Update on vascular disrupting agents for cancer therapy

Tumor vascular networks supply cancer cells with essential nutrients and oxygen. Since 1971, when Judah Folkman proposed the hypothesis of therapeutic targeting tumor angiogenesis, multiple compounds with anti-angiogenic activities have been discovered, some of which have demonstrated clear clinical benefits for patients with cancer. Tumor angiogenesis became a new target in combating the growth and metastasis of solid tumors. Apart from inhibitors of tumor angiogenesis, which affect the formation of new blood vessels, another class of drugs – vascular disrupting agents (VDAs) – that target endothelial cells of the already established tumor vessels are currently under clinical investigation. VDAs are divided into two types: small molecules and ligand-directed VDAs. Small molecules are further subdivided into two classes: the tubulin-binding agents and the synthetic flavonoids, which work to induce local production of cytokines. Ligand-directed VDAs consist of targeting and effector moieties that are combined together and designed to deliver toxic compounds selectively to the tumor vascular network. The review discusses the mechanisms of action of VDAs and their preclinical and clinical results.

KEYWORDS: antiangiogenic drugs ■ imaging of tumor vasculature ■ tumor angiogenesis ■ vascular disrupting agents

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Tumor vasculature is now a well-recognized therapeutic target [1]. Blood vessels sustain tumor growth and enable spread and formation of distant metastases [2]. The phenomenon of tumors stimulating formation of their own blood vessels (angiogenesis) was first observed in the 1930s [3]. In 1971, Judah Folkman proposed that tumors cannot grow beyond a certain volume (1–2 mm^3) without support of a system to supply blood and he further hypothesized that blocking tumor angiogenesis would be effective at controlling cancer growth and metastasis [4]. Extensive research has been conducted since then that has focused on the molecular mechanisms of tumor angiogenesis and critical control points that can be targeted therapeutically. Two major concepts regarding therapeutic applications of tumor angiogenesis are to block formation of new vessels (anti-angiogenic strategies) or disrupt tumor vessels that are already established (vascular disrupting strategies) [5,6].

Anti-angiogenic drugs can be divided into two major groups: biologics including monoclonal antibodies (e.g., bevacizumab) and fusion protein constructs (e.g., aflibercept) and small molecular weight drugs such as tyrosine kinase inhibitors (TKIs; e.g., sunitinib). Monoclonal antibodies or fusion proteins target the angiogenic cascade by interfering with ligand–receptor interactions. For example bevacizumab binds to the angiogenic growth factor VEGF and prevents it from binding and activating its cognate signaling receptors (VEGF receptors 1 and 2 [VEGFR1/2]), which are primarily expressed on endothelial cells. TKIs are small molecules that penetrate the cell membrane and block downstream signaling by competing with ATP for the ATP-binding site within the catalytic domain of receptor tyrosine kinases (RTKs). TKIs typically have broader specificity than biologic agents because multiple RTKs share similarities within the ATP-binding site in the kinase domain [7].

Vascular disrupting agents (VDAs) specifically target endothelial cells, pericytes or the vascular basement membrane of established tumor vessels. The idea of targeting the process of endothelial cell proliferation within the existing tumor vessels was advocated in 1980s by Julia Denekamp [8]. Her concept of targeted tumor vessels was different from Judah Folkman's hypothesis. Instead of preventing the formation of new tumor vasculature, Denekamp advocated that existing tumor vessels can be affected by interfering with endothelial proliferation and function that would lead to functional shutdown of the vessels feeding the tumor. This in turn would cause extensive necrosis, as it was observed in animal models after physical obstruction of tumor vessels, mainly due to profound hypoxia [9,10].

