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Review

Vascular-homing peptides for targeted drug delivery and molecular imaging: Meeting the clinical challenges

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ABSTRACT

The vasculature of each organ expresses distinct molecular signatures critically influenced by the pathological status. The heterogeneous profile of the vascular beds has been successfully unveiled by the in vivo phage display, a high-throughput tool for mapping normal, diseased, and tumor vasculature. Specific challenges of this growing field are targeted therapies against cancer and cardiovascular diseases, as well as novel bioimaging diagnostic tools. Tumor vasculature-homing peptides have been extensively evaluated in several preclinical and clinical studies both as targeted-therapy and diagnosis. To date, results from several Phase I and II trials have been reported and many other trials are currently ongoing or recruiting patients. In this review, advances in the identification of novel peptide ligands and their corresponding receptors on tumor endothelium through the in vivo phage display technology are discussed. Emphasis is given to recent findings in the clinical setting of vascular-homing peptides selected by in vivo phage display for the treatment of advanced malignancies and their altered vascular beds.

Contents

Abbreviations: ANXA 1, annexin A1; APA, aminopeptidase A; APN, aminopeptidase N; APP, aminopeptidase P; BBB, blood–brain barrier; CT, computed tomography; CREG1, cellular repressor of E1A-stimulated genes 1; DDL4, delta like ligand 4; DLP, dynamin-1 like protein; Eph-B2, ephrin type-B2; Eph-B4, ephrin type-B4; ERBB2, epidermal growth factor tyrosine kinase receptor 2; ERK1/2, extracellular-signal-regulated kinase 1/2; FGF8b, fibroblast growth factor 8b; aFGF, acidic fibroblast growth factor; bFGFR, fibroblast growth factor receptor b; FN-ED-B, fibronectin extra-domain B; GRP78, glucose regulated protein 78; HGG, high-grade glioma; HSP90, heat-shock protein 90 kDa; IL11R, interleukin 11 receptor; MAP2, microtubule-associated protein 2; MGMT, methylated methylguanosine methyltransferase; NG2, neuron-glial antigen 2; NGR, asparagine-glycine-arginine; NSCLC, non-small cell lung cancer; OPA-1, Optic Atrophy-1; PCR, polymerase chain reaction; PDGFR, platelet derived growth factor receptor; PET, positron emission tomography; PRGD2, pegylated arginine-glycine-aspartic acid dimer; RGD, arginine-glycine-aspartic acid; SCID, severe combined immunodeficiency; SCLC, small cell lung carcinoma; SPECT, single photon emission computed tomography; TEM-5, tumor endothelial marker 5; TEM-8, tumor endothelial marker 8; TF, tissue factor; TNFα, tumor necrosis factor alpha; VCAM, vascular cell adhesion molecules; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor

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1. Introduction

The vascular feature, markedly modified under pathological conditions, is an address system that allows the specific targeting towards blood vessels. The heterogeneous expression of proteins in the vasculature has been profiled by the in vivo phage display technology, a powerful method used to identify peptides homing specifically to normal and diseased vasculature. Vascular homing peptides are optimal ligands for the targeted delivery of imaging agents or drugs for the treatment of cancer, cardiovascular diseases (restenosis, atherosclerosis, hypertension, ischemia), and neurological disorders (stroke, Alzheimer's disease) [1–3]. Importantly, they provide an efficient mean of discriminating between normal cells and tumor-associated endothelial cells, thus, controlling tumor growth independently of the cell type. Tumor vasculature, structurally and functionally different compared to the vasculature of normal tissues, is highly disorganized with vessels strikingly tortuous and leaky. Indeed, neoformed vessels are discontinuous, leaky and present a deregulated expression of a number of molecules such as integrins, endothelial cell growth factor receptors, cell surface proteoglycans, proteases, and extracellular matrix components [1] (Fig. 1). In particular, proteins functionally important for tumor angiogenesis, which represent potential targets for anti-angiogenic therapy, are vascular endothelial growth factor receptor (VEGFR), integrins, delta-like ligand 4, ephrin-B4, ephrin-B2, tumor endothelial markers 5 and 8, annexin A1, and fibronectin extra-domain B [4] (Fig. 1).

In vivo phage display has been widely used to analyze the structural and molecular diversity of normal and tumor vasculature [1,2,5]. The pioneering in vivo phage display study was aimed at discovering brain vasculature targeting peptides [6]. To date, sequences capable of targeting vasculature of normal tissue or organs, such as brain, kidney, lung, muscle, pancreas, thymus, and mammary gland have been discovered [7].

The construction and use of phage libraries include millions of polypeptides expressed within the coat proteins of filamentous bacteriophages, such as protein 3 (pIII), protein 6 (pVI) and protein 8 (pVIII) [7,8]. The phage display of peptide libraries is based on the concept that the epitopes of interest can be targeted with foreign proteins expressed on the phage. Phage particles binding to a certain epitope are isolated and transduced back into bacteria which are then grown to expand the selected phage population [7,8]. A consistent number of peptides with high specificity and affinity have been isolated from phage display libraries using affinity selection (*panning*) and used in different fields [5,9]. To date, several comprehensive reviews describing the phage display technology steps, such as phage libraries, *biopanning* strategies, and animal models are available [1,7,10,11]. Here, we review advances on new candidate peptides identified by in vivo screening phage display libraries and, looking towards clinical challenges, provide an update of current clinical trials evaluating novel targeted drugs and imaging agents.

2. Principles of the in vivo phage display technology

The phage display technology is based on the exposure of combinatorial peptide libraries on the surface of recombinant phages [8]. Two are the main types of phage systems; the phage vector and phagemid vector. Filamentous phage f1, fd and M13 (Ff phages) are the main tools in the phage display as they are very stable under extreme pH and temperature conditions and in the presence of DNAse or proteolytic enzymes [8]. The engineered expression of random peptide libraries on the coat proteins of filamentous bacteriophage consists in the expression of a unique peptide by each phage obtained by cloning a segment of peptide-coding DNA into surface protein genes (pIII and pVIII). Interestingly, the lambda phage engineered to display multiple copies of peptides or large protein domains offers the possibility of dual display of large proteins (antibody fragment and a reported/effector moiety) on the capsid using both the head- and the tail-based display platforms. Such bifunctional phage nanoparticles can be particularly useful for diagnostic and therapeutic delivery [12].

The basic components of the phagemid, filamentous-phage-derived vectors containing the replication origin of a plasmid, include the replication origin of a plasmid, the restriction enzyme recognition sites, the gene of a phage coat protein, the intergenic region, the selective marker, the promoter, the DNA segment encoding a signal peptide, and a molecular tag which facilitates screening of phagemid-based library [13]. Major advantages of phagemid vectors are represented by the small genome that can accommodate a larger foreign DNA fragment, high transformation efficiency, stability under multiple propagations, variety

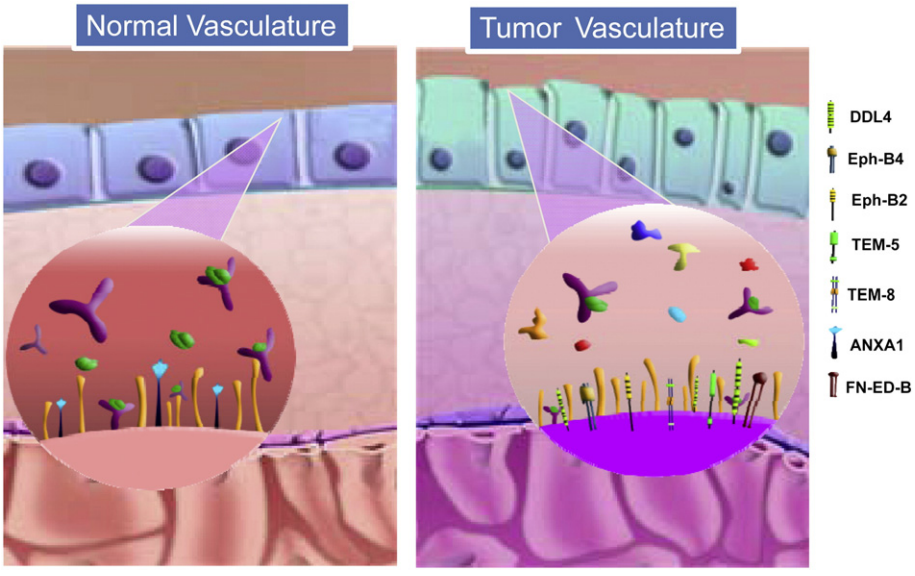

Fig. 1. *Targeting endothelial cells as anticancer strategy*. The molecular diversity of the luminal endothelial cell surface provides the basis for developing targeted molecules using in vivo phage display through which it is possible to decipher the molecular signature of (A) normal vasculature and (B) tumor vasculature. Tumor vessels, structurally and functionally different from normal vasculature, are highly disorganized, strikingly tortuous, and with a leaky architecture. They express molecules that are not present in normal blood vessels, such as delta like ligand 4 (DDL4), ephrin type-B4 (Eph-B4), ephrin type-B2 (Eph-B2), tumor endothelial marker 5 (TEM-5), tumor endothelial marker 8 (TEM-8), annexin A1 (ANXA 1), and fibronectin extra-domain B (FN-ED-B).

of restriction enzyme recognition sites, and possibility to control the expression level of fusion proteins [13].

The main limit of the phage display applied to cell lines is the divergence from the complexity and heterogeneity of living organisms. This problem has been overcome by the in vivo phage display, firstly described by Pasqualini and Ruoslahti [6], in which phage particles were administered directly into a living animal. The in vivo phage display uses various libraries cloned into various phage vectors. Specifically, phage libraries contain random oligonucleotides, cDNA, antibodies, or enzymes [14]. Special features that characterize the phage libraries compared to other types of expression vectors are; i) the physical linkage between the expressed peptide or protein, ii) the small size of the phage particles that enables the screening of high amount of different particles *per* round, iii) the affinity selection of the library (*panning*) which allows an easy identification of a specific phage. Recombinant techniques have been used to develop efficient methods to create highly diverse phage display libraries [10]. The (poly)peptide diversity can be increased by cosmix-plexing, an approach for selection from combinatorial libraries where the primary library is constructed in a phagemid vector and the gene encoding the displayed randomized (poly)peptide is divided into several fragments separated by restriction sites [10].

Vascular mapping of normal endothelium, performed using mice and rat models, has been widely reviewed by Babickova et al. [7]. Briefly, targeting of normal endothelial receptors has been achieved using Balb/c, Balb/c nude mice, and ICR mice. Brain homing peptides, M-cell and Peyer's patches have been identified in Wistar Kyoto rat model, mammary gland targeting peptide in Sprague–Dawley rat model, and retina targeting sequences in Albino rat model [7]. Among the animal models of tumor vasculature, the most successfully used are the severe combined immunodeficiency (SCID)/nude mice of PC-3 prostate carcinomas [15] and the mice bearing xenografts of human cancer tissues which comprise xenografts of human gastric cancer cell lines [16], human renal tumor endothelial cells [17], human breast cancer [18], and human hepatocellular carcinoma cell lines [19].

