Biochimica et Biophysica Acta 1826 (2012) 53–70

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbacan

Review

Angiogenesis inhibition for the improvement of photodynamic therapy: The revival of a promising idea

Andrea Weiss [a], Hubert van den Bergh [a], Arjan W. Griffioen [b,*], Patrycja Nowak-Sliwinska [b,c,**]

[a] Medical Photonics Group, Institute of Bioengineering, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland
[b] Angiogenesis Laboratory, Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands
[c] Urology Department, University Hospital (CHUV), Lausanne, Switzerland

ARTICLE INFO

Article history:
Received 18 December 2011
Received in revised form 13 March 2012
Accepted 14 March 2012
Available online 21 March 2012

Keywords:
Angiogenesis inhibitors
Endothelial cells
Photodynamic therapy
Photosensitizer
Targeting
Vascular normalization

ABSTRACT

Photodynamic therapy (PDT) is a minimally invasive form of treatment, which is clinically approved for the treatment of angiogenic disorders, including certain forms of cancer and neovascular eye diseases. Although the concept of PDT has existed for a long time now, it has never made a solid entrance into the clinical management of cancer. This is likely due to secondary tissue reactions, such as inflammation and neoangiogenesis. The recent development of clinically effective angiogenesis inhibitors has lead to the initiation of research on the combination of PDT with such angiostatic targeted therapies. Preclinical studies in this research field have shown promising results, causing a revival in the field of PDT. This review reports on the current research efforts on PDT and vascular targeted combination therapies. Different combination strategies with angiogenesis inhibition and vascular targeting approaches are discussed. In addition, the concept of increasing PDT selectivity by targeted delivery of photosensitizers is presented. Furthermore, the current insights on sequencing the therapy arms of such combinations will be discussed in light of vascular normalization induced by angiogenesis inhibition.

Contents

Abbreviations: ALA, aminolevulinic acid; AMD, age-related macular degeneration; AP-1, activator protein-1; $AlPcS_4$, tetrasulfonated aluminum phthalocyanine; APRPG, Ala-Pro-Arg-Pro-Gly peptide; ATP, adenosine triphosphate; bFGF, basic fibroblast growth factor; CAM, chorioallantoic membrane; CCH, circumscribed choroidal haemangioma; COX-2, cyclooxygenase-2; DMXAA, 5,6-dimethylxanthenone-4-acetic acid; EGF, epidermal growth factor; GM-CSF, granulocyte-macrophage colony stimulating factor; HeLa, human cervix carcinoma; HIF-1α, hypoxia-inducible factor 1-alpha; ICG, Indocyanine Green; IL-1β, interleukin 1-beta; MMP, matrix metalloproteinase; n.a., not applicable; NF-κB, nuclear factor κB; NPC, nasopharyngeal carcinoma; NPC, nasopharyngeal carcinoma; NSCLC, non-small-cell lung cancer; PCI, photochemical internalization; PDGF(R), platelet derived growth factor (receptor); PDT, photodynamic therapy; PS, photosensitizer; PEG, polyethylene glycol; PGE2, prostaglandin E2; PMN, polymorphonuclear; PNET, Pancreatic Neuroectodermal Tumor; RCH, retinal capillary haemangioma; RGD, Arg-Gly-Asp tri-peptide; RIF, radiation-induced fibrosarcoma; SCC, squamous-cell carcinoma; SIP, small immune protein; TNF-α, tumor necrosis factor alpha; TTT, transpupillary thermotherapy; VDA, vascular disrupting agent; VEGF, vascular endothelial cell growth factor; vWF, von Willebrand factor

* Correspondence to: A.W. Griffioen, Angiogenesis Laboratory, Department of Medical Oncology, VU University Medical Center Amsterdam, Amsterdam, The Netherlands. Tel.: +31 20 4443374; fax: +31 20 4443844.
** Correspondence to: P. Nowak-Sliwinska, Urology Department, University Hospital (CHUV), Lausanne, CH-1011, Switzerland. Tel.: +41 21 6935169; fax: +41 21 6935110.
E-mail addresses: aw.griffioen@vumc.nl (A.W. Griffioen), Patrycja.Nowak-Sliwinska@chuv.ch (P. Nowak-Sliwinska).