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Since the hypothesis was proposed, multiple VDAs with endothelium-specific cytotoxic effects have been tested in preclinical and early clinical settings [6,11]. VDAs are divided into two major types: small molecules and ligand-directed VDAs. Small molecules comprise two classes: flavonoids, which mainly exert their therapeutic function by inducing local cytokine production and the tubulin-binding agents, which mainly interfere with the cytoskeleton of endothelial cells. Ligand-directed VDAs have a targeting moiety that binds to a unique antigen or molecule that is expressed on or around tumor vasculature and is accessible from the intravascular space. The targeting moiety is linked to an effector moiety that exerts its cytotoxic or pro-embolic effect once bound to its target [12,13].

While anti-angiogenic drugs (monoclonal antibodies and TKIs) are already approved for clinical use in a variety of human solid malignancies, vascular targeting agents still remains in the phase of preclinical or early clinical trials.

The VDAs that are the most advanced in clinical development are 5,6-dimethylxanthenone-4-acetic acid (DMXAA; ASA404) and combretastatin A-4 disodium phosphate (CA4P) [14,15]

The aim of this review is to provide an update on the most advanced clinical applications of VDAs in patients with cancer.

Vascular disrupting agents: mechanism of action

Tumor vessels differ from normal vasculature. Generally, tumor vasculature has irregular caliber, is leaky and tortuous with sluggish blood flow and increased vascular resistance when compared with normal vessels [1]. However, even within the same tumor its vasculature is composed of vessels that differ from each other and this heterogeneity has therapeutic implications when agents that target tumor vessels are used. In the majority of solid malignancies, vessels that are present within the tumor center form a chaotic network of thin-walled, leaky, tortuous vessels that are highly dependent on constant survival stimuli from vascular growth factors secreted by tumor, stromal and inflammatory cells [16–20].

Endothelial cells that line the inside of these vessels are characterized by high proliferation index. On the tumor periphery, vessels are more mature (covered with supportive cells such as pericytes and smooth muscle cells), which makes them less dependent on vascular growth factors for survival [21]. This vascular heterogeneity within the tumor has certain implications when either anti-angiogenic or vascular disrupting agents are used.

Anti-angiogenic drugs work through deprivation of pro-angigogenic or prosurvival growth factors, which results in arrest of new vessel formation and regression of immature and leaky vessels in the core of the tumor [22]. VDA can affect vascular endothelium in multiple ways. Small molecule VDAs can be divided into two major groups based on mechanism of action: tubulin binding, microtubule depolymerizing agents (e.g., combretastatins) or agents that cause endothelial cells apoptosis through stimulation of local cytokines production (e.g., DMXAA) [23].

Endothelial cell proliferation and function is dependent on integrity of the tubulin cytoskeleton [24]. VDAs with tubulin-binding properties, interfere with the process of tubulin polymerization and thus induce irreversible changes in the shape and function of vascular endothelial cells. The disruption of the cytoskeleton structure and cell-to-cell junctions induces the series of events that lead to increased permeability and decreased blood flow within the affected vessels. Upon structural changes of endothelium, basement membrane components are exposed to platelets and blood coagulation factors that, together with the sluggish blood flow, accelerate endovascular thrombosis that results in functional vascular shutdown. Interaction of VDA with the structure and function of tubulin within tumor endothelial cells does not fully explain the phenomenon of rapid vascular effects observed *in vivo*. It is very likely that multiple mechanisms rather than a single mechanism are responsible for the observed immediate effects of tubulin-specific VDA. An increase in permeability of the tumor vasculature to macromolecules and the activation cell death pathway mediated by inhibition of PI3K/Akt signaling after disruption of VE-cadherin junctions by CA4P are one of the most common proposed mechanisms [25,26].

Ligand-directed VDAs are composed of the effector moiety (cytotoxic drug or procoagulant) and the targeting moiety that binds to the molecule that is exclusively expressed within the tumor endothelium [13]. The first preclinical evidence that ligand-directed VDA therapy can affect solid tumors comes from using ricin combined with an antibody against major histocompatibility complex (MHC) class II antigen that was selectively expressed within the tumor endothelium [27]. Upon binding to MHC class II

antigen, endothelial cells internalized ricin and the cytotoxic cascade of events caused collapse of the tumor vascular network and thus eradicated the tumor. A similar approach using the same targeting moiety combined with tissue factor (TF) resulted in even more potent selective thrombosis within the tumor vessels followed by tumor regression [28].