In humans, the first in vivo phage display, performed by injecting intravenously the phage library into a comatose patient [20,21], allowed the production of a phage library and the identification of several motifs. Main steps of the in vivo phage display are the intravenous injection of the phage libraries, the removal of whole organs, tumors, or tissue biopsies, and the elution of the bound phages (Fig. 2). The eluted phage can be either plated to isolate clones for sequencing or amplified in vivo and subjected to an additional round of affinity selection, namely *biopanning*

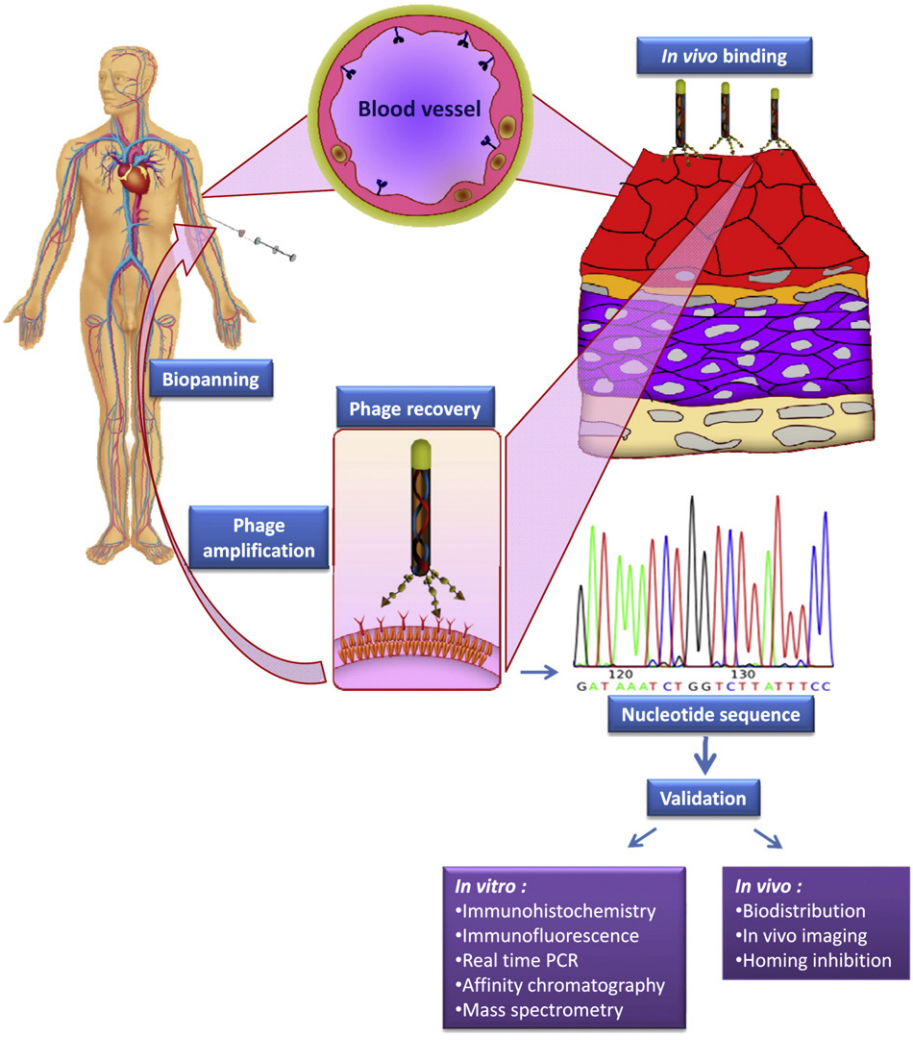

Fig. 2. *Steps of the* in vivo *phage display technology to map human vasculature*. Phage libraries are injected intravenously to identify phage peptides that interact with molecules specifically expressed in the blood vessels of a particular organ or tissue. After target organ removal or tissue biopsies, phages are recovered and amplified for a desired amount of rounds. Amplification is followed by multiple rounds of injection of phage, vascular perfusion, tissue removal, phage isolation, and amplification (*biopanning*) leading to the isolation of the phage population that selectively binds to blood vessels. After the identification of consensus sequences, the identified sequences are translated into protein sequence and subjected to validation either in vitro (immunohistochemistry, immunofluorescence and real time PCR) or in vivo (biodistribution, in vivo imaging, specific inhibition by competitive ligand). The identification of the receptor is achieved by affinity chromatography in combination with mass spectrometry.

(Fig. 2) which allows the preservation of membrane-bound protein conformations, the maintenance of the cell membrane environment necessary for cell-surface receptor function, and the selection of unknown receptors through a combinatorial approach.

Furthermore, sequencing, immunohistochemistry, in vivo imaging, and mass spectrometry analysis are necessary to validate phages and their corresponding targets (Fig. 2) [22–24]. Indeed, immunostaining with anti-phage antibodies and other approaches with fluorescent or radioactive labeling of either phage particles or synthetically manufactured peptide sequence enables the in vivo visualization of the tissue peptide distribution [24] (Fig. 2).

As for the *biopanning*, this process consists of multiple rounds of phage injection, vascular perfusion, tissue removal, phage isolation and amplification (Fig. 3) [22,23]. Basically, after the incubation of the phage library with an immobilized target molecule, the unbound phages are washed away whereas the interacting phages, still bound to the target, are subsequently eluted from the target molecule and used to infect host bacteria for their amplification [22,23]. This process is repeated several times to obtain phages that specifically bind to the target tissue. After multiple rounds of biopanning, phage titering (plaque assay) or immunological assays are used to determine the enrichment of the target-binding phage.

3. Peptides targeting vasculature

Vascular-homing peptides discovered through in vivo phage display libraries are summarized in Table 1. Arginine-glycine-aspartic acid (RGD) and asparagine-glycine-arginine (NGR) peptides, discovered earlier, are the most promising peptides targeting tumor vasculature [18,25–27].

3.1. RGD peptides

RGD peptide homes to tumor vasculature selectively expressing αvβ3 and αvβ5 integrins [18,25]. Integrin αvβ3 and αvβ5 are overexpressed in tumor-associated vasculature and glioma cells and their activation triggers pathways responsible for cell proliferation and tumor-induced angiogenesis [28,29]. Integrin αvβ3 has been involved in the resistance to taxanes and zoledronic acid through the modulation of the bridge protein Cyr61 involved in tumor angiogenesis [30,31]. In fact, integrins play an important role in the regulation of intracellular signaling that protects cancer cells from the antiproliferative effects of anti-tumor drugs. Therefore, the disruption of this protein could be useful also in the sensitization of tumor cells to anti-cancer agents.

On these bases, the antiangiogenic and antitumor effects of peptides containing the RGD motif have been tested in preclinical and clinical models, as well as their efficacy in imaging applications [32–34]. In vitro and in vivo studies demonstrated that targeting αvβ3 and αvβ5 integrins in combination with radiation therapy induces apoptosis in endothelial cells and inhibits tumor growth in U87 glioblastoma xenografts [35,36]. RGD-functionalized protein nanoparticles showed a trophic potential in PC12 pheochromocytoma model promoting protoneurite formation, ERK1/2-dependent cell division, and expression of the neuronal marker microtubule-associated protein 2 (MAP2) [37]. This trophic potential of RGD peptides is of critical relevance in the functionalization of RGD based-nanoparticle-linked drug, highlighting the possibility that the vehicle might antagonize or synergize with the therapeutic cargo [37].

It has to be considered that RGD peptide binds a large number of cellular integrins, including αIIbβ3 on platelets, as well as αvβ3 and α5β1 on endothelial cells so that the development of more specific integrin-binding peptides is required. In this light, the CRRETAWAC peptide

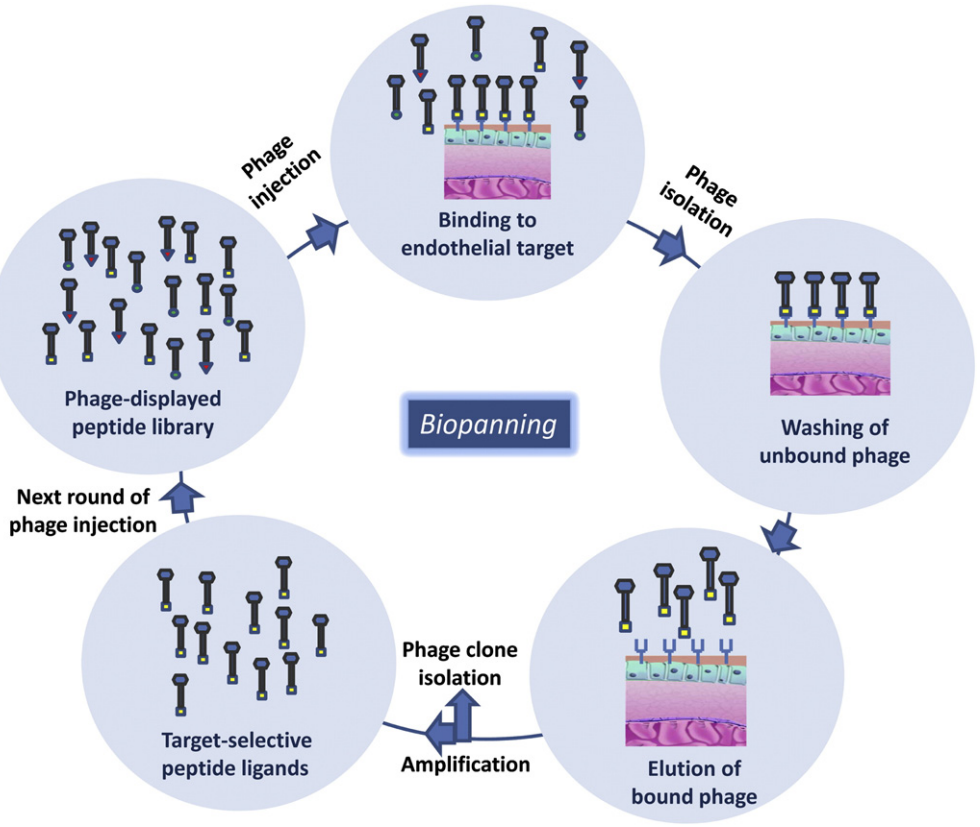

Fig. 3. *Biopanning process.* A typical round of affinity selection process begins with the intravenous phage-displayed peptide library injection and incubation with the target. After organ extraction and homogenization, the unbound phages are washed away whereas the remaining bound phages are eluted from the target molecule and amplified for subsequent rounds of screening.

Table 1
Molecular addresses on vascular endothelium identified by in vivo phage display.

Target	Function	Localization	Homing motif	References
αvβ5-Integrin αvβ3-Integrin	Cell adhesion	Tumor endothelium	CDCRGDCFC (RGD-4C)	[18,28,29]
α5β1-Integrin	Cell adhesion	Normal endothelium	CRRETAWAC	[38]
α4-Annexin A4	Cell adhesion	Normal endothelium	CMRGFRAAC	[50]
VCAM-1	Cell adhesion	Tumor endothelium	VHSPNKK	[52,53]
E-cadherin	Cell adhesion	Normal/tumor endothelium	CSSRTMHHC	[54]
APN/CD13	Protease	Normal/breast tumor endothelium, perycites	CNGRC	[26]
CD13	Protease	Tumor cells	CVLNGRMEC	[27]
APA	Protease	Tumor endothelium	CPRECESIC	[62]
APP	Protease	Breast tumor endothelium	CPGPEGAGC	[5]
MMP2	Protease	Normal/tumor endothelium	TLTYTWS/CTTHWGFTLC	[60,61]
MMP9	Protease	Normal/tumor endothelium	CRRHWGFEFC	[60]
Kallikrein-9 substrate	Protease substrate	Tumor endothelium	CSRPRRSEC	[66]
NG2	Proteoglycan	Tumor endothelium	GLS	[63]
VEGF	Growth factor	Normal/tumor endothelium	WHLPFKC	[64]
VEGFR (neuropilin)	VEGF receptor	Normal/tumor endothelium	CPQPRPLC	[55]
VEGR (Flk-1/KDR)	VEGF receptor	Tumor endothelium	HTMYYHHYQHHL	[56]
VEGR (Flt-1)	VEGF receptor	Tumor endothelium	NGYEIEWYSWVTHGMY	[57]
PDGFR	PDGF receptor	Premalignant angiogenic islet	CRGRRST	[67]
bFGFR	FGF receptor	Tumor endothelium	MQLPLAT	[58]
FGF8b	Growth factor	Tumor endothelium	HSQAAVP	[59]
aFGF	Growth factor	Tumor endothelium	AGNWTPI	[77]
IL-11R	Cytokine receptor	Normal/tumor endothelium	CGRRAGGSC	[48]
GRP78	Heat shock protein	Normal endothelium	HWRR	[65]
Prohibitin	Membrane protein	White fat vasculature	CKGGRAKDC	[71]
Ephrin A2/A4	Receptor tyrosine kinase	Pancreatic islet vessels	CVSNPRWKC/CHVLWSTRC	[74]
Ephrin A5/A4	Receptor tyrosine kinase	Normal/tumor endothelium	CTYYWPLPC	[76]
Ephrin B2/B4	Receptor tyrosine kinase	Normal/tumor endothelium	TNYL-RAW	[75]
ERBB2	Growth factor tyrosine kinase receptors	Breast tumor	MEGPSKCCYSLALSH	[78]
Apo transferrin	Glycoprotein	Central nervous system capillary endothelium	CRTIGPSVC	[72]
N.D.	N.D.	Inflammed endothelium	NTTTH	[86]
N.D.	N.D.	Gastric tumor endothelium	CGNSNPKSC	[85]
N.D.	N.D.	Heart tissue vasculature	DDTRHWG	[8]
N.D.	N.D.	Prostate tumor endothelium	SMSIARL	[20]
N.D.	N.D.	Melanoma vasculature	CVNHPAFAC	[84]
N.D.	N.D.	Ovarian cancer endothelium	WSGPGVWGASVK	[87]
N.D.	N.D.	Brain normal microvasculature	CAGALCY	[81]
N.D.	N.D.	Brain normal endothelium	GLAHSFSDFARDFV/GYRPVHNIRGHWAPG	[91]
N.D.	N.D.	Brain normal endothelium	CTSTSAPYC	[92]
N.D.	N.D.	Normal endothelium of tubules and glomeruli	HTTHREP/HITSLLS	[82]
N.D.	N.D.	Lung cancer tumor endothelium	SVSVGMKPSPRP	[83]
N.D.	N.D.	Thymus vessels	CHAQGSAEC	[73]
N.D.	N.D.	Abnormal retina neovessels	ACSTEALRHCGGGS	[88]
N.D.	[64Cu]DOTA PET tracer	Lung carcinoma endothelium	QFPPKLTNNSML	[80]
Stabilin-2	Transmembrane protein	Atherosclerotic plaques	CRTLTVRKC	[70]
OPA-1, DLP-1	Mitochondrial fusion protein	Ischemic heart tissue vasculature	CSTSMLKAC	[69]
N.D.	N.D.	Aorta/cardiac vasculature	CRPPR/CSGMARTKC	[97]