0304-419X/$ – see front matter © 2012 Published by Elsevier B.V.
doi:10.1016/j.bbcan.2012.03.003

1. Introduction

Photodynamic therapy (PDT) is a minimally invasive treatment that utilizes the combination of a non-toxic dose of light-sensitive molecules, known as a photosensitizer (PS), with the application of light at a wavelength appropriate to excite the PS and tissue oxygen in order to generate tissue damage. The earliest recorded use of a PS and a light source for a medical effect can be found in records originating in ancient Egypt from as early as 3000 years ago. Information on the treatment of vitiligo with sunlight after the application of plant extracts, probably containing psolarens (furanocumarins), can be found in the so-called Ebers Papyrus. This document dates circa 1550 BC, but was likely based on prior knowledge dating back as far as 3400 BC [1]. The use of a topically applied vegetable substance on the skin and subsequent irradiation by sunlight was used to produce photoreactions for skin repigmentation. The first scientific evidence of the use of PSs was reported over 100 years ago, when it was found that acridine orange and the application of light could kill protozoan cells [2]. Shortly after, Jesionek experimented with eosin as a therapeutic compound for the PDT treatment of cancer [3]. In the 1970s, following some intermittent PDT activity, Thomas Dougherty and co-workers clinically tested PDT and were able to demonstrate total and partial eradication of the growth of various malignant tumors, including metastatic melanomas, recurrent colon carcinomas, metastatic breast carcinomas on the chest wall, and recurrent basal cell carcinomas [4]. The latter publication, to a large extent, set off the current field of PDT research.

PDT is based on the generation of cytotoxic reactive oxygen species that cause tissue damage in the treated area [5,6]. In this way, PDT can be used to induce direct damage to tumor cells [7]. In addition, PDT can exert its effect through damage to the endothelium. This can lead to the disruption of the vasculature via endothelial cell (EC) damage, vascular leakage, and induction of thrombocyte aggregation and coagulation, eventually resulting in the occlusion of the vessels [8] and having effects on the immune system [9]. In current medicine, PDT is approved and has been implemented extensively in the treatment of various diseases of the eye, such as age-related macular degeneration (AMD) [5]. For therapy of oncological conditions, however, the success of PDT has been limited with the exception of certain skin conditions [10]. Although approved for some cancers, such as squamous cell carcinoma [11–13] and centrally located unresectable early stage lung cancer [14,15], major applications for the treatment of solid tumors have not yet been developed. This may be due to limitations of PDT for certain indications, such as the local nature of the therapy and the limited depth of light penetration into tissue. However, more important delimiters of the failure so far, may be the inevitable tissue responses to therapy, possibly enhancing and facilitating regrowth or further outgrowth of tumors. PDT has been shown to induce an inflammatory response in the tumor tissue. During and directly following PDT, the release of pro-inflammatory mediators, such as arachidonic acid metabolites, histamine discharged from mast cells, complement anaphylatoxins, cytokines, chemoattractants, leukocyte adhesion molecules and degradative enzymes, help to activate an inflammatory response and to attract neutrophils and other inflammatory cells to the treated site [9]. All of these factors induce tumor vasculature alterations, including increased permeability to allow the transport of blood proteins and pro-adhesiveness to inflammatory cells. Interleukin (IL)-1β and IL-6 have been shown to play critical roles in this PDT-induced inflammatory process [16,17]. Therefore, the modulation of different inflammatory factors can be used to enhance the effects of PDT, as elegantly reviewed by Agostinis et al. [18].

The above mentioned plethora of produced cytokines can also lead to direct tumor cell activation. Such cytokine storms, as well as attracted macrophages, can also stimulate endothelial cells to increase angiogenesis. Additionally, initial damage to the tumor tissue causes hypoxia and oxidative stress in the treated area [19], which induces hypoxia-inducible factor 1-alpha (HIF-1α) expression leading to VEGF production. These processes are interpreted by the body as localized acute trauma [18], which will then lead to more unwanted angiogenesis, possibly leading to the induction of tumor recurrence and maybe even the acceleration of tumor growth [20–25]. Due to these secondary effects following PDT, it was realized that the use of combination therapies which attempt to counteract these tissue responses may improve the final outcome of PDT. The main therapeutic modalities that are tested in this respect are chemotherapy [26], pro-oxidant therapies (e.g. erythropoietin) [27,28], anti-inflammation [18] and anti-angiogenesis therapies [29].

