyright notice: Images were adapted and reprinted from (A) Nuklearmedizin 2019, 58, 309–18. Müller et al., Preclinical evaluation of peptide-based radiotracers for integrin $\alpha\beta6$ -positive pancreatic carcinoma. [97] © Georg Thieme Verlag KG. (B,E) Nat. Commun. 2019, 10, 4673. Kimura et al., Evaluation of integrin $\alpha\beta6$ cystine knot PET tracers to detect cancer and idiopathic pulmonary fibrosis [93]; (C) Eur. J. Nucl. Med. Mol. Imaging 2021, 48, 4107–4108. Quigley et al., PET/CT imaging of pancreatic carcinoma targeting the “cancer integrin” $\alpha\beta6$ [106]; and (F) Eur. J. Nucl. Med. Mol. Imaging 2021, doi:10.1007/s00259-021-05559-x. Quigley et al., PET/CT imaging of head-and-neck and pancreatic cancer in humans by targeting the “Cancer Integrin” $\alpha\beta6$ with Ga-68-Trivehexin [105]; under Creative Commons CC BY 4.0. (D) This research was originally published in JNM. Feng et al., Clinical Translation of a ⁶⁸Ga-Labeled Integrin $\alpha\beta6$ -Targeting Cyclic Radiotracer for PET Imaging of Pancreatic Cancer. J. Nucl. Med. 2020;61:1461–1467. [98] © SNMMI.

The most promising field of application arguably is the imaging of pancreatic cancer, because PET imaging of this entity is not reliably possible with the standard tracer [¹⁸F]FDG [111]. This applies all the more because PDAC is one of the carcinomas with the worst prognosis, and treatment options are still limited as compared to other malignant cancers, such as prostate carcinoma. A powerful agent for imaging of PDAC might therefore not only be important for diagnostics, e.g., to improve planning of surgery, but also implicates a perspective for future development of targeted radiotherapeutics. An overview of $\alpha\beta6$ -integrin targeted PET/CT with different tracers (Figure 6) corroborates the feasibility of this approach, not only for imaging of PDAC primaries but also of small liver metastases thereof (Figure 6F).

The hitherto reported clinical $\alpha\beta6$ -integrin PET data comprise only a few cases and thus, limited conclusions can be drawn at this stage. The available images of cancers nonetheless clearly indicate a high potential and might, therefore, boost the research in this field, and, above all, trigger the development of the respective radiotherapeutics labeled with the commercially available beta emitter ¹⁷⁷Lu, or emerging alpha emitters like ²²⁵Ac [46]. This, in turn, is a hopeful perspective for many of pancreatic cancer patients

who, in view of a very short overall survival after diagnosis, are in desperate need for novel, improved therapies.

4. $\alpha\nu\beta 8$ -Integrin PET—A Solution without a Problem?

The integrin subunit $\beta 8$ was discovered as the last among the five β subunits which exclusively dimerize with $\alpha\nu$ [112]. $\alpha\nu\beta 8$ -Integrin appears to be the odd one out, as considerably less is known about this dimer than about the other $\alpha\nu$ integrins [5,19]. It is predominantly expressed on astrocytes and, just like $\alpha\nu\beta 6$, is an activator of TGF- β , although by a different mechanism. In contrast to $\alpha\nu\beta 6$ -integrin which releases TGF- β by exerting a pulling force on LAP (vide supra), $\alpha\nu\beta 8$ also recognizes and deforms LAP, but without cleaving the SLC. Instead, the binding of $\alpha\nu\beta 8$ leads to activation of TGF- β without releasing it from the LAP, by exposing the receptor-binding sites of TGF- β [113]. Irrespective of the activation mode, one would thus expect comparable diagnostic and therapeutic implications for $\alpha\nu\beta 6$ and $\alpha\nu\beta 8$, i.e., a strong connection to TGF- β -driven disease patterns. Expression and functions of $\alpha\nu\beta 8$ -integrin in cancer and fibrosis has been discussed as well, but there is less evidence for a clinical relevance than in the case of $\alpha\nu\beta 6$ -integrin [19]. A high proportion of $\beta 8$ positive tumor cells was recently detected in various carcinomas (ovarian, uterine endometrioid, skin, in situ breast ductal, gastric adenocarcinoma, and, particularly, oral squamous cell carcinoma) by histological methods, but the small numbers of patient specimens (3–22 per entity) still leave some doubt whether $\alpha\nu\beta 8$ -integrin could actually be useful for cancer imaging or theranostics [114]. A very intriguing discovery was recently made by Takasaka and colleagues, who found that up-regulation of $\alpha\nu\beta 8$ -integrin is apparently another mechanism for immune evasion of tumor cells. Blockade of this integrin has been shown to potentiate a cytotoxic T cell response in tumors, independently of the PD1–PD-L1 axis, thus suggesting $\alpha\nu\beta 8$ -integrin as a target for immune checkpoint therapy [114].

Since the wealth of information about $\alpha\nu\beta 3$ -integrin is most likely a result of the long-term availability of small-molecule inhibitors, i.e., cRGD peptides [10], the comparably low amount of knowledge about $\alpha\nu\beta 8$ -integrin might, likewise, mainly be a result of a lack of respective ligands. This situation has changed recently with the discovery of a small, selective, and stable ligand for $\alpha\nu\beta 8$ [115]. The cyclic octapeptide with the sequence c(GLRGDLp(NMe)K) was subsequently—in analogy to the above-mentioned ^{68}Ga -Trivehexin—trimerized on the TRAP chelator scaffold, in order to generate a practicable PET radiopharmaceutical named ^{68}Ga -Triveoctin [116]. Trimerization [6] again greatly increased the affinity, and the encouraging preclinical results prompted a clinical translation.

Figure 7 shows the first—and, currently, the only— $\alpha\nu\beta 8$ -integrin PET image acquired in human. There is apparently neither a strong specific nor a non-specific uptake in any major organ, apart from a strong signal in kidneys and bladder due to renal excretion. The notion of $\beta 8$ -integrin expression on neural cells (astrocytes) might nonetheless be connected to a substantial uptake in the plexus choroideus and -coeliacus [115]. However, due to a lack of pertinent immunohistochemistry data, no definitive statement can currently be made concerning this observation, and any conclusion would be premature. This situation is quite typical for the entire field of $\alpha\nu\beta 8$ -integrin imaging—many hypotheses are not yet supported with enough data and experience. It is nevertheless expected that the knowledge about the in vivo expression patterns of this integrin will substantially grow in the near future, because an imaging tool is now available.

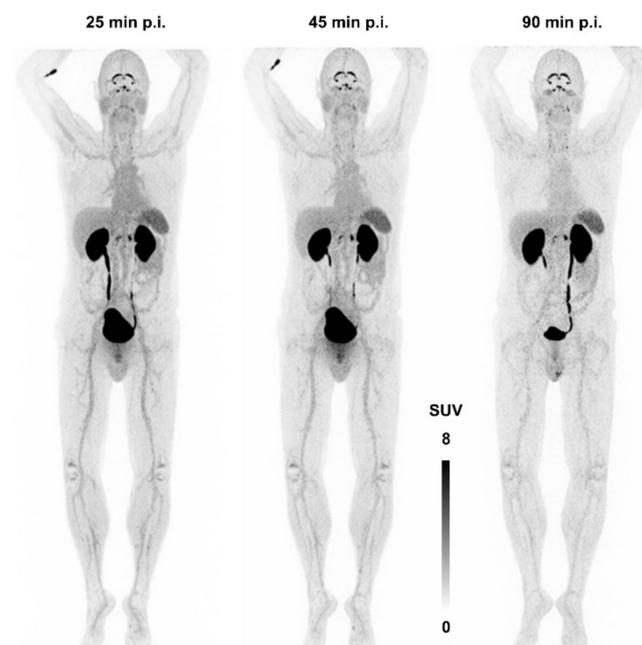


Figure 7. $\alpha v\beta 8$ -integrin PET imaging (maximum intensity projection) in human using ^{68}Ga -Triveoctin. Copyright notice: Figure reprinted from *EJNMMI Res.* 2020, 10, 133. Quigley et al., Tracking a TGF- β activator in vivo: sensitive PET imaging of $\alpha v\beta 8$ -integrin with the Ga-68-labeled cyclic RGD octapeptide trimer Ga-68-Triveoctin [116]; under Creative Commons CC BY 4.0.