N.D.: not determined.

has been shown to selectively block α5β1 integrin-mediated human cell binding to fibronectin surfaces and seems to be promising in the setting of cardiovascular implants with a platelet resistant coating [38,39]. In vitro evidence showed that the vascular grafts coated with CRRETAWAC peptide prevented platelet adhesion and increased the duration of the graft [40]. However, data from in vivo implantation in an animal model to demonstrate their possible clinical feasibility are still expected. In this regard, results on the capability of CRRETAWAC peptide to cross-react with porcine endothelial cells are promising [41]. So far, only few non-RGD recognition-based integrin inhibitors have been developed. Among these, peptides against $\alpha_M\beta 2$ integrins, DDGW or CPCLLGCC [42,43], expressed only by leukocytes, prevented in vitro leukocyte attachment and in vivo inflammation. However, these peptides did not show any effect on leukemia and lymphoma burden in mice [44].

3.2. NGR peptides

NGR peptides specifically bind to CD13 expressed by tumor vasculature [26,27]. These peptides, CNGRC, acetylated-CNGRC, CVLNGRMEC, CNGRG, and NGRAHA, have been largely investigated for tumor targeting delivery of chemotherapeutic drugs, cytokines, virus, liposomes and anti-angiogenic compounds showing enhancement of tumor growth inhibition [45,46]. Recently, the NGR peptide radiolabeled with 99mTc has been shown as a promising diagnostic candidate, particularly efficient for lung cancer imaging [47]. Indeed, although it showed a similar uptake in ovarian and lung tumor cells, imaging studies indicated its highest efficacy in the visualization of lung tumor [47].

3.3. Other vascular-homing peptides

A consistent number of other identified vascular-homing peptides are reported in Table 1 and, as shown, for some of them the endothelial target is still unknown. A peptide with consensus sequence for interleukin-11, found to home into prostate, has been confirmed as the ligand for IL-11 receptor alpha [23,48] with specific tumor uptake in tibias of PC-3 xenografts injected in nude mice (Table 1) [49]. Biopanning of the phage library injected in two human subjects, followed by sequencing of phage particles, yielded fifteen putative ligand/receptor couples. Experimental validation was achieved for four of them which resulted to be annexin A2/prohibitin, integrin α4 subunit/annexin A4, advanced glycation end products/specific receptor with leukocyte proteinase 3,

and cathepsin B/apolipoprotein E3 [50]. The injection of a phage peptide library into breast cancer or melanoma cancer patients allowed the identification of several peptides [51]. Interestingly, the peptides identified from a patient with breast cancer shared homology with breast carcinoma amplified sequence 3, CREG1 protein precursor, neurogenic locus notch homologue protein 2 precursor, prostaglandin E2 receptor, integrin α6 protein, and zinc finger homeobox protein 2. One peptide isolated from a patient with malignant melanoma shared a homology motif with human multiple epidermal growth factor-like domain protein 7 [51].

Other identified vascular molecular addresses include vascular cell adhesion molecule (VCAM) [52,53], E-cadherin [54], vascular endothelial growth factor receptor (VEGFR) [55–57], fibroblast growth factor receptor b (bFGFR) [58], fibroblast growth factor 8b (FGF8b) [59], matrix metalloproteinase 2/9 (MMP-2/MMP-9) [60,61], aminopeptidase N (APN) [26], aminopeptidase A (APA) [62], aminopeptidase P (APP) [5], proteoglycan (NG2) [63], vascular endothelial growth factor (VEGF) [64], heat shock protein [65], kallikrein-9 substrate [66], and platelet derived growth factor receptor (PDGFR) [67,68]. Moreover, other peptides have been identified in hearth vasculature [8,69], atherosclerotic plaques [70], white fat vasculature [71], central nervous system capillary endothelium [72], and thymus vessels [73] (Table 1).

Peptides reported to act as ligands of ephrin receptors, a family of tyrosine kinase receptors that regulates a variety of physiological and pathological processes during the formation of tumor blood vessels, are the ephrin A2/A4 peptide CVSNPRWKC and CHVLWSTRC [74], the ephrin B4 peptide TNYL-RAW [75], and the newly discovered cyclic peptide CTYYWPLPC which binds to ephrin A4 but not to ephrin B4 [76].

A novel peptide, AGNWTPI, showing high affinity for the acidic FGF (aFGF)-binding site of the FGFR1 has been recently selected by phage display after four rounds of *biopanning* [77]. This peptide, which acts as an aFGF antagonist, inhibits the aFGF-stimulated cell proliferation, arrests the cell cycle at the G0/G1 phase, and blocks the aFGF-induced activation of Erk1/2 in breast cancer cells and vascular endothelial cells [77]. With the aim of improving the early detection of breast carcinomas, the pharmacokinetic properties of an ERBB2-targeted radioimaging peptide based on a KCCYSL core sequence showed encouraging results [78]. Indeed, evaluation of the phages and corresponding peptides (MEGPSKCCYSLALSH and SGTKSKCCYSLRRSS) indicated that the peptide MEGPSKCCYSLALSH, radiolabeled with [111In]DOTA, possesses a rapid clearance and higher ERBB2-specific binding capacity compared to the first-generation peptide KCCYSL [78].

A combination of in vitro and in vivo functional screening protocols led to the identification of the QFPPKLTNNSML peptide, selective for Lewis lung carcinoma endothelium, able to deliver molecular cargo to sites of ongoing angiogenesis for non-invasive positron emission tomography (PET) imaging [79,80].

Several vascular-homing peptides with unknown targets have been identified in endothelium of blood–brain barrier (BBB), kidney, prostate cancer, ovarian cancer, melanoma, gastric cancer, abnormal retina neovessels, and inflamed endothelium [20,81–89]. More in details, the CGNSNPKSC peptide conjugated to the recombinant mutant human TNFα (rmhTNFα) was found to selectively bind to the gastric tumor vasculature with a higher anti-tumor activity and lower systemic toxicity than the rmhTNFα alone [90]. As for the brain microvasculature, a high affinity delivery to human brain endothelial cells has been achieved with selective homing peptides, GLAHSFSDFARDFV, GYRPVHNIRGHWAPG, CAGALCY, and CTSTSAPYC [7,91,92] (Table 1). However, the binding sites and potential uptake mechanisms for GLA- and GYR-peptides are unknown. Results from the negative selection performed by perfusion through the heart indicated that the binding site is likely the brain endothelium, with the GYR peptide showing high binding to the brain. Probably, these peptides interact with a not yet identified receptor suitable for specific targeting to the BBB [91]. The CAGALCY cyclic peptide expressed as a GST-fusion protein resulted an effective inhibitor of platelet adhesion to brain microvasculature [81]. Similarly, also the cyclic conformation of the CTSTSAPYC peptide plays a critical role in maintaining the selectivity and affinity for the brain, suggesting its possible application for brain drug delivery when the respective molecular sites of interaction on the BBB will be identified [92].

Peptides able to recognize tumor vasculature but not normal blood vessels of SCID mice bearing human tumors have been described [93]. These tumor-homing peptides (SNPFSKPYGLTV and YPHYSLPGSSTL), validated on different types of human xenografts (H460, HCT 116, BT483, PC3, PaCa-2 and Mahlavu) in SCID mice, following conjugation with liposomal doxorubicin, showed enhanced therapeutic efficacy against human cancer xenografts by decreasing tumor angiogenesis and increasing cancer cell apoptosis [93]. Administration of phage display library has been used into athymic nude mice with LS174T colorectal cancer cell line to identify peptides selectively homing to tumors treated with bevacizumab, but not to untreated tumors [94]. Liposome-entrapped doxorubicin targeting APN displayed enhanced anti-tumor effects and prolonged survival in human neuroblastoma-bearing mice. Interestingly, mice treated with a combination of APA and APN liposome-entrapped doxorubicin showed a significant increase in lifespan compared to each treatment administered separately [95]. Finally, in vivo phage display performed in stroke-prone spontaneously hypertensive rats and normotensive Wistar Kyoto rats led to identification of the peptide DDTRHWG, CRPPR, and CSGMARTKC homing to heart tissue, aorta, and cardiac vasculature, respectively [8,96,97].

4. In vivo applications of vascular homing peptides

4.1. RGD peptides

The RGD peptide, widely used in nanomedicine for the targeted delivery of drugs or as a tool for imaging [32,33], has been associated with chemicals, self-assembling peptides, liposomes, carbon nanotubes, magnetic nanoparticles, polymers, and other delivery agents [37,98].

To date, in vivo studies have demonstrated the potential value of targeting αvβ3 and αvβ5 integrins with cilengitide (EMD 121974; Merck KgaA, Darmstadt, Germany) [34], a cyclized RGD-containing pentapeptide that showed encouraging results in patients with glioblastoma [34,99,100]. Specifically, when tested in combined therapy with standard radiotherapy and temozolomide for the treatment of newly diagnosed glioblastoma, it enhanced survival of patients with methylated methylguanosine methyltransferase (MGMT) promoter [34,99,100]. In vitro studies demonstrated that cilengitide targets pediatric glioma and neuroblastoma cells directly and efficiently [101], enhances radiation response in preclinical models of breast cancer [102], blocks the differentiation of pancreatic tumor-initiating cells by vitronectin [103], inhibits pivotal factors of all compartments of bone metastases, vasculature and bone microenvironment in animal models [104], and augments tumor response to melphalan [105]. However, the results of clinical trials have not fully confirmed its effectiveness. Indeed, outcomes of a Phase II study by the Children's Oncology Group ACNS0621, aimed at evaluating the efficacy of cilengitide as a single agent in the treatment of children with refractory high-grade glioma (HGG), did not show any significant beneficial effect [106]. Cilengitide, both as a single agent and in combination with other chemotherapeutic agents, has also been evaluated for the treatment of prostate, pancreatic and head and neck cancers (Phase I/II trials). However, although safe and well tolerated, it did not demonstrate any clear survival advantage [34,107–109]. Above all, the impression derived form clinical data is that cilengitide, in combination with radiotherapy, could be useful in the crossing of BBB and in the delivery of drugs to the brain for the treatment of glioblastoma.