Although very attractive and promising in animal studies, ligand-directed VDAs have lagged behind small molecule VDAs in clinical development due in part to specificity concerns [29]. A unique genetic expression profile of tumor endothelial cells when compared with endothelial cells in healthy tissue is recognized and has been documented [30]. The list of antigens and molecules that are selectively expressed on tumor endothelium is long and still growing. In Table 1 some of the most promising clinically vascular targets are presented. However, the lack of a target on tumor endothelium that would not be expressed anywhere else within the healthy tissue remains a major obstacle in introducing these agents into clinical practice.

In animal models, VDA administration results in dramatic and abrupt changes within the core of the tumor where the majority of vessels do not have pericyte coverage. Functional impairment of the vessels within the tumor center results in nearly immediate marked central thrombosis and onset of necrosis. Unfortunately, vessels at the border of the tumor are more mature and stable, and thus less sensitive to VDA action. This so-called 'viable rim' of well perfused tumor tissue persists after administration of VDAs and new vessels will re-grow from this area into the tumor core when VDAs are withdrawn [30]. Combination therapy that includes a ligand-directed VDA along with cytotoxic chemotherapy can be effective at complete tumor eradication [28,31].

Table 1. Potential markers of tumor blood vessels.

Antigen	Location of marker	Ref.
VEGF:VEGFR	Angiogenic BVs	[92–95]
Integrin $\alpha_3\beta_1$	Angiogenic BVs	[96]
Integrin $\alpha_6\beta_1$	Angiogenic BVs	[96]
p30.5	Proliferating ECs	[97]
CD105 (endoglin)	Proliferating ECs	[98–103]
Endosialin	Proliferating ECs	[104]
VCAM-1	Activated ECs	[105]
E-selectin, CD62E	Activated ECs	[105]
H-5–2, Lewis^y-6	Activated ECs	[106]
CD44	Activated ECs	[107]
Hyaluronan	Activated ECs	[108]
Integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$	Activated ECs	[80,109,110]
Integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$	Activated ECs	[111,112]
Integrin $\alpha_5\beta_1$	Activated ECs	[113]
Phosphatidylserine	Activated ECs	[105]
FN	Basement membrane	[114]
ED-B isoform of FN	Basement membrane	[115–118]
Denaturated collagens	Proteolyzed basement membrane	[119,120]
NG2 proteoglycan	Pericytes	[121]
CD13/APN	Tumor ECs	[122]

APN: Aminopeptidase N; BV: Blood vessel; EC: Endothelial cell; FN: Fibronectin; VEGF:VEGFR: Complex of VEGF and its receptor.

Clinical applications of small molecule VDAs

Synthetic flavonoids that induce local cytokine production and tubulin-binding agents constitute the major classes of small molecule VDAs [6]. The most clinically advanced small molecule VDA is DMXAA, also known as ASA0404. It is a potent cytokine inducer with a selective antivascular effect in preclinical mouse studies [14]. The exact mode of action of DMXAA is not fully understood. Substantial experimental evidence exists for activation of NFκB transcription factor upon DMXAA administration that leads to upregulation of multiple cytokines, including TNF-α, within tumor cells and in inflammatory and endothelial cells [32–34]. DMXAA has been tested in Phase I and II clinical trials in patients with solid malignancies. The drug was well tolerated at 1200 mg/m^2 dose given weekly during 20 min infusion [35]. Currently there are two ongoing trials (one in Phase III) investigating clinical benefit of DMXAA in combination with platinum-based chemotherapy in patients with advanced (stage IIIB/IV) non-small-cell lung cancer (NSCLC; clinical trials numbers: NCT00738387; NCT01057342 [201]). The oncology community awaits results of the Phase III study that has overall survival as an end point.