5. Translation of Radiopharmaceuticals Targeting Other Integrins

The largest part of radioligands was reported for the integrins discussed above, and comparably few attempts have been made to establish other integrins as targets for radio-theranostics. These include the laminin receptors $\alpha 6\beta 1$, $\alpha 6\beta 4$, and $\alpha 3\beta 1$ [117], the collagen receptor $\alpha 2\beta 1$ [118], as well as the leucocyte-specific receptors $\alpha 4\beta 1$ [119–121] and $\alpha M\beta 2$ [122,123]. The laminin receptors $\alpha 6\beta 4$ and $\alpha 6\beta 1$ arguably have the largest potential next to RGD receptors, because they are expressed by several cancers such as colorectal, squamous cell, small cell lung, breast, pancreatic, and prostate carcinoma, while their expression has been linked to poor survival [124]. $\alpha 6\beta 4$ signaling in malignant cells cooperates with growth factor receptors and amplification of well-known tumor-promoting pathways, such as PI3K, AKT, and MAPK, thus resulting in, for example, proliferative signaling, tumor invasion, metastasis, evasion of apoptosis, and stimulation of angiogenesis. Expression of $\alpha 6$ -integrin in general, and the dimer $\alpha 6\beta 1$ in particular, has been linked to poor prognosis of breast cancer and hepatocellular carcinoma [125].

An ^{18}F -labeled derivative of the peptide sequence CRWYDENAC (referred to as RWY) was reported as the first $\alpha 6$ -integrin PET tracer and preclinically characterized in mouse models of hepatocellular cancer [126]. Of note, this peptide is apparently not selective for a single integrin but binds to both $\alpha 6\beta 4$ and $\alpha 6\beta 1$, which somewhat limits the strength of the rationale required for a meaningful interpretation of in vivo signals. It can be assumed that selective targeting has not yet been achieved due to structural similarity of both integrins' binding sites and/or the conformational flexibility of the linear amino acid sequence. This peptide was nonetheless applied for first-in-human imaging in form of the monomeric SPECT tracer $^{99\text{m}}\text{Tc}$ -RWY, which was able to clearly delineate tumor lesions in two breast cancer patients [127]. Compared to the single-digit nanomolar and even low picomolar affinity of other clinically applied integrin-targeted radioligands, the affinities of the investigated $\alpha 6$ -tracers were, however, fairly low (0.2–1.5 μM). Furthermore, no data on $\alpha 6\beta 4$ vs. $\alpha 6\beta 1$ selectivity were reported. It thus remains unclear to what extent the observed tumor uptake is causally related to expression of $\alpha 6$ -integrins at all and, if so, to which of the two possible dimers. Improved versions of the same peptide were developed

as well [128], but their affinity for $\alpha 6$ still remained in the micromolar range (1354 nM) and in-human data has, to the best of our knowledge, not been reported yet.

A similar situation is encountered for radiotracers targeting $\alpha 5\beta 1$ -integrin. The bis-specific cyclic peptide c(phg-isoD-G-R-k) [129], which shows a high affinity for both of the functionally different integrins $\alpha 5\beta 1$ [7] and $\alpha v\beta 6$ [59], was recently labeled with ^{99m}Tc via the HYNIC approach and tested for SPECT imaging of pancreatic carcinoma in humans [130]. Further studies are required to firmly establish its clinical value for cancer imaging—but the intrinsic problems of a weak biochemical rationale, i.e., the inability of the tracer to distinguish between both addressed integrins and, thus, between fundamentally different tumorigenic pathways, will remain. Interestingly, it was reported already several years ago that *N*-methylation of this peptide at the D-Lys position eliminated its $\alpha v\beta 6$ -integrin affinity, resulting in the $\alpha 5\beta 1$ -integrin specific peptide c(phg-isoD-G-R-(NMe)k) [131]. A respective ^{68}Ga -labeled conjugate showed promise for $\alpha 5\beta 1$ -integrin targeted PET imaging in rodents [131], but similar to other highly specific $\alpha 5\beta 1$ -integrin radiotracers such as ^{68}Ga -AQUIBEPRIN [132,133], no first-in-human data were reported yet.

6. Conclusions

A thorough look at the latest translational research in the area of integrin-targeting radiopharmaceuticals reveals that we are currently witnessing a paradigm change. During the last two decades, the pertinent studies were strongly focused on the subtype $\alpha v\beta 3$ and its most popular biochemical characteristics—its presence on endothelial cells and overexpression in the course of angiogenesis. It appears that the focus of interest is now moving away from $\alpha v\beta 3$ -integrin and angiogenesis towards other integrin subtypes which are involved in a large variety of tumorigenic pathways. Peptidic and non-peptidic radioligands for the integrins $\alpha 5\beta 1$, $\alpha v\beta 6$, $\alpha v\beta 8$, $\alpha 6\beta 1$, $\alpha 6\beta 4$, $\alpha 3\beta 1$, $\alpha 4\beta 1$, and $\alpha M\beta 2$ were synthesized and characterized in rodent models. Integrin tracers targeting the subtypes $\alpha v\beta 6$, $\alpha v\beta 8$, and $\alpha 6\beta 1/\beta 4$ were tested in humans. The largest number of recent first-in-human applications has been reported for radiolabeled compounds targeting $\alpha v\beta 6$ -integrin, owing to its well-established role as a biomarker for malignancy and invasiveness of carcinomas as well as its known overexpression by the most deadly cancers. Of all integrins, the subtype $\alpha v\beta 6$ is arguably the most promising target structure for radiotheranostics, because its clinical scope is not restricted to oncological applications but also encompasses fibrotic diseases such as idiopathic pulmonary fibrosis (IPF), and probably even COVID-19 related syndromes.

Author Contributions: Conceptualization, S.K. and J.N.; writing, S.K., A.J.B. and J.N.; All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the Deutsche Forschungsgemeinschaft (CRC 824).

Conflicts of Interest: J.N. is a co-inventor of patents related to Ga-68-Trivehexin. In addition, J.N. is a shareholder and Chief Science Officer of TRIMT GmbH (Radeberg, Germany), and a member of the Scientific Advisory Board of Radiopharm Theranostics (Carlton, Australia). S.K. receives research funding from TRIMT GmbH. A.J.B. declares that there are no conflicts of interest.