The combination of PDT with anti-angiogenic strategies is the focus of the current review. The relationship between PDT-induced damage, the activation of angiogenic pathways and subsequent vascular proliferation were confirmed by the landmark study of Ferrario et al. [25]. In this study, Photofrin®-mediated PDT was performed *in vitro* on breast carcinoma cells showing increased VEGF expression which may have been associated with treatment-induced hypoxia and to a lesser extent with treatment-induced oxidative stress [25]. In addition, this study examined the effects of PDT followed by the administration of anti-angiogenic drugs *in vivo* on tumor bearing mice. This report showed that the combination therapy resulted not only in a significant reduction in PDT-induced VEGF expression, but also elicited a significant increase in the tumoricidal activity of PDT, as measured by tumor cure rates. The results of this study have since been confirmed and reproduced by an assortment of groups using various combinations of different PSs and anti-angiogenic or anti-vascular drugs on many different cancer cell lines and tumor models. This review will focus on what is known of this combination strategy and will give a critical view of its future applications. The different approaches of angiogenesis inhibition and vascular targeting, as well as the timing and sequencing of therapeutic strategies will be discussed. In addition, the enhancement of the selectivity of PDT through the molecular targeting of PSs will be reviewed and discussed. The importance of the subject of this review is reflected by the view of researchers and clinicians that the combination of PDT with angiostasis may allow for a revival in the field of PDT and holds promise for the application of PDT in cancer.

2. PDT and its vascular effects

Research over the past few decades investigating the mechanisms and cellular effects of PDT has revealed that the vascular damage caused by PDT is largely responsible for the therapeutic benefit of the treatment, depending on the timing and the PS administered

[30–32]. It has also been demonstrated that tumor cure rates are strongly dependent on vessel damage in and around the treated tumor [33]. Tissue damage from PDT depends on the photosensitizing agent and the treatment regimen used. The vascular effects of PDT, which eventually lead to vascular occlusion, will be described in more detail. Early damage following the injection and illumination of a PS is mostly observed in the endothelial and subendothelial cells [34]. Endothelial cell damage, originating at the endothelial luminal surface, begins with the influx of calcium into the cells [35]. An increase in the cytosolic calcium concentration leads to conformational changes in adhesion molecules and alterations in cytoskeletal components, including the depolarization of cytoplasmic microtubules. This induces changes in cell shape, such as swelling, rounding up and contraction, as well as fragmentation, resulting in complete eradication of the endothelial lining [36]. Contraction of endothelial cells results in the loss of tight junctions between cells and the exposure of the vascular basement membrane [37] to circulating blood. The interaction of blood platelets with the denudated vascular wall induces the activation and aggregation of platelets. Platelets have also been shown to accumulate PSs and upon irradiation are damaged, losing serotonin and ATP proportional to the PS dose administered [38,39]. Taken together, platelet damage and the interaction of platelets with pro-coagulant extracellular matrix components eventually leads to vessel constriction and a thrombus formation [36,40,41].

The adhesion of granulocytes has also been observed following endothelial cell damage, which can induce increased vascular permeability and edema [42]. Damage to platelets further stimulates the release of thromboxane (a platelet pro-aggregation compound) and leukotriene B4, both of which further contribute to enhanced vascular permeability and the disruption of the endothelial cell lining of exposed blood vessels [43,44]. These leukocytes have been shown to bind to the vascular endothelium in PDT-treated normal tissue [45], but not to PDT-treated tumor microvasculature, indicating that tumor vasculature damage following PDT is not related to leukocyte adherence [46]. It is likely that the anti-adhesive properties of tumor endothelial cells, as have been previously described, explain these observations [47,48]. Endothelial cells may also influence the blood clotting balance through the release of clotting factors [49], including von Willebrand factor (vWf) [35]. All of these biochemical changes disrupt the balance of platelet pro-aggregatory/constricting compounds and anti-aggregatory/vasodilating agents (prostacyclins) [50,51], resulting in smooth muscle constriction and further platelet aggregation. In a parallel mechanism, PDT-induced damage to membrane lipids elicits the release of arachidonic acid, which initiates a series of reactions, also ending in the release of thromboxane. Vessel constriction is further supported by the inhibition of nitric oxide production in endothelial cells [52]. Finally, a blood clot within the vessel lumen could potentially cause obstruction to blood flow, leading to the termination of vascular function and an increase in intratumoral interstitial fluid pressure [53]. A simplified scheme of the PDT-vascular effects is shown in Fig. 1.

3. PDT in combination with anti-angiogenesis

Major problems associated with the application of PDT for cancer are the secondary angiogenic and inflammatory responses to treatment, which result in the revascularization of treated lesions and contribute to tumor recurrence [25,32]. Tumor angiogenesis is a robust physiological process, regulated by a variety of endogenous pro- and anti-angiogenic factors. The process starts in many cases with hypoxia in a malignantly transformed cell mass. This forces the angiogenic switch in these cells, which will then express angiogenic growth factors, such as vascular endothelial growth factor (VEGF). Endothelial cells in preexisting blood vessels will then migrate into the growth factor gradient, proliferate and form new vascular sprouts. These initially immature blood vessels form a new basement membrane and attract accessory cells to form mature blood vessels that can transport blood. The angiogenesis cascade is schematically presented and summarized in Fig. 2.