Another in vivo application of RGD peptide, the RGD modified adenovirus (Ad5-Δ24-RGD), resulted to be well tolerated in patients with ovarian cancer (Phase I) and fourteen out of twenty-one patients showed stable disease one month after the treatment [32].

Finally, three fusion proteins, RGD/tTF, chTNT-3/tTF, and chTV-1/tTF, tested for their antitumor effects, have shown the ability to induce thrombosis [110]. The protein tTF is the truncated form of tissue factor (TF), a cell membrane receptor protein that is the initiator of the extrinsic pathway of the blood coagulation cascade, normally released from damaged tissues. By substituting the attachment site with a tumor delivery agent, the tTF can be targeted to the tumor where it occludes tumor's blood supply and causes rapid tumor destruction. The fusion protein chTNT-3/tTF targets extracellular DNA, the fusion protein chTV-1/tTF targets fibronectin which is located in the basement membrane of vessels but only accessible in leaky tumor endothelium, and the fusion protein RGD/tTF targets endothelial $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins exposed in tumor vessels. In vivo studies in mice bearing established MAD109 lung and colon carcinomas revealed that the chTV-1/tTF and RGD/tTF fusion proteins induced thrombosis in small and medium sized tumor vessels, whereas the chTNT-3/tTF was more effective in relatively larger vessels [110].

4.2. NGR peptides

A wide evaluation of the tripeptide motif NGR for targeted cancer therapy and imaging has been performed when linked to human tumor necrosis factor alpha (NGR-hTNFα) [111]. Indeed, NGR-hTNFα has been tested in Phase II trials for the treatment of malignant pleural mesothelioma [112], advanced solid tumors [113], liver cancer [114], and metastatic colorectal cancer [115]. As directed drug for the treatment of mesothelioma and liver cancer, the NGR-hTNFα therapy was well tolerated and showed a good median disease free progression time [112,114]. On the other hand, the results in metastatic colorectal cancer did not show improvements in both median disease free progression time and overall survival [115]. The administration of NGR-mTNFα in combination with chemotherapy gave also promising results. Specifically, when administered in combination with doxorubicin and melphalan in mice with implanted B16F10 melanoma cells or Thy-1.1 cDNA-transfected RMA lymphoma cells, NGR-mTNFα showed a synergistic effect in inducing tumor regression [116]. These results were supported also by a Phase I clinical trial in the treatment of refractory/resistant solid tumors by the combination of doxorubicin and NGR-hTNFα [113] and by a Phase II clinical trial in relapsed ovarian cancer [117]. Moreover, the combination of NGR-hTNFα with capecitabine and oxaliplatin for the treatment of colorectal cancer patients not responding to standard treatment [118] showed that 42% of patients of the first cohort had a median progression-free time of 4.6 months and 50% patients of the second cohort had a progression-time of 3.3 months [118].

5. Vascular peptides under clinical evaluation

The therapeutic application of vascular homing peptides represents an important milestone of the in vivo phage display technology that, over the years, has been surrounded by unbelief regarding its real effectiveness and, above all, its safety. Overall, improvements of specific tissue delivery of known drugs and the encouraging results of some clinical investigations demonstrated that the identification of specific ligands by in vivo phage display is extremely valuable for the development of novel therapeutic approaches. As a demonstration of the progress achieved in this field, the number of clinical trials evaluating vascular targeting peptides is continuously rising and for some of them results are expected (Tables 2 and 3).

The fusion protein NGR-hTNFα, which selectively alters drug penetration barriers, is currently under evaluation in several trials (Table 2). Indeed, two ongoing clinical trials are assessing the progression free survival in patients with advanced or metastatic malignant pleural mesothelioma treated with NGR-hTNFα as a single agent (Phase II) and its efficacy, at a weekly low dose, in patients previously treated with a pemetrexed-based chemotherapy regimen (Phase III). Another Phase II trial is recruiting patients to evaluate the efficacy of NGR-hTNFα administered as a maintenance treatment in malignant pleural mesothelioma. Phase II and III studies aimed at treating small cell lung carcinoma (SCLC) with NGR-hTNFα in combination with doxorubicin and at treating non-small cell lung cancer (NSCLC) in combination with cisplatin, gemcitabin, and pemetrexed, are currently ongoing (Table 2). The administration of NGR-hTNFα in combination with doxorubicin is currently under evaluation for the treatment of ovarian cancer

Table 2
NGR peptides under clinical evaluation (http://clinicaltrials.gov/).

Condition	Status	Intervention	Description	Phase	Code
Malignant pleural mesothelioma	Recruiting	NGR-hTNFα	To evaluate the efficacy of NGR-hTNF administered as maintenance treatment in advanced malignant pleural mesothelioma	II	NCT01358084
Malignant pleural mesothelioma	Ongoing	NGR-hTNFα	To test the progression free survival in advanced or metastatic malignant pleural mesothelioma patients treated with NGR-hTNF as single agent	II	NCT00484276
Malignant pleural mesothelioma	Ongoing	NGR-hTNFα plus best investigator choice	To document the efficacy of NGR-hTNF administered at low dose weekly in patients previously treated with a pemetrexed-based chemotherapy regimen	III	NCT01098266
Solid tumors	Completed	NGR-hTNFα Doxorubicin	To document the safety of NGR-hTNF in combination with doxorubicin	I	NCT00305084
Solid tumors	Ongoing	NGR-hTNFα Cisplatin	To study the efficacy of NGR-hTNF in combination with cisplatin.	I	NCT00483093
Solid tumors	Completed	NGR-hTNFα	To explore the safety and biological activity of NGR-hTNF and to define an optimal biological dose	I	NCT00419328
Solid tumors	Ongoing	NGR-hTNFα	To document the safety and antivascular effect of escalating doses of NGR-hTNF in patients not amenable of standard therapies	I	NCT00878111
Colorectal cancer	Ongoing	NGR-hTNFα	To document the progression free survival in advanced or metastatic colorectal cancer patients treated with NGR-hTNF as single agent	II	NCT00483080
Colorectal cancer	Ongoing	NGR-hTNFα Oxaliplatin Capecitabine	To document the safety of NGR-hTNF administered in combination with a standard oxaliplatin based regimen	II	NCT00675012
Non-small cell lung cancer (NSCLC)	Ongoing	NGR-hTNFα Cisplatin Gemcitabine Pemetrexed	To demonstrate superiority in progression-free survival when NGR-hTNF is added to standard chemotherapy regimen	II	NCT00994097
Small cell lung cancer (SCLC)	Ongoing	NGR-hTNFα Doxorubicin	To evaluate the efficacy of NGR-hTNF in combination with doxorubicin	III	NCT00483509
Ovarian cancer	Recruiting	NGR-hTNFα Pegylated liposomal-doxorubicin Doxorubicin	To compare progression-free survival in patients randomized to NGR-hTNF plus anthracycline treatment	II	NCT01358071
Ovarian cancer	Ongoing	NGR-hTNFα Doxorubicin	To document the response rate in patients treated with NGR-hTNF in combination with doxorubicin	II	NCT00484432
Soft-tissue sarcoma	Recruiting	NGR-hTNFα Doxorubicin	To document the preliminary antitumor activity of two doses of NGR-hTNF administered either alone or in combination with doxorubicin	II	NCT00484341
Hepatocellular carcinoma	Ongoing	NGR-hTNFα	To test the progression free survival after treatment with NGR-hTNF as single agent	II	NCT00484211

Table 3
Clinical trials evaluating RGD peptides (http://clinicaltrials.gov/).

Condition	Status	Intervention	Description	Phase	Code
Diffuse intrinsic pontine glioma	Recruiting	Cilengitide radiotherapy	To determine the safety profile of the Cilengitide and the 6 months progression-free time and overall survival	I	NCT01165333
Glioma and diffuse intrinsic pontine glioma	Recruiting	Cilengitide temozolomide	To evaluate the efficacy of a combined treatment with cilengitide and temozolomide after diagnosis of relapse or tumor progression in children and adolescents	II	NCT01517776
Childhood high-grade glioma	Completed	Cilengitide	To evaluate effects of cilengitide in the treatment of younger patients with recurrent or progressive high-grade glioma not responding to standard therapy	II	NCT00679354
Childhood refractory primary brain tumors	Completed	Cilengitide	To study the side effects and best dose of cilengitide in treating children with recurrent, progressive, or refractory primary brain tumors	I	NCT00063973
Glioblastoma	Completed	Cilengitide temozolomide radiotherapy	To test the efficacy of cilengitide, temozolomide, and radiation therapy in treating patients with newly diagnosed glioblastoma and unmethylated gene promoter status (CORE)	II	NCT00813943
Glioblastoma	Completed	Cilengitide temozolomide radiotherapy	To assess the efficacy and safety of cilengitide in combination with standard treatment in newly diagnosed glioblastoma subjects with a methylated promoter of the O6-methylguanine-deoxyribonucleic acid methyltransferase gene in the tumor tissue (CENTRIC)	III	NCT00689221
Advanced solid tumors or glioblastoma multiforme	Ongoing	Cilengitide	Pilot study on the efficacy of cilengitide together with sunitinib malate in patients with advanced solid tumors or glioblastoma multiforme		NCT01122888
Newly diagnosed non-methylated glioblastoma multiforme grade 4	Unknown	Cilengitide	To test the safety and efficacy of cilengitide in combination with concurrent chemotherapy and radiotherapy followed by protracted daily low dose of temozolomide and low dose of procarbazine D1-20 in newly diagnosed glioblastoma without methylation of the MGMT promoter gene (ExCentric)	II	NCT01124240
Recurrent or progressive glioblastoma multiforme	Completed	Cilengitide	To evaluate cilengitide efficacy in the treatment of patients undergoing surgery for recurrent or progressive glioblastoma multiforme	II	NCT00112866
Glioblastoma multiforme	Ongoing	Cilengitide temozolomide radiation therapy	A safety run-in/randomized trial of cilengitide in conjunction with concomitant and adjuvant temozolomide with radiation therapy in patients with newly diagnosed glioblastoma multiforme	I II	NCT00085254
Glioblastoma multiforme	Completed	Cilengitide	Investigation on the clinical activity, safety, and tolerability of cilengitide administered as a single agent	II	NCT00093964
Brain and central nervous system tumors	Completed	Cilengitide	To study the effectiveness of cilengitide in treating patients who have progressive or recurrent malignant glioma	I II	NCT00006093
Renal impairment	Completed	Cilengitide	To investigate the pharmacokinetics of a single intravenous dose of cilengitide in subjects with mild, moderate or severe renal impairment	I	NCT01504165
Squamous cell cancer of the head and neck	Ongoing	Cilengitide cetuximab 5FU cisplatin	To evaluate the safety and efficacy of the combination of different regimens of cilengitide added to cisplatin, 5FU, and cetuximab	I II	NCT00705016
Solid tumors	Recruting	Cilengitide paclitaxel	To evaluate the side effects and the best dose of cilengitide given together with paclitaxel for the treatment of patients with advanced solid tumors	I	NCT01276496
Advanced solid tumors or lymphoma	Completed	Cilengitide	To study the effect of continuous infusion of cilengitide in patients with solid tumors or lymphoma	I	NCT00077155
Solid tumors	Completed	Cilengitide	To study the effectiveness of cilengitide in treating patients with advanced solid tumors	I	NCT00022113
Non-small cell lung cancer (NSCLC)	Completed	Cilengitide cilengitide cetuximab and platinum-based chemotherapy	To investigating 2 cilengitide regimens in combination with cetuximab and platinum-based chemotherapy in the treatment of patients with advanced NSCLC	I II	NCT00842712
Prostate cancer	Completed	Cilengitide	Evaluation of EMD121974 (NSC 707544, cilengitide) in asymptomatic patients with metastatic androgen independent prostate cancer	II	NCT00103337
Leukemia, lymphoma, multiple myeloma and plasma cell neoplasm, myelodysplastic syndromes, precancerous non-malignant condition, small intestine cancer, unspecified adult solid tumor	Completed	Cilengitide	To study the effectiveness of cilengitide in the treatment of patients with locally advanced or metastatic cancer	I	NCT00004258
Ovarian cancer	Completed	Ad5-Δ24RGD	To determine the maximally tolerated dose and spectrum of toxicities encountered with intraperitoneal delivery of a RGD modified conditionally replicative adenovirus (Ad5-Δ24RGD)	I	NCT00562003
Ovarian cancer	Completed	Ad5.SSTR/TK.RGD Ganciclovir	To determine the maximally tolerated dose and toxicities associated with intraperitoneal delivery of adenovirus that expresses a suicide gene and a gene that allows imaging of gene transfer	I	NCT00964756
Brain tumor glioblastoma	Unknown	Δ24-RGD	To test the safety and tolerability of adenovirus Δ24-RGD administered by convection-enhanced delivery to the tumor and the surrounding infiltrated brain	I II	NCT01582516