References

1. Barczyk, M.; Carracedo, S.; Gullberg, D. Integrins. *Cell Tissue Res.* **2010**, *339*, 269–280. [[CrossRef](#)]
2. Kechagia, J.Z.; Ivaska, J.; Roca-Cusachs, P. Integrins as biomechanical sensors of the microenvironment. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 457–473. [[CrossRef](#)]
3. Berman, A.E.; Kozlova, N.I.; Morozovich, G.E. Integrins: Structure and signaling. *Biochemistry* **2003**, *68*, 1284–1299. [[CrossRef](#)]
4. Campbell, I.D.; Humphries, M.J. Integrin structure, activation, and interactions. *Cold Spring Harb. Perspect. Biol.* **2011**, *3*, a004994. [[CrossRef](#)]
5. Ludwig, B.S.; Kessler, H.; Kossatz, S.; Reuning, U. RGD-binding integrins revisited: How recently discovered functions and novel synthetic ligands (Re-)shape an ever-evolving field. *Cancers* **2021**, *13*, 1711. [[CrossRef](#)]
6. Steiger, K.; Quigley, N.G.; Groll, T.; Richter, F.; Zierke, M.A.; Beer, A.J.; Weichert, W.; Schwaiger, M.; Kossatz, S.; Notni, J. There is a world beyond $\alpha v\beta 3$ -integrin: Multimeric ligands for imaging of the integrin subtypes $\alpha v\beta 6$, $\alpha v\beta 8$, $\alpha v\beta 3$, and $\alpha 5\beta 1$ by positron emission tomography. *EJNMMI Res.* **2021**, *11*, 106. [[CrossRef](#)]

7. Kim, S.; Bell, K.; Mousa, S.A.; Varner, J.A. Regulation of angiogenesis in vivo by ligation of integrin $\alpha 5\beta 1$ with the central cell-binding domain of fibronectin. *Am. J. Pathol.* **2000**, *156*, 1345–1362. [\[CrossRef\]](#)
8. Brown, N.F.; Marshall, J.F. Integrin-mediated TGF β activation modulates the tumour microenvironment. *Cancers* **2019**, *11*, 1221. [\[CrossRef\]](#)
9. Ha, T. Growth factor rattled out of its cage. *Nature* **2017**, *542*, 40–41. [\[CrossRef\]](#)
10. Schottelius, M.; Laufer, B.; Kessler, H.; Wester, H.J. Ligands for mapping $\alpha_v\beta_3$ -integrin expression in vivo. *Acc. Chem. Res.* **2009**, *42*, 969–980. [\[CrossRef\]](#)
11. Gärtner, F.C.; Kessler, H.; Wester, H.J.; Schwaiger, M.; Beer, A.J. Radiolabelled RGD peptides for imaging and therapy. *Eur. J. Nucl. Med. Mol. Imaging* **2012**, *39*, S126–S138. [\[CrossRef\]](#)
12. Haubner, R.; Maschauer, S.; Prante, O. PET radiopharmaceuticals for imaging integrin expression: Tracers in clinical studies and recent developments. *Biomed. Res. Int.* **2014**, *2014*, 871609. [\[CrossRef\]](#)
13. Aumailley, M.; Gurrath, M.; Müller, G.; Calvete, J.; Timpl, R.; Kessler, H. Arg-Gly-Asp constrained within cyclic pentapeptides—strong and selective inhibitors of cell-adhesion to vitronectin and laminin fragment-P1. *FEBS Lett.* **1991**, *291*, 50–54. [\[CrossRef\]](#)
14. Pierschbacher, M.D.; Ruoslahti, E. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature* **1984**, *309*, 30–33. [\[CrossRef\]](#)
15. Brooks, P.C.; Clark, R.A.F.; Cheres, D.A. Requirement Of vascular integrin $\alpha_v\beta_3$ For angiogenesis. *Science* **1994**, *264*, 569–571. [\[CrossRef\]](#)
16. Avraamides, C.J.; Garmy-Susini, B.; Varner, J.A. Integrins in angiogenesis and lymphangiogenesis. *Nat. Rev. Cancer* **2008**, *8*, 604–617. [\[CrossRef\]](#)
17. Liu, Z.; Wang, F.; Chen, X. Integrin $\alpha_v\beta_3$ -targeted cancer therapy. *Drug Dev. Res.* **2008**, *69*, 329–339. [\[CrossRef\]](#)
18. Marelli, U.K.; Rechenmacher, F.; Sobahi, T.R.A.; Mas-Moruno, C.; Kessler, H. Tumor targeting via integrin ligands. *Front. Oncol.* **2013**, *3*, 222. [\[CrossRef\]](#)
19. Nieberler, M.; Reuning, U.; Reichart, F.; Notni, J.; Wester, H.J.; Schwaiger, M.; Weinmüller, M.; Räder, A.; Steiger, K.; Kessler, H. Exploring the role of RGD-recognizing integrins in cancer. *Cancers* **2017**, *9*, 116. [\[CrossRef\]](#)
20. Eo, J.S.; Jeong, J.M. Angiogenesis imaging using Ga-68-RGD PET/CT: Therapeutic implications. *Semin. Nucl. Med.* **2016**, *46*, 419–427. [\[CrossRef\]](#)