3.1. Growth factor targeted agents

Over the last few decades, many angiogenesis mechanisms have been delineated and treatment strategies against cancer, based on angiogenesis inhibition, have been developed. The most well-developed strategy of angiogenesis inhibition is the intervention with angiogenic growth factor signaling. Both growth factor neutralizing antibodies or antibody-based constructs and inhibitors of growth factor receptor signaling, which act through the inhibition of tyrosine kinase activity, have been developed. Other drug targets which are currently being clinically assessed as modulators of angiogenesis, include circulating endothelial progenitor cells, the cytoskeleton (see Section 5), cell adhesion molecules (intergrins, see Section 6.2), hypoxia (Section 3.1), MMPs and cathepsins (Section 3.2). Procoagulant pathways are also upregulated in tumor vasculature, a pathway which can be blocked with anti-thrombotic drugs (fragmin). Platelets are often overstimulated by IL-6 in tumors and provide a major source of VEGF. Additionally, the enzyme thymidine phosphorylase is often upregulated in tumors and produces angiogenic metabolites. The inhibition of its action may therefore potentially lead to effective angiogenesis inhibition (Fig. 3).

Central to the growth factor targeting approach is the VEGF signaling axis. VEGF is a rather specific mitogenic endothelial growth factor, which is able to stimulate all steps in the angiogenesis cascade, from the activation of the endothelium to produce proteases, to the stimulation of migration and proliferation, and the maturation and attraction of pericytes. In addition, its role in cancer is of pivotal importance, as reflected by the overexpression of VEGF in practically all types of cancer. The importance of the VEGF signaling axis is further evidenced by the large interest within the field of PDT regarding combination therapies that seek to inhibit this pathway. Ferrario et al. performed the initial study that confirmed a link between PDT-induced vascular damage, the activation of angiogenic pathways, and tumor revascularization. This study showed that this combination treatment in a breast cancer model reduced VEGF expression and increased tumor cure rates from 39% with PDT alone to 80–90% in the combination group [25]. An overview of such targeted combination studies will be given below, where a distinction will be made between VEGF and other growth factor (−receptor) targeted approaches and agents that directly target endothelial cell function (Fig. 3B).

3.1.1. Vascular endothelial growth factor (VEGF) axis targeting agents

The first angiogenesis inhibitor approved for the treatment of cancer was the monoclonal antibody based compound bevacizumab (Avastin®, Genentech). This drug, the activity of which is the neutralization of VEGF, is currently approved as a first-line treatment, in combination with chemotherapy, for patients suffering from advanced metastatic colorectal cancer [54]. The initial success of clinical trials based on the use of bevacizumab set a milestone in the field of anti-angiogenic cancer therapy [55,56]. The combination of bevacizumab with PDT is approved for the treatment of AMD [57], and has also become of interest in the treatment of various forms of cancer [58].

Following their landmark study in 2000, the group of Ferrario reported on the combination of Photofrin®-mediated PDT with bevacizumab, applied immediately after PDT, in human Kaposi's sarcoma xenografts in nude mice [59]. This study confirmed the angiogenic response induced by PDT through the detection of increased expression of HIF-1α, VEGF, prostaglandin E2 (PGE2), tumor necrosis factor alpha (TNF-α) and interleukin 1-beta (IL-1β) following PDT treatment. More interestingly, a significant increase in long-term tumor