VDAs that belong to the tubulin-binding class comprise multiple different compounds that function similarly to colchicine as they bind to tubulin and cause disruption of the endothelial cell cytoskeleton [36]. The combretastatin family of drugs are derived from the African shrub *Combretum caffrum* [37,38]. Among compounds that are the most promising are CA4P (fosfbretabulin tromethamine), combretastatin A-1 diphosphate (CA1P; Oxi4503), ABT-751 and NPI-2358 (plinabulin). There are multiple clinical trials investigating efficacy of these agents as single agents and in combination with cytotoxic chemotherapy. Four reports from Phase I/II clinical trials that included

heterogenous group of patients with refractory solid malignancies showed encouraging results when CA4P was combined with platinum-based chemotherapy [39–41]. Abnormal angiogenesis is recognized in renal cell carcinoma and this is the only malignancy that has shown single-agent efficacy with anti-angiogenic therapy [7]. The idea of combined anti-angiogenic and vascular-targeted approaches was evaluated in a mouse model of renal cell carcinoma and showed synergistic activity of this regimen [42]. Results of a Phase I study in patients with various solid tumors treated with bevacizumab and CA4P are awaited (clinical trial number: NCT00395434 [201]). The FALCON trial investigated the efficacy of CA4P combined with a carboplatin/paclitaxel/bevacizumab regimen in 50 patients with stage IIIB/IV NSCLC who were randomized to chemotherapy/bevacizumab/CA4P versus chemotherapy/bevacizumab/placebo. Preliminary data suggest a survival benefit in the group treated with CA4P; however, mature data is not available [43]. The ADVANCE study randomized patients with stage IIIB/IV NSCLC who progressed on prior chemotherapy to docetaxel alone or in combination with plinabulin [44]. Although recruitment is still ongoing, some preliminary analysis showed promising results of 22% partial response rate in combination group versus 5% in docetaxel only group. It is also recognized that CA4P may potentiate the effects of radiotherapy. This has been evaluated clinically in a small number of patients who received a relatively small dose of CA4P (50 mg/m^2) after fractional radiation (4.5 Gy) was delivered to the tumor [45].

Future studies will show how clinically meaningful and safe these new combinations are.

Ligand-directed VDAs

Ligand-directed VDAs (LD-VDAs) are the most exciting and clinically challenging class of VDA. Elegant animal studies have demonstrated remarkable results with these agents [27,28,46]. For LD-VDAs to work, two crucial elements have to be combined together: effector moiety (cytotoxic, radioactive or procoagulant agent) and targeting moiety (antibody or peptide) that ensure activation of LD-VDA within the tumor vascular bed. In the search for specific endothelial markers of tumor angiogenesis, multiple approaches have been used [47–49] and a number of potential targets for LD-VDAs have been identified. Despite encouraging results, LD-VDAs remain in the preclinical phase of the development. Major obstacles for these agents to be introduced into the clinic are specificity and their safety profile.

However, some tumor endothelial markers identified so far are being investigated in the clinical setting. Prostate surface membrane antigen (PSMA) expression is elevated on the surface of prostate cancer cells as well as on the tumor vascular endothelium [50]. Anti-PSMA antibody (J591) conjugated with radioactive compound (for imaging purposes) in patients with melanoma, kidney, bladder, lung, breast, colorectal and pancreatic cancers showed excellent targeting efficacy of radiologically confirmed sites of metastases [51]. Multiple studies with J591 combined with a radioactive agent as an effector moiety in patients with prostate cancer are ongoing at present.

NGR-TNF is a LD-VDA that uses a tumor-homing peptide (cNGRCG) that selectively binds to CD13, which is expressed on tumor neovasculature. Preclinical data showed that NGR-TNF at low dose changes vascular permeability and is a potent VDA at high doses. Tumor-specific delivery of TNF-α was investigated in a Phase I study in patients with solid malignancies and it was well tolerated [52]. Currently, there are ongoing clinical trials investigating the combination of NGR-TNF with chemotherapy in patients with variety of solid malignancies.