21. Bader, B.L.; Rayburn, H.; Crowley, D.; Hynes, R.O. Extensive vasculogenesis, angiogenesis, and organogenesis precede lethality in mice lacking all α_v integrins. *Cell* **1998**, *95*, 507–519. [\[CrossRef\]](#)
22. Reynolds, L.E.; Wyder, L.; Lively, J.C.; Taverna, D.; Robinson, S.D.; Huang, X.; Sheppard, D.; Hynes, R.O.; Hodivala-Dilke, K.M. Enhanced pathological angiogenesis in mice lacking β_3 integrin or β_3 and β_5 integrins. *Nat. Med.* **2002**, *8*, 27–34. [\[CrossRef\]](#)
23. Reynolds, A.R.; Reynolds, L.E.; Nagel, T.E.; Lively, J.C.; Robinson, S.D.; Hicklin, D.J.; Bodary, S.C.; Hodivala-Dilke, K.M. Elevated Flk1 (vascular endothelial growth factor receptor 2) signaling mediates enhanced angiogenesis in β_3 -integrin deficient mice. *Cancer Res.* **2004**, *64*, 8643–8650. [\[CrossRef\]](#)
24. Brown, E.J. Integrins of macrophages and macrophage-like cells. In *The Macrophage as Therapeutic Target, Handbook of Experimental Pharmacology*; Gordon, S., Ed.; Springer: Berlin/Heidelberg, Germany, 2003; Volume 158, pp. 111–130.
25. Sofias, A.M.; Bjørkøy, G.; Ochando, J.; Sønstevold, L.; Hegvik, M.; de Lange Davies, C.; Haraldseth, O.; Lammers, T.; Mulder, W.J.M.; Hak, S. Cyclic arginine–glycine–aspartate-decorated lipid nanoparticle targeting toward inflammatory lesions involves hitchhiking with phagocytes. *Adv. Sci.* **2021**, *8*, 2100370. [\[CrossRef\]](#)
26. Heckmann, D.; Laufer, B.; Marinelli, L.; Limongelli, V.; Novellino, E.; Zahn, G.; Stragies, R.; Kessler, H. Breaking the dogma of the metal-coordinating carboxylate group in integrin ligands: Introducing hydroxamic acids to the MIDAS to tune potency and selectivity. *Angew. Chem. Int. Ed.* **2009**, *48*, 4436–4440. [\[CrossRef\]](#)
27. Rechenmacher, F.; Neubauer, S.; Polleux, J.; Mas-Moruno, C.; De Simone, M.; Cavalcanti-Adam, E.A.; Spatz, J.P.; Fässler, R.; Kessler, H. Functionalizing $\alpha_v\beta_3$ - or $\alpha_5\beta_1$ -selective integrin antagonists for surface coating: A method to discriminate integrin subtypes in vitro. *Angew. Chem. Int. Ed.* **2013**, *52*, 1572–1575. [\[CrossRef\]](#)
28. Kapp, T.G.; Rechenmacher, F.; Neubauer, S.; Maltsev, O.V.; Cavalcanti-Adam, E.A.; Zarka, R.; Reuning, U.; Notni, J.; Wester, H.-J.; Mas-Moruno, C.; et al. A Comprehensive evaluation of the activity and selectivity profile of ligands for RGD-binding integrins. *Sci. Rep.* **2017**, *7*, 39805. [\[CrossRef\]](#)
29. Kossatz, S.; Notni, J. NIR fluorescence imaging of colon cancer with cRGD-ZW800-1—Letter. *Clin. Cancer Res.* **2021**, *27*, 4937. [\[CrossRef\]](#)
30. Chen, H.; Niu, G.; Wu, H.; Chen, X. Clinical application of radiolabeled RGD peptides for PET imaging of integrin $\alpha_v\beta_3$. *Theranostics* **2016**, *6*, 78–92. [\[CrossRef\]](#)
31. Atkinson, S.J.; Ellison, T.S.; Steri, V.; Gould, E.; Robinson, S.D. Redefining the role(s) of endothelial $\alpha_v\beta_3$ -integrin in angiogenesis. *Biochem. Soc. Trans.* **2014**, *42*, 1590–1595. [\[CrossRef\]](#)
32. Hennrich, U.; Kopka, K. Lutathera[®]: The first FDA- and EMA-approved radiopharmaceutical for peptide receptor radionuclide therapy. *Pharmaceuticals* **2019**, *12*, 114. [\[CrossRef\]](#)
33. Meyer, A.; Auernheimer, J.; Modlinger, A.; Kessler, H. Targeting RGD recognizing integrins: Drug development, biomaterial research, tumor imaging and targeting. *Curr. Pharm. Des.* **2006**, *12*, 2723–2747. [\[CrossRef\]](#)
34. Haubner, R.; Wester, H.J.; Reuning, U.; Senekowitsch-Schmidtke, R.; Diefenbach, B.; Kessler, H.; Stöcklin, G.; Schwaiger, M. Radiolabeled $\alpha_v\beta_3$ integrin antagonists: A new class of tracers for tumor targeting. *J. Nucl. Med.* **1999**, *40*, 1061–1071.