Table 3 (*continued*)

Condition	Status	Intervention	Description	Phase	Code
Malignant gliomas	Ongoing	Δ24-RGD-4C	To find the highest tolerable dose of Δ 24-RGD-4C that can be injected directly into brain tumors and into the surrounding brain tissue where tumor cells can multiply	I	NCT00805376
Breast cancer colon/rectum cancer non-squamous non-small cell lung cancer	Completed	[18F]RGD-K5	To explore the usefulness of [18F]RGD-K5 PET/CT to predict efficacy or early response to the anti-angiogenesis drug plus chemotherapy treatment before the full course of treatment is completed	II	NCT00988936
Sarcoma melanoma lung cancer breast cancer high grade glioma	Completed	[18F]RGD-K5	To evaluate biodistribution and dosimetry of [18F]RGD-K5 and determine its uptake in angiogenic tumor	0	NCT00743353
Head and neck neoplasm	Enrolling by invitation	[18F]RGD-K5	To determine the relationship between [18F]RGD-K5 distribution and angiogenesis in head and neck cancer patients		NCT01447134
Renal carcinoma	Not open for recruitment	[18F]RGD-PET-CT Perfusion CT	Prospective study of [18F]-RGD PET-CT in assessment of response to antiangiogenic treatment	II	NCT01492192
Glioblastoma	Recruiting	Chemoradiation Adjuvant Therapy [18F] Galacto-RGD PET	To explore whether potential image biomarkers correlate with efficacy of bevacizumab combined with conventional therapy in newly diagnosed glioblastoma		NCT01939574
Solid tumors	Recruiting	[18F]F-PPRGD2	To study [18F]2-fluoropropionyl-labeled pegylated dimeric RGD peptide, [18F]F-PPRGD2, PET/CT in predicting early response in patients with cancer receiving anti-angiogenesis therapy	I II	NCT01806675
Kidney neoplasm	Recruiting	[18F]Fluciclatide containing the RGD	Evaluation of [18F]Fluciclatide PET for the prediction of response to pazopanib in patients with metastatic renal cell carcinoma	II	NCT01961583
Lung cancer glioma	Recruiting	[68Ga]-BNOTA-PRGD2	To investigate the diagnostic performance and evaluation efficacy of 68Ga-BNOTA-PRGD2	0	NCT01527058 NCT01801371
High-grade glioma lung cancer head & neck cancer sarcoma melanoma	Completed	[18F] labeled cyclic RGD peptide	To assess the ability to detect tumors and angiogenesis with PET imaging		NCT00565721

(Phase II), soft tissue sarcoma (Phase II), and solid tumors (Phase I). Moreover, safety, biological activity, and optimal biological dose of NGR-hTNFα are also under clinical evaluation in patients with solid tumors (Phase I, completed) and in patients affected by advanced or metastatic solid tumors not amenable to standard therapies (Phase I) (Table 2). Finally, the efficacy of the administration of NGR-hTNFα alone (Phase II) or in combination with oxaliplatin and capecitabine (Phase II) is currently tested in patients with advanced or metastatic colorectal cancer and hepatocellular carcinoma (Table 2).

The RGD motif, the most investigated peptide in clinical trials, is currently tested for its antiangiogenic and antitumor effects and for its application in diagnostic imaging. A detailed summary of the clinical trials evaluating the RGD motif is shown in Table 3. The efficacy and safety of cilengitide, administered alone and in combination with other drugs and radiation therapy, is mostly under evaluation for the treatment of brain and central nervous system tumors, including glioblastoma, diffuse intrinsic pontine glioma, and high-grade childhood glioma (Table 3). In particular, Phase II and III studies are investigating two cilengitide regimens in combination with standard treatment (temozolomide with concomitant radiation therapy, followed by temozolomide maintenance therapy) in subjects with newly diagnosed glioblastoma showing un-methylated MGMT gene promoter (CORE trial) and in subjects with a methylated promoter of the O_6-methylguanine-deoxyribonucleic acid methyltransferase gene in the tumor tissue (CENTRIC trial). Moreover, Phase I and II studies are testing the effectiveness of cilengitide for the treatment of solid tumors, lymphoma, leukemia, multiple myeloma, squamous cell cancer of the head and neck, non-small cell lung cancer, small intestine cancer, and prostate cancer. Although most of them are completed, results are not yet posted on the ClinicalTrials.gov website.

As for diagnostic application, the compound [18F]RGD-K5 is currently tested in the assessment of the response to antiangiogenic treatment in patients with head and neck neoplasm, and renal carcinoma (Phase II) (Table 3). [18F]RGD-K5, radiotracer composed of a cyclic triazole-containing the RGD peptide (RGD-K5) radiolabeled with 18F, possesses a potential αvβ3-integrin imaging activity for PET. Upon administration, the RGD moiety of [18F]RGD-K5 selectively binds to αvβ3-integrin, thus allowing the visualization of αvβ3integrin-expressing tumor cells and evaluation of the tumor angiogenesis degree (Table 3).

For further interests in clinical translation, the compound [68Ga] NODAGA-RGD, a peptide that combines easy accessibility with high stability and good imaging properties, is currently used for imaging of αvβ3integrin expression [119].

Another RGD-based radiopharmaceutical agent, [68Ga]BNOTA-PRGD2, is under assessment for imaging of lung cancer, glioma, myocardial infarction, stroke, and rheumatoid arthritis (Table 3). This agent comprises a pegylated-RGD dimer (PRGD2) labeled with 68Ga, with potential αvβ3integrin imaging activity for PET or single photon emission computed tomography (SPECT). Compared to other radiolabeled RGD-containing peptides, this agent shows increased affinity to αvβ3integrin, enhanced tumor uptake, and improved pharmacokinetics (Table 3).

Finally, two clinical trials aimed at evaluating the efficacy of Ad5-Δ24-RGD for the treatment of recurrent glioblastoma and malignant glioma are currently recruiting participants (Phase I/II) or ongoing (Phase I) (Table 3). Instead, another Phase I study, aimed at determining the maximally tolerated dose and spectrum of toxicities encountered with intraperitoneal delivery of Ad5-Δ24-RGD in patients with ovarian cancer, has been completed and results are expected (Table 3). Other expected clinical outcomes relate to a Phase I/II study in ovarian cancer patients for the evaluation of the maximally tolerated dose and toxicities associated with intraperitoneal delivery of Ad5.SSTR/TK.RGD ganciclovir, an adenovirus with enhanced infectivity that expresses a suicide gene and a gene that allows imaging of gene transfer (Table 3).

6. Concluding remarks and future directions

The field of in vivo phage display has consistently widened in these last years opening the way to diagnostic and therapeutic strategies based on the targeted delivery of therapeutics/diagnostics to the vasculature. Since 1996 [6], numerous vascular-homing peptides have been

discovered and tested, suggesting their usefulness as excellent alternative targeting agents for cancer. However, encouraging preclinical data on the use of integrin inhibitors has not been fully confirmed by clinical studies, which, unexpectedly, have shown only limited survival benefits, probably due to the high genetic instability and heterogeneity of some tumors, and to the poor tumor-penetrating properties of the homing peptides. It is worthy to point out that tumor microenvironment shows common molecular features with inflammatory vasculature, as it is rich in inflammatory mediators involved in tumor angiogenesis, such as cytokines, chemokines, and growth factors [120]. This could explain why targeted-tumor peptides, such as RGD and NGR, can also home vasculature in the course of inflammatory diseases. On the other hand, when RGD- or NGR-based therapy fails, highly selective tumor-homing peptides could represent a more suitable therapeutic strategy. In this regard, nanoparticles combined with tumor-homing peptides represent a promising future area of investigation for tumor-targeted diagnosis and therapy, as they will combine the limited toxicity and the good penetrating ability of nanoparticles with the highly specific selectivity of the vascular peptides [121]. A recent combination method of phage display with a liposomal nanocarrier for targeted delivery of doxorubicin in a rodent model showed improved efficiency and reduced hepatotoxicity, opening new prospective for future clinical studies addressing breast cancer [122].

Another key point in this field is that the development of vectors with improved specificity, safety, and efficiency for gene transfer is still required, as well as the identification of novel labeling vascular-homing peptides for the setting of non-invasive diagnostic procedures. Within this scenario, vascular-targeted peptides combined with nanoparticles and, at the same time, the identification of novel molecular vascular "addresses" which allows the selection of patients suitable for combined treatments are the next frontier of this field. The achievement of these major steps will lead to vascular-targeted therapies more efficient, safe and specific, opening up a new path towards a personalized treatment of the patient.