Phosphatidylserine (PS) is an anionic phospholipid that is normally present in the inner part of the cell membrane bilayer. However, in tumor endothelium, PS is present in the outer aspect of the cell membrane bilayer, making it accessible for vascular targeting agents [53]. Monoclonal antibody targeting of PS inhibits tumor growth as a single agent and potentiates the anti-tumor effects of chemotherapeutic drugs in animal models of human breast and pancreatic cancer [54,55]. Furthermore, treatment with anti-PS monoclonal antibodies combined with radiotherapy in animal models of lung cancer and glioblastoma showed synergistic therapeutic effect [56]. Bavituximab is a chimeric monoclonal antibody that binds PS via β2 glycoprotein 1 and is currently undergoing Phase II clinical evaluation in patients with solid malignancies. Promising results from an earlier Phase II study of bavitixumab in combination with platinum-based chemotherapy showed improvement in progression free survival by 2.4 months and in response rate by 28% in chemotherapy/bavitixumab versus chemotherapy/placebo group. It is anticipated that these results will translate into clinically meaningful

benefit in future studies. Although bavituximab is not conjugated with the effector moiety, its therapeutic effect, as it was presented in animal studies, may be related to an antibody-dependent cell-mediated cytotoxicity (ADCC) response within the tumor.

Toxicity of VDAs

One of the characteristic features of VDAs is abrupt and nearly immediate post administration tumor vascular shutdown. It is hard to imagine, that this profound effect within the tumor vasculature has no implications within other vascular beds in normal tissue. Indeed, the common toxicity profile of small molecule VDAs is directly related to vascular function with episodes of hypo- and hyper-tension, transient cardiac ischemia, changes in heart rate, vasovagal syncope, hot flushes and tumor pain. One of the rare toxicities of DMXAA described in the literature is transient visual disturbances. Visual symptoms included blurring, flickering, fragmentation, alteration of colors and contrast and photosensitivity. All symptoms usually started during the infusion and resolved completely within 1 h after infusion. One possible explanation for these side effects, except regional blood flow impairment within the retina, is nonspecific phosphodiesterase inhibition [35].

The toxicity profile of LD-VDAs is less-well understood because fewer clinical studies have been completed. The major concern regarding the side effects of these compounds is that they could exert their potent cytotoxic or prothrombotic effects within the normal vasculature. Animal studies using recombinant fusion protein-targeting tissue factor (TF) targeted to vascular cell adhesion molecule 1 (VCAM-1; CD106) on tumor endothelium, failed to detect the evidence of systemic activation of coagulation cascade [46]. The rationale underlying this lack of toxicity led in part to identification of externalized PS as a cofactor in the induction of VDA-direct thrombosis [53].

Imaging applications of vascular targeting compounds

Selective expression of certain antigens and molecules on the surface of tumor endothelium provides an opportunity for targeted therapy using ligand-directed VDAs and for tumor specific imaging [47]. Targeted imaging of tumor angiogenesis using both invasive (i.e., intravital microscopy) or noninvasive (i.e., contrast ultrasound; US) methods showed very promising results in animal models. However, no targeted imaging methods are yet available for clinical use. The majority of imaging techniques that evaluate tumor effects of antivascular (both anti-angiogenic and vascular disrupting) agents use perfusion studies. Tumor blood flow is a surrogate marker of an activity of antivascular agent, assuming that blood flow within the tumor changes due to changes in the structure and function of tumor vessels [57–59]. The most clinically advanced imaging technique for assessment of vascular hemodynamic function is perfusion (dynamic contrast-enhanced; DCE) MRI [60].