35. Haubner, R.; Wester, H.J.; Burkhart, F.; Senekowitsch-Schmidtke, R.; Weber, W.; Goodman, S.L.; Kessler, H.; Schwaiger, M. Glycosylated RGD-containing peptides: Tracer for tumor targeting and angiogenesis imaging with improved biokinetics. *J. Nucl. Med.* **2001**, *42*, 326–336.
36. Beer, A.J.; Haubner, R.; Göbel, M.; Luderschmidt, S.; Spilker, M.E.; Wester, H.J.; Weber, W.A.; Schwaiger, M. Biodistribution and pharmacokinetics of the $\alpha_v\beta_3$ -selective tracer ^{18}F -galacto-RGD in cancer patients. *J. Nucl. Med.* **2005**, *46*, 1333–1341.
37. Beer, A.J.; Haubner, R.; Sarbia, M.; Göbel, M.; Luderschmidt, S.; Grosu, A.L.; Schnell, O.; Niemeyer, M.; Kessler, H.; Wester, H.J.; et al. Positron emission tomography using ^{18}F -Galacto-RGD identifies the level of integrin $\alpha_v\beta_3$ expression in man. *Clin. Cancer Res.* **2006**, *12*, 3942–3949. [[CrossRef](#)]
38. Haubner, R.; Weber, W.A.; Beer, A.J.; Vabuliene, E.; Reim, D.; Sarbia, M.; Becker, K.F.; Goebel, M.; Hein, R.; Wester, H.J.; et al. Noninvasive visualization of the activated $\alpha_v\beta_3$ integrin in cancer patients by positron emission tomography and [^{18}F]Galacto-RGD. *PLoS Med.* **2005**, *2*, 244–252. [[CrossRef](#)]
39. Zhou, Y.; Gao, S.; Huang, Y.; Zheng, J.; Dong, Y.; Zhang, B.; Zhao, S.; Lu, H.; Liu, Z.; Yu, J.; et al. A pilot study of ^{18}F -alfatide PET/CT imaging for detecting lymph node metastases in patients with non-small cell lung cancer. *Sci. Rep.* **2017**, *7*, 2877. [[CrossRef](#)]
40. Kang, F.; Wang, Z.; Li, G.; Wang, S.; Liu, D.; Zhang, M.; Zhao, M.; Yang, W.; Wang, J. Inter-heterogeneity and intra-heterogeneity of $\alpha_v\beta_3$ in non-small cell lung cancer and small cell lung cancer patients as revealed by ^{68}Ga -RGD₂ PET imaging. *Eur. J. Nucl. Med. Mol. Imaging* **2017**, *44*, 1520–1528. [[CrossRef](#)]
41. Wu, J.; Wang, S.; Zhang, X.; Teng, Z.; Wang, J.; Yung, B.C.; Niu, G.; Zhu, H.; Lu, G.; Chen, X. ^{18}F -alfatide II PET/CT for identification of breast cancer: A preliminary clinical study. *J. Nucl. Med.* **2018**, *59*, 1809–1816. [[CrossRef](#)]
42. Baum, R.P.; Kulkarni, H.R.; Carreras, C. Peptides and receptors in image-guided therapy: Theranostics for neuroendocrine neoplasms. *Semin. Nucl. Med.* **2012**, *42*, 190–207. [[CrossRef](#)]
43. Beer, A.J.; Schwarzenböck, S.M.; Zantl, N.; Souvatzoglou, M.; Maurer, T.; Watzlowik, P.; Kessler, H.; Wester, H.J.; Schwaiger, M.; Krause, B.J. Non-invasive assessment of inter- and inpatient variability of integrin expression in metastasized prostate cancer by PET. *Oncotarget* **2016**, *7*, 28151–28159. [[CrossRef](#)]
44. Beer, A.J.; Kessler, H.; Wester, H.J.; Schwaiger, M. PET imaging of integrin $\alpha_v\beta_3$ expression. *Theranostics* **2011**, *1*, 48–57. [[CrossRef](#)]
45. Liu, S. Radiolabeled cyclic RGD peptides as integrin $\alpha_v\beta_3$ -targeted radiotracers: Maximizing binding affinity via bivalency. *Bioconjugate Chem.* **2009**, *20*, 2199–2213. [[CrossRef](#)]
46. Notni, J.; Wester, H.J. Re-thinking the role of radiometal isotopes: Towards a future concept for theranostic radiopharmaceuticals. *J. Label. Compd. Radiopharm.* **2018**, *61*, 141–153. [[CrossRef](#)]
47. Slack, R.J.; Macdonald, S.J.F.; Roper, J.A.; Jenkins, R.G.; Hatley, R.J.D. Emerging therapeutic opportunities for integrin inhibitors. *Nat. Rev. Drug Discov.* **2021**, *20*. [[CrossRef](#)]
48. Curley, D.; Plaza, B.L.; Shah, A.M.; Botnar, R. Molecular imaging of cardiac remodelling after myocardial infarction. *Basic Res. Cardiol.* **2018**, *113*, 10. [[CrossRef](#)]
49. Sherif, H.M.; Saraste, A.; Nekolla, S.G.; Weidl, E.; Reder, S.; Tapfer, A.; Rudelius, M.; Higuchi, T.; Botnar, R.M.; Wester, H.J.; et al. Molecular imaging of early $\alpha_v\beta_3$ integrin expression predicts long-term left-ventricle remodeling after myocardial infarction in rats. *J. Nucl. Med.* **2012**, *53*, 318–323. [[CrossRef](#)]
50. Laitinen, I.; Notni, J.; Pohle, K.; Rudelius, M.; Farrell, E.; Nekolla, S.G.; Henriksen, G.; Neubauer, S.; Kessler, H.; Wester, H.J.; et al. Comparison of cyclic RGD peptides for $\alpha_v\beta_3$ integrin detection in a rat model of myocardial infarction. *EJNMMI Res.* **2013**, *3*, 38. [[CrossRef](#)]
51. Makowski, M.R.; Rischpler, C.; Ebersberger, U.; Keithahn, A.; Kasel, M.; Hoffmann, E.; Rassaf, T.; Kessler, H.; Wester, H.J.; Nekolla, S.G.; et al. Multiparametric PET and MRI of myocardial damage after myocardial infarction: Correlation of integrin $\alpha_v\beta_3$ expression and myocardial blood flow. *Eur. J. Nucl. Med. Mol. Imaging* **2021**, *48*, 1070–1080. [[CrossRef](#)]
52. Beer, A.J.; Pelisek, J.; Heider, P.; Saraste, A.; Reeps, C.; Metz, S.; Seidl, S.; Kessler, H.; Wester, H.J.; Eckstein, H.H.; et al. PET/CT imaging of integrin $\alpha_v\beta_3$ expression in human carotid atherosclerosis. *JACC Cardiovasc. Imaging* **2014**, *7*, 178–187. [[CrossRef](#)]
53. Lowin, T.; Straub, R.H. Integrins and their ligands in rheumatoid arthritis. *Arthritis Res. Ther.* **2011**, *13*, 244. [[CrossRef](#)]
54. Zhu, Z.; Yin, Y.; Zheng, K.; Li, F.; Chen, X.; Zhang, F.; Zhang, X. Evaluation of synovial angiogenesis in patients with rheumatoid arthritis using ^{68}Ga -PRGD₂ PET/CT: A prospective proof-of-concept cohort study. *Ann. Rheum. Dis.* **2014**, *73*, 1269–1272. [[CrossRef](#)]
55. Niu, J.; Li, Z. The roles of integrin $\alpha_v\beta_6$ in cancer. *Cancer Lett.* **2017**, *403*, 128–137. [[CrossRef](#)]
56. Worthington, J.J.; Klementowicz, J.E.; Travis, M.A. TGF β : A sleeping giant awoken by integrins. *Trends Biochem. Sci.* **2011**, *36*, 47–54. [[CrossRef](#)]
57. Blobel, G.C.; Schiemann, W.P.; Lodish, H.F. Role of transforming growth factor β in human disease. *N. Engl. J. Med.* **2000**, *34*, 1350–1358. [[CrossRef](#)]
58. Caja, L.; Dituri, F.; Mancarella, S.; Caballero-Diaz, D.; Moustakas, A.; Giannelli, G.; Fabregat, I. TGF- β and the Tissue Microenvironment: Relevance in Fibrosis and Cancer. *Int. J. Mol. Sci.* **2018**, *19*, 1294. [[CrossRef](#)]
59. Shi, M.; Zhu, J.; Wang, R.; Chen, X.; Mi, L.; Walz, T.; Springer, T.A. Latent TGF- β structure and activation. *Nature* **2011**, *474*, 343–349. [[CrossRef](#)]
60. Khan, Z.; Marshall, J.F. The role of integrins in TGF β activation in the tumour stroma. *Cell Tissue Res.* **2016**, *365*, 657–673. [[CrossRef](#)]