References

[1] M. Trepel, R. Pasqualini, W. Arap, Screening phage-display peptide libraries for vascular targeted peptides, Methods Enzymol. 445 (2008) 83–106.
[2] P. Laakkonen, L. Zhang, E. Ruoslahti, Peptide targeting of tumor lymph vessels, Ann. N. Y. Acad. Sci. 1131 (2008) 37–43.
[3] F. de Nigris, M.L. Balestrieri, C. Napoli, Targeting c-Myc, Ras and IGF cascade to treat cancer and vascular disorders, Cell Cycle 5 (2006) 1621–1628.
[4] T.C. Johannessen, M. Wagner, O. Straume, R. Bjerkvig, H.P. Eikesdal, Tumor vasculature: the Achilles' heel of cancer? Expert Opin. Ther. Targets 7 (2013) 7–20.
[5] M.L. Balestrieri, C. Napoli, Novel challenges in exploring peptide ligands and corresponding tissue-specific endothelial receptors, Eur. J. Cancer 43 (2007) 1242–1250.
[6] R. Pasqualini, E. Ruoslahti, Organ targeting in vivo using phage display peptide libraries, Nature 380 (1996) 364–366.
[7] J. Bábíčková, L. Tóthová, P. Boor, P. Celec, In vivo phage display — a discovery tool in molecular biomedicine, Biotechnol. Adv. 31 (2013) 1247–1259.
[8] C.G. Nicol, L. Denby, O. Lopez-Franco, R. Masson, C.A. Halliday, S.A. Nicklin, A. Kritz, L.M. Work, A.H. Baker, Use of in vivo phage display to engineer novel adenoviruses for targeted delivery to the cardiac vasculature, FEBS Lett. 583 (2009) 2100–2107.
[9] S. Ueberberg, S. Schneider, Phage library-screening: a powerful approach for generation of targeting-agents specific for normal pancreatic islet-cells and islet-cell carcinoma in vivo, Regul. Pept. 160 (2010) 1–8.
[10] T. Bratkovic, Progress in phage display: evolution of the technique and its applications, Cell. Mol. Life Sci. 67 (2010) 749–767.
[11] P. Molek, B. Strukelj, T. Bratkovic, Peptide phage display as a tool for drug discovery: targeting membrane receptors, Molecules 16 (2011) 857–887.
[12] E. Pavoni, P. Vaccaro, V. D'Alessio, R. De Santis, O. Minenkova, Simultaneous display of two large proteins on the head and tail of bacteriophage lambda, BMC Biotechnol. 13 (Sep 30 2013) 79, http://dx.doi.org/10.1186/1472-6750-13-79.
[13] H. Qi, H. Lu, H.J. Qiu, V. Petrenko, A. Liu, Phagemid vectors for phage display: properties, characteristics and construction, J. Mol. Biol. 3417 (2012) 129–143.
[14] M. Hust, A. Frenzel, T. Meyer, T. Schirrmann, S. Dubel, Construction of human naive antibody gene libraries, Methods Mol. Biol. 907 (2012) 85–107.
[15] J.R. Newton, K.A. Kelly, U. Mahmood, R. Weissleder, S.L. Deutscher, In vivo selection of phage for the optical Imaging of PC-3 human prostate carcinoma in mice, Neoplasia 8 (2006) 772–780.
[16] N. Akita, F. Maruta, L.W. Seymour, D.J. Kerr, A.L. Parker, T. Asai, N. Oku, J. Nakayama, S. Miyagawa, Identification of oligopeptides binding to peritoneal tumors of gastric cancer, Cancer Sci. 97 (2006) 1075–1081.
[17] B. Bussolati, C. Grange, L. Tei, M.C. Deregibus, M. Ercolani, S. Aime, G. Camussi, Targeting of human renal tumor-derived endothelial cells with peptides obtained by phage display, J. Mol. Med. (Berl.) 85 (2007) 897–906.
[18] W. Arap, R. Pasqualini, E. Ruoslahti, Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model, Science 279 (1998) 377–380.
[19] W.D. Jia, H.C. Sun, J.B. Zhang, Y. Xu, Y.B. Qian, J.Z. Pang, L. Wang, L.X. Qin, Y.K. Liu, Z. Y. Tang, A novel peptide that selectively binds highly metastatic hepatocellular carcinoma cell surface is related to invasion and metastasis, Cancer Lett. 247 (2007) 234–242.
[20] W. Arap, M.G. Kolonin, M. Trepel, J. Lahdenranta, M. Cardò-Villa, R.J. Giordano, P.J. Mintz, P.U. Ardelt, V.J. Yao, C.I. Vidal, L. Chen, A. Flamm, H. Valtanen, L.M. Weavind, M.E. Hicks, R.E. Pollock, G.H. Botz, C.D. Bucana, E. Koivunen, D. Cahill, P. Troncoso, K.A. Baggerly, R.D. Pentz, K.A. Do, C.J. Logothetis, R. Pasqualini, Steps toward mapping the human vasculature by phage display, Nat. Med. 8 (2002) 121–127.
[21] W. Arap, R. Pasqualini, The human vascular mapping project. Selection and utilization of molecules for tumor endothelial targeting, Haemostasis 31 (2001) 30–31.
[22] G. Castel, M. Chtéoui, B. Heyd, N. Tordo, Phage display of combinatorial peptide libraries: application to antiviral research, Molecules 16 (2011) 3499–3518.
[23] A.J. Zurita, P. Troncoso, M. Cardo-Vila, C.J. Logothetis, R. Pasqualini, W. Arap, Combinatorial screenings in patients: the interleukin-11 receptor alpha as a candidate target in the progression of human prostate cancer, Cancer Res. 64 (2004) 435–439.
[24] K.N. Sugahara, T. Teesalu, P.P. Karmali, V.R. Kotamraju, L. Agemy, O.M. Girard, D. Hanahan, R.F. Mattrey, E. Ruoslahti, Tissue penetrating delivery of compounds and nanoparticles into tumors, Cancer Cell 16 (2009) 510–520.
[25] R. Pasqualini, E. Koivunen, E. Ruoslahti, Alpha v integrins as receptors for tumor targeting by circulating ligands, Nat. Biotechnol. 15 (1997) 542–546.
[26] F. Curnis, A. Sacchi, L. Borgna, F. Magni, A. Gasparri, A. Corti, Enhancement of tumor necrosis factor a antitumor immunotherapeutic properties by targeted delivery to aminopeptidase N (CD13), Nat. Biotechnol. 18 (2000) 1185–1190.
[27] R. Soudy, S. Ahmed, K. Kaur, NGR peptide ligands for targeting CD13/APN identified through peptide array screening resemble fibronectin sequences, ACS Comb. Sci. 14 (2012) 590–599.
[28] M.A. Dechantsreiter, E. Planker, B. Mathä, E. Lohof, G. Hölzemann, A. Jonczyk, S.L. Goodman, H. Kessler, N-Methylated cyclic RGD peptides as highly active and selective alpha(V)beta(3) integrin antagonists, J. Med. Chem. 42 (1999) 3033–3040.
[29] L. Belvisi, T. Riccioni, M. Marcellini, L. Vesci, I. Chiarucci, D. Efrati, D. Potenza, C. Scolastico, L. Manzoni, K. Lombardo, M.A. Stasi, A. Orlandi, A. Ciucci, B. Nico, D. Ribatti, G. Giannini, M. Presta, P. Carminati, C. Pisano, Biological and molecular properties of a new alpha(v)beta3/alpha(v)beta5 integrin antagonist, Mol. Cancer Ther. 4 (2005) 1670–1680.
[30] M. Marra, D. Santini, G. Meo, B. Vincenzi, S. Zappavigna, A. Baldi, M. Rosolowski, G. Tonini, M. Loeffler, R. Lupu, S.R. Addeo, A. Abbruzzese, A. Budillon, M. Caraglia, Cyr61 downmodulation potentiates the anticancer effects of zoledronic acid in androgen-independent prostate cancer cells, Int. J. Cancer 125 (2009) 2004–2013.
[31] J.A. Menendez, L. Vellon, I. Mehmi, P.K. Teng, D.W. Griggs, R. Lupu, A novel CYR61-triggered 'CYR61-alphavbeta3 integrin loop' regulates breast cancer cell survival and chemosensitivity through activation of ERK1/ERK2 MAPK signaling pathway, Oncogene 24 (2005) 761–779.
[32] K.J. Kimball, M.A. Preuss, M.N. Barnes, M. Wang, G.P. Siegal, W. Wan, H. Kuo, S. Saddekni, C.R. Stockard, W.E. Grizzle, R.D. Harris, R. Aurigemma, D.T. Curiel, R.D. Alvarez, A phase I study of a tropism-modified conditionally replicative adenovirus for recurrent malignant gynecologic diseases, Clin. Cancer Res. 16 (2010) 5277–5287.
[33] P. Laverman, J.K. Sosabowski, O.C. Boerman, W.J. Oyen, Radiolabelled peptides for oncological diagnosis, Eur. J. Nucl. Med. Mol. Imaging 39 (2012) S78–S92.
[34] C. Scaringi, R.M. Enrici, G. Minniti, Combining molecular targeted agents with radiation therapy for malignant gliomas, Onco Targets Ther. 6 (2013) 1079–1095.
[35] S. Ning, Z. Chen, A. Dirks, B. Husbeck, M. Hsu, B. Bedogni, M. O'Neill, M.B. Powell, S.J. Knox, Targeting integrins and PI3K/Akt-mediated signal transduction pathways enhances radiation-induced anti-angiogenesis, Radiat. Res. 168 (2007) 125–133.
[36] A. Abdollahi, D.W. Griggs, H. Zieher, A. Roth, K.E. Lipson, R. Saffrich, H.J. Gröne, D.E. Hallahan, R.A. Reisfeld, J. Debus, A.G. Niethammer, P.E. Huber, Inhibition of alpha(v)beta3 integrin survival signaling enhances antiangiogenic and antitumor effects of radiotherapy, Clin. Cancer Res. 11 (2005) 6270–6279.
[37] J. Domingo-Espin, V. Petegnief, N. de Vera, O. Conchillo-Solé, P. Saccardo, U. Unzueta, E. Vazquez, J. Cedano, L. Negro, X. Daura, H. Peluffo, A.M. Planas, A. Villaverde, N. Ferrer-Miralles, RGD-based cell ligands for cell-targeted drug delivery act as potent trophic factors, Nanomedicine 8 (2012) 1263–1266.
[38] E. Koivunen, B. Wang, E. Ruoslahti, Isolation of a highly specific ligand for the alpha 5 beta 1 integrin from a phage display library, J. Cell Biol. 124 (1994) 373–380.
[39] C.C. Larsen, F. Kligman, C. Tang, K. Kottke-Marchant, R.E. Marchant, A biomimetic peptide fluorosurfactant polymer for endothelialization of ePTFE with limited platelet adhesion, Biomaterials 28 (2007) 3537–3548.
[40] S.R. Meyers, D.J. Kenan, X. Khoo, M.W. Grinstaff, Bioactive stent surface coating that promotes endothelialization while preventing platelet adhesion, Biomacromolecules 12 (2011) 533–539.
[41] L.A. Dudash, F.L. Kligman, J.M. Bastijanic, K. Kottke-Marchant, R.E. Marchant, Cross-reactivity of cell-selective CRRETAWAC peptide with human and porcine endothelial cells, J. Biomed. Mater. Res. A (Sep 30 2013), http://dx.doi.org/10.1002/jbm.a.34960.
[42] E. Koivunen, T.M. Ranta, A. Annila, S. Taube, A. Uppala, M. Jokinen, G. van Willigen, E. Ihanus, C.G. Gahmberg, Inhibition of beta(2) integrin-mediated leukocyte cell adhesion by leucine–leucine–glycine motif-containing peptides, J. Cell Biol. 153 (2001) 905–916.
[43] M. Stefanidakis, M. Bjorklund, E. Ihanus, C.G. Gahmberg, E. Koivunen, Identification of a negatively charged peptide motif within the catalytic domain of progelatinases