DCE-MRI uses paramagnetic tracers like low-molecular-weight gadolinium and is the standard method for vascular function measurement in clinical trials of anti-angiogenic drugs [61]. Tissue perfusion and permeability, contrast concentration and the extravascular space volume determine signal enhancement obtained by DCE-MRI [62]. The results of imaging studies performed with the use of DCE-MRI in patients who received therapy with VDA confirm the usefulness of this imaging modality in clinical assessment of VDA effects within tumor vasculature [63]. US is an imaging technique used worldwide. Since blood and the surrounding tissue have similar echogenicity, US is not very useful for small blood vessels imaging. However, introduction of microbubbles (MBs) as US contrast agents markedly expanded application of US in the area of vascular imaging. MBs enable clear distinction of vascular structures from the surrounding tissue due to enhancement of blood echogenicity after intravenous injection. MBs are 1–10 μm particles that are truly intravascular tracers. They circulate freely in the bloodstream and do not extravasate unless there is structural damage to the vessel wall. They consist of a gaseous core and a lipid or protein shell [64]. MBs are used in echocardiography in the evaluation of ejection fraction and myocardial blood flow [65–67]. Contrast enhanced US imaging was used successfully in imaging of subcentimeter liver metastases and it was shown to have a comparable sensitivity to DCE-MRI technique and confirmed the usefulness in blood flow assessement [68–72].

Microbubbles behave hemodynamically like red blood cells, they circulate freely after injection and are small enough to reach the capillary microcirculation [73]. However, they can also be targeted actively to specific vascular beds by conjugation of targeting moieties (e.g., antibodies or peptides) to the MB shell [74–76]. Thus, tumor vasculature is an attractive subject for imaging with targeted MB and US [77]. One of the first studies of contrast US combined

with ligand-specific MB used $\alpha_v\beta_3$ integrin as a target [78]. $\alpha_v\beta_3$ integrin-specific MB homed specifically to tumor blood vessels and were detectable only within vascular areas in the tumor. Furthermore, signal enhancement from the targeted MB correlated with immunohistochemical staining of $\alpha_v\beta_3$ integrin in the same tumor samples [79]. $\alpha_v\beta_3$ integrin has also been used as a tumor vasculature-specific target in studies that use MRI and paramagnetic targeted nanoparticles as contrast agents [80,81].

Multiple animal studies validated contrast US using targeted microbubbles as a feasible method to visualize tumor blood vessels and also to monitor changes of the expression levels of variety of vascular markers (i.e., VEGFR2, CD105, VEGF:VEGFR complex and intercellular adhesion molecule-1 [ICAM-1]) in response to antivascular therapy [82–85]. Technically MBs can be designed to target more than one ligand [86]. Using MBs that bind selectively to more than one target is an attractive idea of increasing specificity to distinguish blood vessels in tumors versus nontumor tissue. In a mouse model of ovarian cancer, MBs that were conjugated with antibodies specific for VEGFR2 and $\alpha_v\beta_3$ integrin enhanced the US signal when compared with MB that were conjugated with a single targeting antibody. Dual-targeted MB-enhanced US was proven to increase sensitivity and specificity of imaging tumor angiogenesis [87]. Hopefully, these encouraging results from preclinical studies will be introduced into routine clinical imaging techniques and help to better monitor effects of vascular-targeted therapies in cancer patients.

Future perspective

Therapeutic targeting of tumor vasculature is a clinical reality. Despite the substantial advances in understanding tumor angiogenesis, the exact mode of action of anti-angiogenic drugs including VDAs is not yet fully understood. The clinical benefit of this class of drugs is rather disappointing, especially after some promising results from animal studies and early clinical Phase I/II studies. It is clear that randomized Phase III clinical trials fail to show a clinical benefit of VDAs when compared with standard of care treatments in patients with advanced solid malignant tumors. As we continue to

Executive summary

Vascular disrupting agents: mechanism of action

- Vascular disrupting agents (VDAs) can affect the vascular endothelium in multiple ways. Small molecule VDAs can be divided into two major groups based on mechanism of action: tubulin binding, microtubule depolymerizing agents (e.g., combretastatins) or agents that cause endothelial cells apoptosis through stimulation of local cytokines production (e.g., 5,6-dimethylxanthenone-4-acetic acid [DMXAA]).
- Ligand-directed VDAs are composed of the effector moiety (cytotoxic drug or procoagulant) and the targeting moiety that binds to the molecule that is exclusively expressed within the tumor endothelium.