61. Dong, X.; Zhao, B.; Iacob, R.E.; Zhu, J.; Koksai, A.C.; Lu, C.; Engen, J.R.; Springer, T.A. Force interacts with macromolecular structure in activation of TGF- β . *Nature* **2017**, *542*, 55–59. [[CrossRef](#)]
62. Ahmed, S.; Bradshaw, A.D.; Geta, S.; Dewan, M.Z.; Xu, R. The TGF β /Smad4 signalling pathway in pancreatic carcinogenesis and its clinical significance. *J. Clin. Med.* **2017**, *6*, 5. [[CrossRef](#)]
63. Adorno, M.; Cordenonsi, M.; Montagner, M.; Dupont, S.; Wong, C.; Hann, B.; Solari, A.; Bobisse, S.; Rondina, M.B.; Guzzardo, V.; et al. A Mutant-p53/Smad complex opposes p63 to empower TGF β -induced metastasis. *Cell* **2009**, *137*, 87–98. [[CrossRef](#)]
64. Inman, G.J. Switching TGF β from a tumor suppressor to a tumor promoter. *Curr. Opin. Genet. Dev.* **2011**, *21*, 93–99. [[CrossRef](#)]
65. Gorelik, L.; Flavell, R.A. Immune-mediated eradication of tumors through the blockade of transforming growth factor- β signaling in T cells. *Nat. Med.* **2001**, *7*, 1118–1122. [[CrossRef](#)]
66. Nieto, M.A.; Huang, R.Y.; Jackson, R.A.; Thiery, J.P. EMT: 2016. *Cell* **2016**, *166*, 21–45.
67. Bates, R.C.; Bellovin, D.I.; Brown, C.; Maynard, E.; Wu, B.; Kawakatsu, H.; Sheppard, D.; Oettgen, P.; Mercurio, A.M. Transcriptional activation of integrin $\beta 6$ during the epithelial-mesenchymal transition defines a novel prognostic indicator of aggressive colon carcinoma. *J. Clin. Investig.* **2005**, *115*, 339–347. [[CrossRef](#)]
68. Koopman Van Aarsen, L.A.; Leone, D.R.; Ho, S.; Dolinski, B.M.; McCoon, P.E.; LePage, D.J.; Kelly, R.; Heaney, G.; Rayhorn, P.; Reid, C.; et al. Antibody-mediated blockade of integrin $\alpha_v\beta_6$ Inhibits tumor progression in vivo by a transforming growth factor- β -regulated mechanism. *Cancer Res.* **2008**, *68*, 561–570. [[CrossRef](#)]
69. Anderton, M.J.; Mellor, H.R.; Bell, A.; Sadler, C.; Pass, M.; Powell, S.; Steele, S.J.; Roberts, R.R.; Heier, A. Induction of heart valve lesions by small-molecule ALK5 inhibitors. *Toxicol. Pathol.* **2011**, *39*, 916–924. [[CrossRef](#)]
70. Vitsky, A.; Waire, J.; Pawliuk, R.; Bond, A.; Matthews, D.; Lacasse, E.; Hawes, M.L.; Nelson, C.; Richards, S.; Piepenhagen, P.A.; et al. Homeostatic role of transforming growth factor-beta in the oral cavity and esophagus of mice and its expression by mast cells in these tissues. *Am. J. Pathol.* **2009**, *174*, 2137–2149. [[CrossRef](#)]
71. Raab-Westphal, S.; Marshall, J.F.; Goodman, S.L. Integrins as therapeutic targets: Successes and cancers. *Cancers* **2017**, *9*, 110. [[CrossRef](#)]
72. Reader, C.S.; Vallath, S.; Steele, C.W.; Haider, S.; Brentnall, A.; Desai, A.; Moore, K.M.; Jamieson, N.B.; Chang, D.; Bailey, P.; et al. The integrin $\alpha_v\beta_6$ drives pancreatic cancer through diverse mechanisms and represents an effective target for therapy. *J. Pathol.* **2019**, *249*, 332–342. [[CrossRef](#)]
73. Henderson, N.C.; Arnold, T.D.; Katamura, Y.; Giacomini, M.M.; Rodriguez, J.D.; McCarty, J.H.; Pellicoro, A.; Raschperger, E.; Betsholtz, C.; Ruminiski, P.G.; et al. Targeting of α_v integrin identifies a core molecular pathway that regulates fibrosis in several organs. *Nat. Med.* **2013**, *19*, 1617–1624. [[CrossRef](#)]
74. Sheppard, D. The role of integrins in pulmonary fibrosis. *Eur. Respir. Rev.* **2008**, *17*, 157–162. [[CrossRef](#)]
75. John, A.E.; Graves, R.H.; Tao Pun, K.; Vitulli, G.; Forty, E.J.; Mercer, P.M.; Morrell, J.L.; Barrett, J.W.; Rogers, R.F.; Hafeji, M.; et al. Translational pharmacology of an inhaled small molecule $\alpha_v\beta_6$ integrin inhibitor for idiopathic pulmonary fibrosis. *Nat. Commun.* **2020**, *11*, 4659. [[CrossRef](#)]
76. Moore, K.M.; Thomas, G.J.; Duffy, S.W.; Warwick, J.; Gabe, R.; Chou, P.; Ellis, I.O.; Green, A.R.; Haider, S.; Brouillette, K.; et al. Therapeutic targeting of integrin $\alpha_v\beta_6$ in breast cancer. *J. Natl. Cancer Inst.* **2014**, *106*, dju169. [[CrossRef](#)]
77. Moore, K.M.; Desai, A.; Delgado, B.d.L.; Trabulo, S.M.D.; Reader, C.; Brown, N.F.; Murray, E.R.; Brentnall, A.; Howard, P.; Masterson, L.; et al. Integrin $\alpha_v\beta_6$ -specific therapy for pancreatic cancer developed from foot-and-mouth-disease virus. *Theranostics* **2020**, *10*, 2930–2942. [[CrossRef](#)]
78. Steiger, K.; Schlitter, A.M.; Weichert, W.; Esposito, I.; Wester, H.J.; Notni, J. Perspective of $\alpha_v\beta_6$ -integrin imaging for clinical management of pancreatic carcinoma and its precursor lesions. *Mol. Imaging* **2017**, *16*, 1536012117709384. [[CrossRef](#)]
79. Cantor, D.I.; Cheruku, H.R.; Nice, E.C.; Baker, M.S. Integrin $\alpha_v\beta_6$ sets the stage for colorectal cancer metastasis. *Cancer Met. Rev.* **2015**, *34*, 715–734. [[CrossRef](#)]
80. Bugatti, K. $\alpha_v\beta_6$ -integrin: An intriguing target for COVID-19 and related diseases. *ChemBioChem* **2021**, *22*, 2516–2520. [[CrossRef](#)]
81. Hausner, S.H.; DiCara, D.; Marik, J.; Marshall, J.F.; Sutcliffe, J.L. Use of a peptide derived from foot-and-mouth disease virus for the Noninvasive Imaging of human cancer: Generation and evaluation of 4- ^{18}F fluorobenzoyl A20FMDV2 for in vivo imaging of integrin $\alpha_v\beta_6$ expression with positron emission tomography. *Cancer Res.* **2007**, *67*, 7833–7840. [[CrossRef](#)]
82. Hausner, S.H.; Abbey, C.K.; Bold, R.J.; Gagnon, M.K.; Marik, J.; Marshall, J.F.; Stanecki, C.E.; Sutcliffe, J.L. Targeted in vivo imaging of integrin $\alpha_v\beta_6$ with an improved radiotracer and its relevance in a pancreatic tumor model. *Cancer Res.* **2009**, *69*, 5843–5850. [[CrossRef](#)]
83. Hausner, S.H.; Bauer, N.; Hu, L.Y.; Knight, L.M.; Sutcliffe, J.L. The effect of Bi-terminal PEGylation of an integrin $\alpha_v\beta_6$ -targeted ^{18}F -peptide on pharmacokinetics and tumor uptake. *J. Nucl. Med.* **2015**, *56*, 784–790. [[CrossRef](#)]
84. Hausner, S.H.; Carpenter, R.D.; Bauer, N.; Sutcliffe, J.L. Evaluation of an integrin $\alpha_v\beta_6$ -specific peptide labeled with ^{18}F fluorine by copper-free, strain-promoted click chemistry. *Nucl. Med. Biol.* **2013**, *233*, 233–239. [[CrossRef](#)]
85. Hausner, S.H.; Bauer, N.; Sutcliffe, J.L. In vitro and in vivo evaluation of the effects of aluminum ^{18}F fluoride radiolabeling on an integrin $\alpha_v\beta_6$ -specific peptide. *Nucl. Med. Biol.* **2014**, *41*, 43–50. [[CrossRef](#)]
86. Hausner, S.H.; Bold, R.J.; Cheuy, L.Y.; Chew, H.K.; Daly, M.E.; Davis, R.A.; Foster, C.C.; Kim, E.J.; Sutcliffe, J.L. Preclinical development and first-in-human imaging of the integrin $\alpha_v\beta_6$ with ^{18}F $\alpha_v\beta_6$ -binding peptide in metastatic carcinoma. *Clin. Cancer Res.* **2019**, *25*, 1206–1215. [[CrossRef](#)]