that mediates binding to leukocyte beta 2 integrins, J. Biol. Chem. 278 (2003) 34674–34684.
[44] J. Suojanen, J. Reunanen, T.M. Ranta, O. Peñate-Medina, T. Salo, P. Saris, T. Sorsa, Peptides against mac-1 do not sufficiently target leukemia or lymphoma in vivo, Anticancer Res. 34 (2014) 645–650.
[45] A. Corti, F. Curnis, Tumor vasculature targeting through NGR peptide-based drug delivery systems, Curr. Pharm. Biotechnol. 12 (2011) 1128–1134.
[46] W. Jiang, G. Jin, D. Ma, F. Wang, T. Fu, X. Chen, K. Jia, F.M. Marikar, Z. Hua, Modification of cyclic NGR tumor neovasculature-homing motif sequence to human plasminogen kringle 5 improves inhibition of tumor growth, PLoS One 7 (2012) e37132.
[47] B.L. Faintuch, E.A. Oliveira, R.C. Targino, A.M. Moro, Radiolabeled NGR phage display peptide sequence for tumor targeting, Appl. Radiat. Isot. 86 (2014) 41–45.
[48] M. Cardo-Vila, A.J. Zurita, R.J. Giordano, J. Sun, R. Rangel, L. Guzman-Rojas, C.D. Anobom, A.P. Valente, F.C. Almeida, J. Lahdenranta, M.G. Kolonin, W. Arap, R. Pasqualini, A ligand peptide motif selected from a cancer patient is a receptor-interacting site within human interleukin- 11, PLoS One 3 (2008) e3452.
[49] W. Qinghua, X. Jianli, L. Lu, Y. Zexuan, T. Guansheng, G. Hailin, J. Jianwei, A radiolabeled nonapeptide probe targeting PC-3 cells and bone metastases of prostate cancer in mice, Contrast Media Mol. Imaging 7 (2012) 223–230.
[50] F.I. Staquicini, M. Cardó-Vila, M.G. Kolonin, M. Trepel, J.K. Edwards, D.N. Nunes, A. Sergeeva, E. Efstathiou, J. Sun, N.F. Almeida, S.M. Tu, G.H. Botz, M.J. Wallace, D.J. O'Connell, S. Krajewski, J.E. Gershenwald, J.J. Molldrem, A.L. Flamm, E. Koivunen, R.D. Pentz, E. Dias-Neto, J.C. Setubal, D.J. Cahill, P. Troncoso, K.A. Do, C.J. Logothetis, R.L. Sidman, R. Pasqualini, W. Arap, Vascular ligand-receptor mapping by direct combinatorial selection in cancer patients, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 18637–18642.
[51] D.N. Krag, G.S. Shukla, G.P. Shen, S. Pero, T. Ashikaga, S. Fuller, D.L. Weaver, S. Burdette-Radoux, C. Thomas, Selection of tumor binding ligands in cancer patients with phage display libraries, Cancer Res. 66 (2006) 7724–7733.
[52] A. Dienst, A. Grunow, M. Unruh, B. Rabausch, J.E. Nor, J.W. Fries, C. Gottstein, Specific occlusion of murine and human tumor vasculature by VCAM-1-targeted recombinant fusion proteins, J. Natl. Cancer Inst. 97 (2005) 733–747.
[53] K.A. Kelly, J.R. Allport, A. Tsourkas, V.R. Shinde-Patil, L. Josephson, R. Weissleder, Detection of vascular adhesion molecule-1 expression using a novel multimodal nanoparticle, Circ. Res. 96 (2005) 327–336.
[54] A.L. Matsuo, A.S. Tanaka, M.A. Juliano, E.G. Rodrigues, L.R. Travassos, A novel melanoma-targeting peptide screened by phage display exhibits antitumor activity, J. Mol. Med. 88 (2010) 1255–1264.
[55] R.J. Giordano, C.D. Anobom, M. Cardo-Vila, J. Kalil, A.P. Valente, R. Pasqualini, F.C. Almeida, W. Arap, Structural basis for the interaction of a vascular endothelial growth factor mimic peptide motif and its corresponding receptors, Chem. Biol. 12 (2005) 1075–1083.
[56] L. Hetian, A. Ping, S. Shumei, L. Xiaoying, H. Luowen, W. Jian, M. Lin, L. Meisheng, Y. Junshan, S. Chengchao, A novel peptide isolated from a phage display library inhibits tumor growth and metastasis by blocking the binding of vascular endothelial growth factor to its kinase domain receptor, J. Biol. Chem. 277 (2002) 43137–43142.
[57] M. El-Mousawi, L. Tchistiakova, L. Yurchenko, G. Pietrzynski, M. Moreno, D. Stanimirovic, D. Ahmad, V. Alakhov, A vascular endothelial growth factor high affinity receptor 1-specific peptide with antiangiogenic activity identified using a phage display peptide library, J. Biol. Chem. 278 (2003) 46681–46691.
[58] F. Maruta, A.L. Parker, K.D. Fisher, M.T. Hallissey, T. Ismail, D.C. Rowlands, L.A. Chandler, D.J. Kerr, L.W. Seymour, Identification of FGF receptor-binding peptides for cancer gene therapy, Cancer Gene Ther. 9 (2002) 543–552.
[59] W. Wang, X. Chen, T. Li, Y. Li, R. Wang, D. He, W. Luo, X. Li, X. Wu, Screening a phage display library for a novel FGF8b-binding peptide with anti-tumor effect on prostate cancer, Exp. Cell Res. 319 (2013) 1156–1164.
[60] A. Hajitou, R. Pasqualini, W. Arap, Vascular Targeting: recent advances and therapeutic perspectives, Trends Cardiovasc. Med. 16 (2006) 80–88.
[61] J. Mueller, F.C. Gaertner, B. Blechert, K.P. Janssen, M. Essler, Targeting of tumor blood vessels: a phage-displayed tumor-homing peptide specifically binds to matrix metalloproteinase-2-processed collagen IV and blocks angiogenesis in vivo, Mol. Cancer Res. 7 (2009) 1078–1085.
[62] S. Marchio`, J. Lahdenranta, R.O. Schlingemann, D. Valdembri, P. Wesseling, M.A. Arap, A. Hajitou, M.G. Ozawa, M. Trepel, R.J. Giordano, D.M. Nanus, H.B. Dijkman, E. Oosterwijk, R.L. Sidman, M.D. Cooper, F. Bussolino, R. Pasqualini, W. Arap, Aminopeptidase A is a functional target in angiogenic blood vessels, Cancer Cell 5 (2004) 151–162.
[63] M.A. Burg, R. Pasqualini, W. Arap, NG2 proteoglycan-binding peptides target tumor neovasculature, Cancer Res. 59 (1999) 2869–2874.
[64] B. Erdag, K.B. Balcioglu, A. Kumbasar, O. Celikbicak, G. Zeder-Lutz, D. Altschuh, B. Salih, K. Baysal, Novel short peptides isolated from phage display library inhibit vascular endothelial growth factor activity, Mol. Biotechnol. 35 (2007) 51–63.
[65] A. Raiter, C. Weiss, Z. Bechor, I. Ben-Dor, A. Battler, B. Kaplan, B. Hardy, Activation of GRP78 on endothelial cell membranes by an ADAM15-derived peptide induces angiogenesis, J. Vasc. Res. 47 (2010) 399–411.
[66] J.A. Hoffman, E. Giraudo, M. Singh, L. Zhang, M. Inoue, K. Porkka, D. Hanahan, E. Ruoslahti, Progressive vascular changes in a transgenic mouse model of squamous cell carcinoma, Cancer Cell 4 (2003) 383–391.
[67] J.A. Joyce, P. Laakkonen, M. Bernasconi, G. Bergers, E. Ruoslahti, D. Hanahan, Stage-specific vascular markers revealed by phage display in a mouse model of pancreatic islet tumorigenesis, Cancer Cell 4 (2003) 393–403.
[68] A. Hajitou, Targeted systemic gene therapy and molecular imaging of cancer contribution of the vascular-Targeted AAAVP vector, Adv. Genet. 69 (2010) 65–82.
[69] S. Kanki, D.E. Jaalouk, S. Lee, A.Y. Yu, J. Gannon, R.T. Lee, Identification of targeting peptides for ischemic myocardium by in vivo phage display, J. Mol. Cell. Cardiol. 50 (2011) 841–848.
[70] G.Y. Lee, J.H. Kim, G.T. Oh, B.H. Lee, I.C. Kwon, I.S. Kim, Molecular targeting of atherosclerotic plaques by a stabilin-2-specific peptide ligand, J. Control. Release 155 (2011) 211–217.
[71] M.G. Kolonin, P.K. Saha, L. Chan, R. Pasqualini, W. Arap, Reversal of obesity by targeted ablation of adipose tissue, Nat. Med. 10 (2004) 625–632.
[72] F.I. Staquicini, M.G. Ozawa, C.A. Moya, W.H. Driessen, E.M. Barbu, H. Nishimori, S. Soghomonyan, L.G. Flores II, X. Liang, V. Paolillo, M.M. Alauddin, J.P. Basilion, F.B. Furnari, O. Bogler, F.F. Lang, K.D. Aldape, G.N. Fuller, M. Höök, J.G. Gelovani, R.L. Sidman, W.K. Cavenee, R. Pasqualini, W. Arap, Systemic combinatorial peptide selection yields a non-canonical iron-mimicry mechanism for targeting tumors in a mouse model of human glioblastoma, J. Clin. Invest. 121 (2011) 161–173.
[73] Y. Yang, W. Zizheng, D. Tongxin, Mouse thymus targeted peptide isolated by in vivo phage display can inhibit bioactivity of thymus output in vivo, J. Biomol. Screen. 13 (2008) 968–974.
[74] V.J. Yao, M.G. Ozawa, M. Trepel, W. Arap, D.M. McDonald, R. Pasqualini, Targeting pancreatic islets with phage display assisted by laser pressure catapult microdissection, Am. J. Pathol. 166 (2005) 625–636.
[75] M. Koolpe, R. Burgess, M. Dail, E.B. Pasquale, EphB receptor-binding peptides identified by phage display enable design of an antagonist with ephrin-like affinity, J. Biol. Chem. 280 (2005) 17301–17311.
[76] X. Han, Y. Xu, Y. Yang, J. Xi, W. Tian, S. Duggineni, Z. Huang, J. An, Discovery and characterization of a novel cyclic peptide that effectively inhibits ephrin binding to the EphA4 receptor and displays anti-angiogenesis activity, PLoS One 8 (2013) e80183.
[77] X. Dai, C. Cai, F. Xiao, Y. Xiong, Y. Huang, Q. Zhang, Q. Xiang, G. Lou, M. Lian, Z. Su, Q. Zheng, Identification of a novel aFGF-binding peptide with anti-tumor effect on breast cancer from phage display library, Biochem. Biophys. Res. Commun. 12 (Feb 12 2014), http://dx.doi.org/10.1016/j.bbrc.2014.02.022 (pii: S0006-291X(14)00274-5).
[78] B.M. Larimer, W.D. Thomas, G.P. Smith, S.L. Deutscher, Affinity maturation of an ERBB2-targeted SPECT imaging peptide by in vivo phage display, Mol. Imaging Biol. (Feb 19 2014), http://dx.doi.org/10.1007/s11307-014-0724-5.
[79] A.N. Veleva, D.B. Nepal, C.B. Frederick, J. Schwab, P. Lockyer, H. Yuan, D.S. Lalush, C. Patterson, Efficient in vivo selection of a novel tumor-associated peptide from a phage display library, Molecules 16 (2011) 900–914.
[80] C. Patterson, C.B. Frederick, H. Yuan, L.A. Dyer, P. Lockyer, D.S. Lalush, A.N. Veleva, Development of a new positron emission tomography tracer for targeting tumor angiogenesis: synthesis, small animal imaging, and radiation dosimetry, Molecules 18 (2013) 5594–5610.
[81] X. Fan, R. Venegas, R. Fey, H. van der Heyde, M.A. Bernard, E. Lazarides, C.M. Woods, An in vivo approach to structure activity relationship analysis of peptide ligands, Pharm. Res. 24 (2007) 868–879.
[82] L. Denby, L.M. Work, D.J. Von Seggern, E. Wu, J.H. McVey, S.A. Nicklin, A.H. Baker, Development of renal-targeted vectors through combined in vivo phage display and capsid engineering of adenoviral fibers from serotype 19p, Mol. Ther. 15 (2007) 1647–1654.
[83] T.Y. Lee, C.T. Lin, S.Y. Kuo, D.K. Chang, H.C. Wu, Peptide-mediated targeting to tumor blood vessels of lung cancer for drug delivery, Cancer Res. 67 (2007) 10958–10965.
[84] A.L. Matsuo, M.A. Juliano, C.R. Figueiredo, W.L. Batista, A.S. Tanaka, L.R. Travassos, A new phage-display tumor-homing peptide fused to antiangiogenic peptide generates a novel bioactive molecule with antimelanoma activity, Mol. Cancer Res. 9 (2011) 1471–1478.
[85] M. Zhi, K.C. Wu, L. Dong, Z.M. Hao, T.Z. Deng, L. Hong, S.H. Liang, P.T. Zhao, T.D. Qiao, Y. Wang, X. Xu, D.M. Fan, Characterization of a specific phage-displayed peptide binding to vasculature of human gastric cancer, Cancer Biol. Ther. 3 (2004) 1232–1235.
[86] M. Yang, C. Liu, M. Niu, Y. Hu, M. Guo, J. Zhang, Y. Luo, W. Yuan, M. Yang, M. Yun, L. Guo, J. Yan, D. Liu, J. Liu, Y. Jiang, Phage-display library biopanning and bioinformatic analysis yielded a high-affinity peptide to inflamed vascular endothelium both in vitro and in vivo, J. Control. Release 174 (2014) 72–80.
[87] C. Ma, G. Yin, D. Yan, X. He, L. Zhang, Y. Wei, Z. Huang, A novel peptide specifically targeting ovarian cancer identified by in vivo phage display, J. Pept. Sci. 19 (2013) 730–736.
[88] M. Staniszewska, X. Gu, C. Romano, A. Kazlauskas, A phage display-based approach to investigate abnormal neovessels of the retina, Invest. Ophthalmol. Vis. Sci. 3 (2012) 4371–4379.
[89] M.L. Balestrieri, C. Fiorito, E. Crimi, F. Felice, C. Schiano, L. Milone, A. Casamassimi, A. Giovane, V. Grimaldi, V. del Giudice, P.B. Minucci, F.P. Mancini, L. Servillo, F.P. D'Armiento, B. Farzati, C. Napoli, Effect of red wine antioxidants and minor polyphenolic constituents on endothelial progenitor cells after physical training in mice, Int. J. Cardiol. 126 (2008) 295–297.
[90] B. Chen, S. Cao, Y. Zhang, X. Wang, J. Liu, X. Hui, Y. Wan, W. Du, L. Wang, K. Wu, D. Fan, A novel peptide (GX1) homing to gastric cancer vasculature inhibits angiogenesis and cooperates with TNF alpha in anti-tumor therapy, BMC Cell Biol. 10 (2009) 63.
[91] I. van Rooy, S. Cakir-Tascioglu, P.O. Couraud, I.A. Romero, B. Weksler, G. Storm, W.E. Hennink, R.M. Schiffelers, E. Mastrobattista, Identification of peptide ligands for targeting to the blood–brain barrier, Pharm. Res. 27 (2010) 673–682.
[92] J. Li, Q. Zhang, Z. Pang, Y. Wang, Q. Liu, L. Guo, X. Jiang, Identification of peptide sequences that target to the brain using in vivo phage display, Amino Acids 42 (2012) 2373–2381.
[93] D.K. Chang, C.Y. Chiu, S.Y. Kuo, W.C. Lin, A. Lo, Y.P. Wang, P.C. Li, H.C. Wu, Antiangiogenic targeting liposomes increase therapeutic efficacy for solid tumors, J. Biol. Chem. 284 (2009) 12905–12916.
[94] Q. Cao, S. Liu, G. Niu, K. Chen, Y. Yan, Z. Liu, X. Chen, Phage display peptide probes for imaging early response to bevacizumab treatment, Amino Acids 41 (2011) 1103–1112.