Clinical applications of small molecule VDAs

- The most clinically advanced small molecule VDA is DMXAA, also known as ASA0404.
- DMXAA has been tested in Phase I and II clinical trials in patients with solid malignancies.
- Among the most promising tubulin-binding compounds are combretastatin A-4 disodium phosphate (CA4P; fosfbretabulin tromethamine), combretastatin A-1 diphosphate (CA1P; Oxi4503), ABT-751 and NPI-2358 (plinabulin).

Ligand-directed VDAs

- For ligand-directed (LD) VDAs to work, two crucial elements have to be combined together: effector moiety (cytotoxic, radioactive or procoagulant agent) and targeting moiety (antibody or peptide), that ensure activation of LD-VDA within the tumor vascular bed.
- Despite encouraging preclinical results, LD-VDAs remain in the preclinical phase of the development. Major obstacles for these agents to overcome before introduction into the clinic are specificity and the safety profile.

Toxicity of VDAs

- The common toxicity profile of clinically tested small molecule VDAs is directly related to vascular function with episodes of hypo- and hyper-tension, transient cardiac ischemia, changes in heart rate, vasovagal syncope, hot flushes and tumor pain.

Imaging applications of vascular targeting compounds

- Selective expression of certain antigens and molecules on the surface of tumor endothelium provides an opportunity for targeted therapy using ligand-directed VDAs and for tumor specific imaging.
- The most advanced clinical imaging technique for assessment of vascular hemodynamic function is perfusion (dynamic contrast-enhanced; DCE) MRI.
- Contrast-enhanced ultrasound imaging using microbubbles targeted to specific antigens expressed on the tumor endothelium is an attractive new tumor imaging modality that may be utilized in the clinic in the future.

Future perspective

- Although Phase III randomized clinical trials with VDAs are disappointing, new combinations of these drugs as well as disease-specific applications may prove clinical benefit.

study compounds with anti-angiogenic activity, unexpected complications of these drugs are being discovered, with some reports suggesting accelerated tumor metastasis and growth after its administration [88,89]. However, it is an exciting new avenue that opens new opportunities for the therapeutic management of cancer.

Vascular disrupting agents, due to their different mode of action when compared with anti-angiogenic drugs such as monoclonal antibodies or TKIs, can help to increase the overall clinical response to the antiangiogenic treatments. Following VDA treatment, tumors experience significant ischemic insult that results in severe and abrupt tumor cell hypoxia. This creates the unique opportunity for these drugs to combine them with prodrugs that can be activated within the tumor in the presence of hypoxic conditions (bioreductive drugs) [90,91].

What becomes more apparent in the therapeutic approach to cancer patients is a focus on personalized medicine and targeted therapies. It is recognized that to improve the outcomes, patients need to be selected based on the molecular and genetic make-up of their tumors. This concept is already proven to be clinically relevant for patients with different solid (i.e., breast, lung and colorectal cancer) and haematological (i.e., chronic myelogenous leukemia) malignancies. Vascular targeting agents offer unique opportunity to enhance the therapeutic effects of classic cytotoxic agents by interfering with the tumor vascular network that is a universal feature of malignancy. Unfortunately, we do not know if we can select patients for anti-angiogenic and vascular-targeted/disrupting therapies using biomarkers that would predict clinical benefit for the individual cancer patient. As research in tumor angiogenesis progresses, we hope that in the future it may be possible to better select patients who benefit from therapeutic targeting of tumor vasculature.

Financial & competing interests disclosure

RA Brekken consults and has stock ownership or options for Peregrine Pharmaceuticals a company that produces and develops vascular targeting agents. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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