87. Lukey, P.T.; Coello, C.; Gunn, R.; Parker, C.; Wilson, F.J.; Saleem, A.; Garman, N.; Costa, M.; Kendrick, S.; Onega, M.; et al. Clinical quantification of the integrin $\alpha v \beta 6$ by [^{18}F]FB-A20FMDV2 positron emission tomography in healthy and fibrotic human lung (PETAL Study). *Eur. J. Nucl. Med. Mol. Imaging* **2020**, *47*, 967–979. [[CrossRef](#)]
88. Maher, T.M.; Simpson, J.K.; Porter, J.C.; Wilson, F.J.; Chan, R.B.; Eames, R.; Cui, Y.; Siederer, S.; Parry, S.; Kenny, J.; et al. A positron emission tomography imaging study to confirm target engagement in the lungs of patients with idiopathic pulmonary fibrosis following a single dose of a novel inhaled $\alpha v \beta 6$ integrin inhibitor. *Respir. Res.* **2020**, *21*, 75. [[CrossRef](#)]
89. Foster, C.C.; Davis, R.A.; Hausner, S.H.; Sutcliffe, J.C. $\alpha v \beta 6$ -Targeted molecular PET/CT imaging of the lungs after SARS-CoV-2 Infection. *J. Nucl. Med.* **2020**, *61*, 1717–1719. [[CrossRef](#)]
90. Kimura, R.H.; Cheng, Z.; Gambhir, S.S.; Cochran, J.R. Engineered knottin peptides: A new class of agents for imaging integrin expression in living subjects. *Cancer Res.* **2009**, *69*, 2435–2442. [[CrossRef](#)]
91. Kimura, R.H.; Teed, R.; Hackel, B.J.; Pysz, M.A.; Chuang, C.Z.; Sathirachinda, A.; Willmann, J.K.; Gambhir, S.S. Pharmacokinetically stabilized cystine knot peptides that bind $\alpha v \beta 6$ Integrin with single-digit nanomolar affinities for detection of pancreatic cancer. *Clin. Cancer Res.* **2012**, *18*, 839–849. [[CrossRef](#)]
92. Hackel, B.J.; Kimura, R.H.; Miao, Z.; Liu, H.G.; Sathirachinda, A.; Cheng, Z.; Chin, F.T.; Gambhir, S.S. F-18-Fluorobenzoate-labeled cystine knot peptides for PET imaging of integrin $\alpha v \beta 6$. *J. Nucl. Med.* **2013**, *54*, 1101–1105. [[CrossRef](#)]
93. Kimura, R.H.; Wang, L.; Shen, B.; Huo, L.; Tummers, W.; Filipp, F.V.; Guo, H.W.H.; Haywood, T.; Abou-Elkacem, L.; Baratto, L.; et al. Evaluation of integrin $\alpha v \beta 6$ cystine knot PET tracers to detect cancer and idiopathic pulmonary fibrosis. *Nat. Commun.* **2019**, *10*, 4673. [[CrossRef](#)]
94. Altmann, A.; Sauter, M.; Roesch, S.; Mier, W.; Warta, R.; Debus, J.; Dyckhoff, G.; Herold-Mende, C.; Haberkorn, U. Identification of a novel ITG $\alpha v \beta 6$ -binding peptide using protein separation and phage display. *Clin. Cancer Res.* **2017**, *23*, 4170–4180. [[CrossRef](#)]
95. Roesch, S.; Lindner, T.; Sauter, M.; Loktev, A.; Flechsig, P.; Müller, M.; Mier, W.; Warta, R.; Dyckhoff, G.; Herold-Mende, C.; et al. Comparison of the RGD motif-containing $\alpha v \beta 6$ integrin-binding peptides SFLAP3 and SFITGv6 for diagnostic application in HNSCC. *J. Nucl. Med.* **2018**, *59*, 1679–1685. [[CrossRef](#)]
96. Flechsig, P.; Lindner, T.; Loktev, A.; Roesch, S.; Mier, W.; Sauter, M.; Meister, M.; Herold-Mende, C.; Haberkorn, U.; Altmann, A. PET/CT imaging of NSCLC with a $\alpha v \beta 6$ integrin-targeting peptide. *Mol. Imaging Biol.* **2019**, *21*, 973–983. [[CrossRef](#)]
97. Müller, M.; Altmann, A.; Sauter, M.; Lindner, T.; Jäger, D.; Rathke, H.; Marmé, F.; Babich, J.; Mier, W.; Haberkorn, U. Preclinical evaluation of peptide-based radiotracers for integrin $\alpha v \beta 6$ -positive pancreatic carcinoma. *Nuklearmedizin* **2019**, *58*, 309–318. [[CrossRef](#)]
98. Feng, X.; Wang, Y.; Lu, D.; Xu, X.; Zhou, X.; Zhang, H.; Zhang, T.; Zhu, H.; Yang, Z.; Wang, F.; et al. Clinical translation of a ^{68}Ga -labeled integrin $\alpha v \beta 6$ -targeting cyclic radiotracer for PET imaging of pancreatic cancer. *J. Nucl. Med.* **2020**, *61*, 1461–1467. [[CrossRef](#)]
99. Maltsev, O.V.; Marelli, U.K.; Kapp, T.G.; Di Leva, F.S.; Di Maro, S.; Nieberler, M.; Reuning, U.; Schwaiger, M.; Novellino, E.; Marinelli, L.; et al. Stable peptides instead of stapled peptides: Highly potent $\alpha v \beta 6$ -selective integrin ligands. *Angew. Chem. Int. Ed.* **2016**, *55*, 1535–1539. [[CrossRef](#)]
100. Notni, J.; Hermann, P.; Havlíčková, J.; Kotek, J.; Kubíček, V.; Plutnar, J.; Loktionova, N.; Riss, P.J.; Rösch, F.; Lukeš, I. A triazacyclononane-based bifunctional phosphinate ligand for the preparation of multimeric ^{68}Ga tracers for positron emission tomography. *Chem. Eur. J.* **2010**, *16*, 7174–7185. [[CrossRef](#)]
101. Baranyai, Z.; Reich, D.; Vágner, A.; Weineisen, M.; Tóth, I.; Wester, H.J.; Notni, J. A shortcut to high-affinity Ga-68 and Cu-64 radiopharmaceuticals: One-pot click chemistry trimerisation on the TRAP platform. *Dalton Trans.* **2015**, *44*, 11137–11146. [[CrossRef](#)]
102. Notni, J.; Wester, H.J. A practical guide on synthesis of metal chelates for molecular imaging and therapy by means of click chemistry. *Chem. Eur. J.* **2016**, *22*, 11500–11508. [[CrossRef](#)]
103. Notni, J.; Reich, D.; Maltsev, O.V.; Kapp, T.G.; Steiger, K.; Hoffmann, F.; Esposito, I.; Weichert, W.; Kessler, H.; Wester, H.J. In vivo PET imaging of the cancer integrin $\alpha v \beta 6$ using ^{68}Ga -labeled Cyclic RGD nonapeptides. *J. Nucl. Med.* **2017**, *58*, 671–677. [[CrossRef](#)]
104. Färber, S.F.; Wurzer, A.; Reichart, F.; Beck, R.; Kessler, H.; Wester, H.-J.; Notni, J. Therapeutic radiopharmaceuticals targeting integrin $\alpha v \beta 6$. *ACS Omega* **2018**, *3*, 2428–2436. [[CrossRef](#)]
105. Quigley, N.Q.; Steiger, K.; Hoberück, S.; Czech, N.; Zierke, M.A.; Pretze, M.; Richter, F.; Kossatz, S.; Weichert, W.; Pox, C.; et al. PET/CT imaging of head-and-neck and pancreatic cancer in humans by targeting the “Cancer Integrin” $\alpha v \beta 6$ with Ga-68-Trivehexin. *Eur. J. Nucl. Med. Mol. Imaging* **2021**, *48*. [[CrossRef](#)]
106. Quigley, N.G.; Czech, N.; Sendt, W.; Notni, J. PET/CT imaging of pancreatic carcinoma targeting the “cancer integrin” $\alpha v \beta 6$. *Eur. J. Nucl. Med. Mol. Imaging* **2021**, *48*, 4107–4108. [[CrossRef](#)]
107. Di Leva, F.S.; Tomassi, S.; Di Maro, S.; Reichart, F.; Notni, J.; Dangi, A.; Marelli, U.K.; Brancaccio, D.; Merlino, F.; Wester, H.-J.; et al. From a helix to a small cycle: Metadynamics-inspired $\alpha v \beta 6$ integrin selective ligands. *Angew. Chem. Int. Ed.* **2018**, *57*, 14645–14649. [[CrossRef](#)]
108. Quigley, N.G.; Tomassi, S.; Di Leva, F.S.; Di Maro, S.; Richter, F.; Steiger, K.; Kossatz, S.; Marinelli, L.; Notni, J. Click-chemistry (CuAAC) trimerization of an $\alpha v \beta 6$ integrin targeting Ga-68-peptide: Enhanced contrast for in-vivo PET imaging of human lung adenocarcinoma xenografts. *ChemBioChem* **2020**, *21*, 2836–2843. [[CrossRef](#)]
109. Li, S.; Mcguire, M.J.; Lin, M.; Liu, Y.-H.; Oyama, T.; Sun, X.; Brown, K.C. Synthesis and characterization of a high-affinity $\alpha v \beta 6$ -specific ligand for in vitro and in vivo applications. *Mol. Cancer. Ther.* **2009**, *8*, 1239–1249. [[CrossRef](#)]