[95] M. Loi, S. Marchio, P. Becherini, D. Di Paolo, M. Soster, F. Curnis, C. Brignole, G. Pagnan, P. Perri, I. Caffa, R. Longhi, B. Nico, F. Bussolino, C. Gambini, D. Ribatti, M. Cilli, W. Arap, R. Pasqualini, T.M. Allen, A. Corti, M. Ponzoni, F. Pastorino, Combined targeting of perivascular and endothelial tumor cells enhances anti-tumor efficacy of liposomal chemotherapy in neuroblastoma, J. Control. Release 145 (2010) 66–73.
[96] A. Liguori, C. Fiorito, M.L. Balestrieri, E. Crimi, G. Bruzzese, S. Williams-Ignarro, M. D'Amora, L. Sommese, V. Grimaldi, P.B. Minucci, A. Giovane, B. Farzati, L.J. Ignarro, C. Napoli, Functional impairment of hematopoietic progenitor cells in patients with coronary heart disease, Eur. J. Haematol. 80 (2008) 258–264.
[97] J.A. Greig, R. Shirley, D. Graham, L. Denby, A.F. Dominiczak, L.M. Work, A.H. Baker, Vascular-targeting antioxidant therapy in a model of hypertension and stroke, J. Cardiovasc. Pharmacol. 56 (2010) 642–650.
[98] A.L. Dunehoo, M. Anderson, S. Majumdar, N. Kobayashi, C. Berkland, T.J. Siahaan, Cell adhesion molecules for targeted drug delivery, J. Pharm. Sci. 95 (2006) 1856–1872.
[99] R. Stupp, M.E. Hegi, B. Neyns, R. Goldbrunner, U. Schlegel, P.M. Clement, G.G. Grabenbauer, A.F. Ochsenbein, M. Simon, P.Y. Dietrich, T. Pietsch, C. Hicking, J.C. Tonn, A.C. Diserens, A. Pica, M. Hermisson, S. Krueger, M. Picard, M. Weller, Phase I/IIa study of cilengitide and temozolomide with concomitant radiotherapy followed by cilengitide and temozolomide maintenance therapy in patients with newly diagnosed glioblastoma, J. Clin. Oncol. 28 (2010) 2712–2718.
[100] D.A. Reardon, L.B. Nabors, R. Stupp, T. Mikkelsen, Cilengitide: an integrin-targeting arginine–glycine-aspartic acid peptide with promising activity for glioblastoma multiforme, Expert Opin. Investig. Drugs 17 (2008) 1225–1235.
[101] P. Leblond, A. Dewitte, F. Le Tinier, C. Bal-Mahieu, M. Baroncini, T. Sarrazin, E. Lartigau, A. Lansiaux, S. Meignan, Cilengitide targets pediatric glioma and neuroblastoma cells through cell detachment and anoikis induction, Anticancer Drugs 24 (2013) 818–825.
[102] T. Lautenschlaeger, J. Perry, D. Peereboom, B. Li, A. Ibrahim, A. Huebner, W. Meng, J. White, A. Chakravarti, In vitro study of combined cilengitide and radiation treatment in breast cancer cell lines, Radiat. Oncol. 8 (2013) 246.
[103] S.M. Cabarcas, L. Sun, L. Mathews, S. Thomas, X. Zhang, W.L. Farrar, The differentiation of pancreatic tumor-initiating cells by vitronectin can be blocked by cilengitide, Pancreas 42 (2013) 861–870.
[104] M. Bretschi, C. Cheng, H. Witt, A. Dimitrakopoulou-Strauss, L.G. Strauss, W. Semmler, T. Bäuerle, Cilengitide affects tumor compartment, vascularization and microenvironment in experimental bone metastases as shown by longitudinal ^{18}F-FDG PET and gene expression analysis, J. Cancer Res. Clin. Oncol. 139 (2013) 573–583.
[105] T.L. Ten Hagen, A.L. Seynhaeve, G.a. de Wiel-Ambagtsheer, E.A. de Bruijn, S.T. van Tiel, C. Ruegg, M. Meyring, M. Grell, S.L. Goodman, A.M. Eggermont, The αVβ3/αVβ5 integrin inhibitor cilengitide augments tumor response to melphalan isolated limb perfusion in a sarcoma model, Int. J. Cancer 132 (2013) 2694–2704.
[106] T.J. MacDonald, G. Vezina, C.F. Stewart, D. Turner, C.R. Pierson, L. Chen, I.F. Pollack, A. Gajjar, M.W. Kieran, Phase II study of cilengitide in the treatment of refractory or relapsed high-grade gliomas in children: a report from the Children's Oncology Group, Neuro Oncol. 15 (2013) 1438–1444.
[107] J.B. Vermorken, J. Guigay, R. Mesia, J.M. Trigo, U. Keilholz, A. Kerber, U. Bethe, M. Picard, T.H. Brummendorf, Phase I/II trial of cilengitide with cetuximab, cisplatin and 5-fluorouracil in recurrent and/or metastatic squamous cell cancer of the head and neck: findings of the phase I part, Br. J. Cancer 104 (2011) 1691–1696.
[108] C. Manegold, J. Vansteenkiste, F. Cardenal, W. Schuette, P.J. Woll, E. Ulsperger, A. Kerber, J. Eckmayr, J. von Pawel, Randomized phase II study of three doses of the integrin inhibitor cilengitide versus docetaxel as second-line treatment for patients with advanced non-small-cell lung cancer, Invest. New Drugs 31 (2013) 175–182.
[109] A. Alva, S. Slovin, S. Daignault, M. Carducci, R. Dipaola, K. Pienta, D. Agus, K. Cooney, A. Chen, D.C. Smith, M. Hussain, Phase II study of cilengitide (EMD 121974, NSC 707544) in patients with non-metastatic castration resistant prostate cancer, NCI-6735. A study by the DOD/PCF prostate cancer clinical trials consortium, Invest. New Drugs 30 (2012) 749–757.
[110] P. Hu, J. Yan, J. Sharifi, T. Bai, L.A. Khawli, A.L. Epstein, Comparison of three different targeted tissue factor fusion proteins for inducing tumor vessel thrombosis, Cancer Res. 63 (2003) 5046–5053.
[111] R.E. Wang, Y. Niu, H. Wu, J. Cai, Development of NGR-based anti cancer agents for targeted therapeutics and imaging, Anticancer Agents Med. Chem. 12 (2012) 76–86.
[112] V. Gregorc, P.A. Zucali, A. Santoro, G.L. Ceresoli, G. Citterio, T.M. De Pas, N. Zilembo, F. De Vincenzo, M. Simonelli, G. Rossoni, A. Spreafico, M. Grazia Viganò, F. Fontana, F.G. De Braud, E. Bajetta, F. Caligaris-Cappio, P. Bruzzi, A. Lambiase, C. Bordignon, Phase II study of asparagine-glycine-arginine-human tumor necrosis factor alpha, a selective vascular targeting agent, in previously treated patients with malignant pleural mesothelioma, J. Clin. Oncol. 28 (2010) 2604–2611.
[113] V. Gregorc, A. Santoro, E. Bennicelli, C.J. Punt, G. Citterio, J.N. Timmer-Bonte, F. Caligaris Cappio, A. Lambiase, C. Bordignon, C.M. van Herpen, Phase Ib study of NGR-hTNF, a selective vascular targeting agent, administered at low doses in combination with doxorubicin to patients with advanced solid tumours, Br. J. Cancer 101 (2009) 219–224.
[114] A. Santoro, T. Pressiani, G. Citterio, G. Rossoni, G. Donadoni, F. Pozzi, L. Rimassa, N. Personeni, S. Bozzarelli, G. Rossoni, S. Colombi, F.G. De Braud, F. Caligaris-Cappio, A. Lambiase, C. Bordignon, Activity and safety of NGR-hTNF, a selective vascular-targeting agent, in previously treated patients with advanced hepatocellular carcinoma, Br. J. Cancer 10 (2010) 837–844.
[115] A. Santoro, L. Rimassa, A.F. Sobrero, G. Citterio, F. Sclafani, C. Carnaghi, L. Rimassa, N. Personeni, S. Bozzarelli, G. Rossoni, S. Colombi, F.G. De Braud, F. Caligaris-Cappio, A. Lambiase, C. Bordignon, Phase II study of NGR-hTNF, a selective vascular targeting agent, in patients with metastatic colorectal cancer after failure of standard therapy, Eur. J. Cancer 46 (2010) 2746–2752.
[116] F. Curnis, A. Sacchi, A. Corti, Improving chemotherapeutic drug penetration in tumors by vascular targeting and barrier alteration, J. Clin. Invest. 110 (2002) 475–482.
[117] D. Lorusso, G. Scambia, G. Amadio, A. di Legge, A. Pietragalla, R. De Vincenzo, V. Masciullo, M. Di Stefano, G. Mangili, G. Citterio, M. Mantori, A. Lambiase, C. Bordignon, Phase II study of NGR-hTNF in combination with doxorubicin in relapsed ovarian cancer patients, Br. J. Cancer 107 (2010) 37–42.
[118] S. Mammoliti, V. Andretta, E. Bennicelli, F. Caprioni, D. Comandini, G. Fornarini, A. Guglielmi, A. Pessino, S. Sciallero, A.F. Sobrero, G. Mazzola, A. Lambiase, C. Bordignon, Two doses of NGR-hTNF in combination with capecitabine plus oxaliplatin in colorectal cancer patients failing standard therapies, Ann. Oncol. 22 (2011) 973–978.
[119] P.A. Knetsch, M. Petrik, C.M. Griessinger, C. Rangger, M. Fani, C. Kesenheimer, E. von Guggenberg, B.J. Pichler, I. Virgolini, C. Decristoforo, R. Haubner, [68Ga]NODAGA-RGD for imaging αvβ3 integrin expression, Eur. J. Nucl. Med. Mol. Imaging 38 (2011) 1303–1312.
[120] E.V. Ariztia, C.J. Lee, R. Gogoi, D.A. Fishman, The tumor microenvironment: key to early detection, Crit. Rev. Clin. Lab. Sci. 43 (2006) 393–425.
[121] V.A. Petrenko, P.K. Jayanna, Phage protein-targeted cancer nanomedicines, FEBS Lett. 588 (2014) 341–349.
[122] T. Wang, W.C. Hartner, J.W. Gillespie, K.P. Praveen, S. Yang, L.A. Mei, V.A. Petrenko, V.P. Torchilin, Enhanced tumor delivery and antitumor activity in vivo of liposomal doxorubicin modified with MCF-7-specific phage fusion protein, Nanomedicine 10 (2014) 421–430