110. Singh, A.N.; McGuire, M.J.; Li, S.; Hao, G.; Kumar, A.; Sun, X.; Brown, K.C. Dimerization of a phage-display selected peptide for imaging of $\alpha_v\beta_6$ -integrin: Two approaches to the multivalent effect. *Theranostics* **2014**, *4*, 745–760. [[CrossRef](#)]
111. Strobel, O.; Büchler, M.W. Pancreatic cancer: FDG-PET is not useful in early pancreatic cancer diagnosis. *Nat. Rev. Gastroenterol. Hepatol.* **2013**, *4*, 203–205. [[CrossRef](#)]
112. Moyle, M.; Napier, M.A.; McLean, J.W. Cloning and expression of a divergent integrin subunit β_8 . *J. Biol. Chem.* **1991**, *266*, 19650–19658. [[CrossRef](#)]
113. Campbell, M.G.; Cormier, A.; Ito, S.; Seed, R.I.; Bondesson, A.J.; Lou, J.; Marks, J.D.; Baron, J.L.; Cheng, Y.; Nishimura, S.L. Cryo-EM reveals integrin-mediated TGF- β activation without release from latent TGF- β . *Cell* **2020**, *180*, 490–501. [[CrossRef](#)]
114. Takasaka, N.; Seed, R.I.; Cormier, A.; Bondesson, A.J.; Lou, J.; Elattma, A.; Ito, S.; Yanagisawa, H.; Hashimoto, M.; Ma, R.; et al. Integrin $\alpha_v\beta_8$ -expressing tumor cells evade host immunity by regulating TGF- β activation in immune cells. *JCI Insight* **2018**, *3*, e122591. [[CrossRef](#)]
115. Reichart, F.; Maltsev, O.V.; Kapp, T.G.; Räder, A.F.B.; Weinmüller, M.; Marelli, U.K.; Notni, J.; Wurzer, A.; Beck, R.; Wester, H.J.; et al. Selective targeting of integrin $\alpha_v\beta_8$ by a highly active cyclic peptide. *J. Med. Chem.* **2019**, *62*, 2024–2037. [[CrossRef](#)]
116. Quigley, N.G.; Steiger, K.; Richter, F.; Weichert, W.; Hoerück, S.; Kotzerke, J.; Notni, J. Tracking a TGF- β activator in vivo: Sensitive PET imaging of $\alpha_v\beta_8$ -integrin with the Ga-68-labeled cyclic RGD octapeptide trimer Ga-68-Triveoctin. *EJNMMI Res.* **2020**, *10*, 133. [[CrossRef](#)]
117. Li, H.; Yuan, L.; Long, Y.; Fang, H.; Li, M.; Liu, Q.; Xia, X.; Qin, C.; Zhang, Y.; Lan, X.; et al. Synthesis and preclinical evaluation of a Ga-68-radiolabeled peptide targeting very late antigen-3 for PET imaging of pancreatic cancer. *Mol. Pharm.* **2020**, *17*, 3000–3008. [[CrossRef](#)]
118. Huang, C.W.; Hsieh, W.C.; Hsu, S.T.; Lin, Y.W.; Chung, Y.H.; Chang, W.C.; Chiu, H.; Lin, Y.H.; Wu, C.P.; Yen, T.Z.; et al. The use of PET imaging for prognostic integrin $\alpha_2\beta_1$ phenotyping to detect non-small cell lung cancer and monitor drug resistance responses. *Theranostics* **2017**, *7*, 4013–4028. [[CrossRef](#)]
119. Beaino, W.; Nedrow, J.R.; Anderson, C.J. Evaluation of ^{68}Ga - and ^{177}Lu -DOTA-PEG4-LLP2A for VLA-4-targeted PET imaging and treatment of metastatic melanoma. *Mol. Pharm.* **2015**, *12*, 1929–1938. [[CrossRef](#)]
120. Roxin, Á.; Zhang, C.; Huh, S.; Lepage, M.L.; Zhang, Z.; Lin, K.S.; Bénard, F.; Perrin, D.M. Preliminary evaluation of ^{18}F -labeled LLP2A-trifluoroborate conjugates as VLA-4 ($\alpha_4\beta_1$ integrin) specific radiotracers for PET imaging of melanoma. *Nucl. Med. Biol.* **2018**, *61*, 11–20. [[CrossRef](#)]
121. Jiang, M.; Ferdani, R.; Shokeen, M.; Anderson, C.J. Comparison of two cross-bridged macrocyclic chelators for the evaluation of ^{64}Cu -labeled-LLP2A, a peptidomimetic ligand targeting VLA-4-positive tumors. *Nucl. Med. Biol.* **2013**, *40*, 245–251. [[CrossRef](#)]
122. Hoffmann, S.H.L.; Reck, D.L.; Maurer, A.; Fehrenbacher, B.; Sceneay, J.E.; Poxleitner, M.; Öz, H.H.; Ehrlichmann, W.; Reischl, G.; Fuchs, K.; et al. Visualization and quantification of in vivo homing kinetics of myeloid-derived suppressor cells in primary and metastatic cancer. *Theranostics* **2019**, *9*, 5869–5885. [[CrossRef](#)]
123. Liu, G.; Hu, Y.; Xiao, J.; Li, X.; Li, Y.; Tan, H.; Zhao, Y.; Cheng, D.; Shi, H. $^{99\text{m}}\text{Tc}$ -labelled anti-CD11b SPECT/CT imaging allows detection of plaque destabilization tightly linked to inflammation. *Sci. Rep.* **2016**, *6*, 20900. [[CrossRef](#)]
124. Stewart, R.L.; O'Connor, K.L. Clinical significance of the integrin $\alpha_6\beta_4$ in human malignancies. *Lab. Invest.* **2015**, *95*, 976–986. [[CrossRef](#)]
125. Ozaki, I.; Yamamoto, K.; Mizuta, T.; Kajihara, S.; Fukushima, N.; Setoguchi, Y.; Morito, F.; Sakai, T. Differential expression of laminin receptors in human hepatocellular carcinoma. *Gut* **1998**, *43*, 837–842. [[CrossRef](#)]
126. Feng, G.K.; Ye, J.C.; Zhang, W.G.; Mei, Y.; Zhou, C.; Xiao, Y.T.; Li, X.L.; Fan, F.; Wang, F.; Zeng, M.S. Integrin α_6 targeted positron emission tomography imaging of hepatocellular carcinoma in mouse models. *J. Control. Release* **2019**, *310*, 11–21. [[CrossRef](#)]
127. Gao, S.; Jia, B.; Feng, G.; Dong, C.; Du, H.; Bai, L.; Zhong, Q.; Ma, Q.; Zeng, M.; Wang, F. First-in-human pilot study of an integrin α_6 -targeted radiotracer for SPECT imaging of breast cancer. *Signal Transduct. Target. Ther.* **2020**, *5*, 147. [[CrossRef](#)]
128. Luo, Q.; Yang, G.; Gao, H.; Wang, Y.; Luo, C.; Ma, X.; Gao, Y.; Li, X.; Zhao, H.; Jia, B.; et al. An integrin alpha 6-targeted radiotracer with improved receptor binding affinity and tumor uptake. *Bioconjugate Chem.* **2020**, *31*, 1510–1521. [[CrossRef](#)]
129. Bochen, A.; Marelli, U.K.; Otto, E.; Pallarola, D.; Mas-Moruno, C.; Di Leva, F.S.; Böhm, H.; Spatz, J.P.; Novellino, E.; Kessler, H.; et al. Biselectivity of *iso*DGR peptides for fibronectin binding integrin subtypes $\alpha_5\beta_1$ and $\alpha_v\beta_6$: Conformational control through flanking amino acids. *J. Med. Chem.* **2013**, *56*, 1509–1519. [[CrossRef](#)]
130. Zhao, H.; Gao, H.; Luo, C.; Yang, G.; Zhao, X.; Gao, S.; Ma, Q.; Jia, B.; Shi, J.; Wang, F. An integrin- $\alpha_v\beta_6/\alpha_5\beta_1$ -bitargeted probe for the SPECT imaging of pancreatic adenocarcinoma in preclinical and primary clinical studies. *Bioconjugate Chem.* **2021**, *32*, 1298–1305. [[CrossRef](#)]
131. Kapp, T.G.; Di Leva, F.S.; Notni, J.; Räder, A.F.B.; Fottner, M.; Reichart, F.; Reich, D.; Wurzer, A.; Steiger, K.; Novellino, E.; et al. N-Methylation of *iso*DGR peptides: Discovery of a selective $\alpha_5\beta_1$ -integrin ligand as a potent tumor imaging agent. *J. Med. Chem.* **2018**, *61*, 2490–2499. [[CrossRef](#)]
132. Notni, J.; Steiger, K.; Hoffmann, F.; Reich, D.; Kapp, T.G.; Rechenmacher, F.; Neubauer, S.; Kessler, H.; Wester, H.J. Complementary, selective PET imaging of integrin subtypes $\alpha_5\beta_1$ and $\alpha_v\beta_3$ using ^{68}Ga -aquibepin and ^{68}Ga -avebetrin. *J. Nucl. Med.* **2015**, *57*, 460–466. [[CrossRef](#)]
133. Notni, J.; Steiger, K.; Hoffmann, F.; Reich, D.; Schwaiger, M.; Kessler, H.; Wester, H.J. Variation of specific activities of ^{68}Ga -aquibepin and ^{68}Ga -avebetrin enables selective PET-imaging of different expression levels of integrins $\alpha_5\beta_1$ and $\alpha_v\beta_3$. *J. Nucl. Med.* **2016**, *57*, 1618–1624. [[CrossRef](#)]