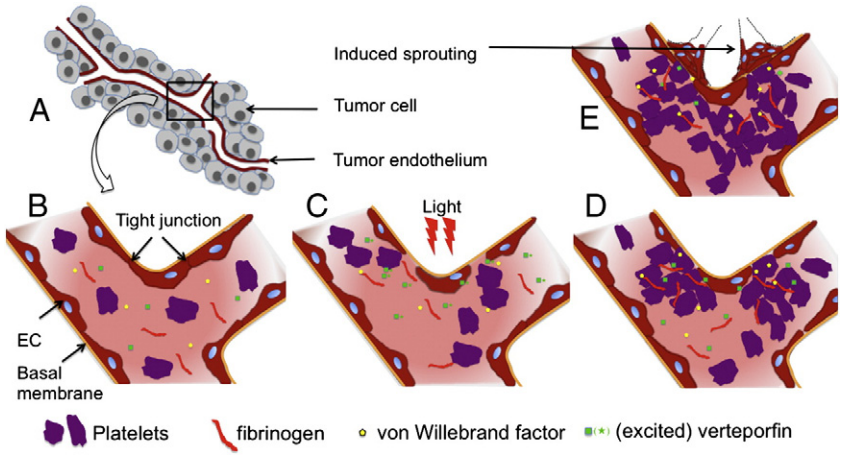

Fig. 1. Tumor endothelial responses after photodynamic therapy (PDT) leading to blood flow stasis. A simplified scheme of the different steps after injection of a photosensitizer and exposure to light. (A) Tumor blood vessel before PDT. (B-E) Magnification of the junction of the tumor vessel. (B) Before PDT endothelial cells are tightly attached to the basement membrane of the vessel wall, lining the blood vessel. Endothelial cells are connected through tight junctions. (C) After injection of photosensitizer and light exposure, cellular stress inside the endothelial cells results in disruption of tight junctions, partial retraction and detachment from the vessel wall. (D) Blood gets in contact with the vessel wall collagen and the clotting cascade is initiated, ultimately leading, through the interaction with fibrinogen, to the formation of a stabilized thrombus, leading to obstruction of the vessel. (E) Due to the angiogenic switch, endothelial cell proliferation, migration and sprout formation is observed.

responses was noted after combination therapy, as compared to either monotherapy (55% of mice saw long-term tumor response in the combination treatment group, compared to 22% and 10% in the PDT-only and bevacizumab-only treated groups). Additionally, there was no significant increase in normal tissue toxicity associated with the better tumor response. These results provided the first proof that bevacizumab could be used to improve PDT and suggested that VEGF inhibitors, in general, may ameliorate the clinical efficacy of PDT in cancer [59].

Shortly after this study, a similar study examined tumor response to hypericin-PDT in combination with bevacizumab on bladder carcinoma xenografts in nude mice [60]. Assessment of tumor volume following treatment showed that combination therapy (with high or low dose PDT) resulted in a significantly better tumor response when compared to control (no treatment) or monotherapy treatment groups. This study also showed that the treatment of bladder carcinoma tumors with PDT and bevacizumab resulted in suppressed expression of VEGF and downregulation in the expression of other angiogenic molecules (angiogenin, bFGF, EGF, and interleukin-6 and -8) as compared to PDT-only treated tumors. These findings strongly support the hypothesis that PDT-induced angiogenesis can be counteracted using subsequent anti-angiogenic therapy.

The same group implemented the use of confocal endomicroscopy to visualize vasculature following the combination approach as described above in order to evaluate the angiogenic responses of vasculature to treatment [61]. This study, which allowed *in vivo* surface and subsurface fluorescence imaging of the tissue, revealed that the damage to tumors in the combination treatment group was significantly more efficient than

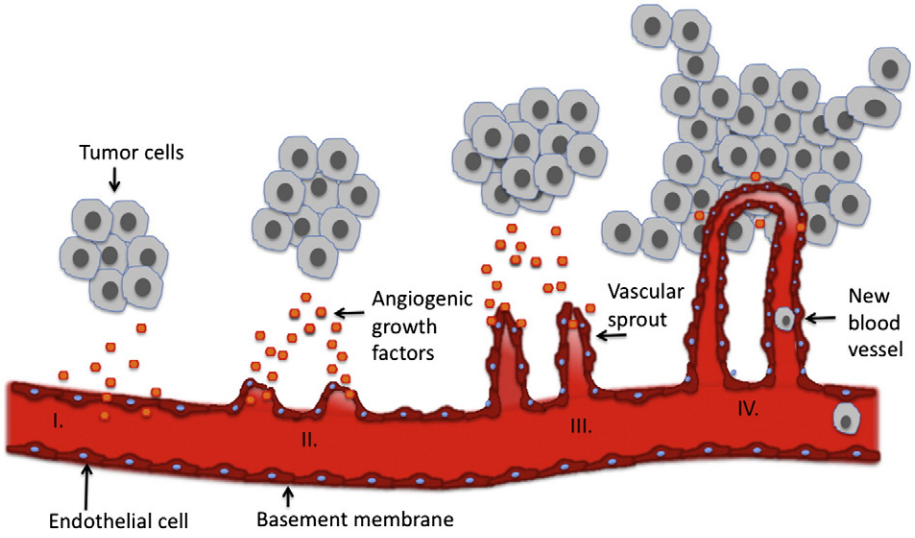

Fig. 2. The different steps of the angiogenic cascade.I. Outgrowth of a tumor will involve generation of hypoxia, leading to the onset of angiogenic genes, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF). II. The secretion of these factors activates endothelial cells of preexisting nearby capillaries to produce matrix metalloproteinases to breakdown the extracellular matrix. This will allow the endothelial cells to start migrating towards the stimulus. III. Endothelial cells proliferate and form vascular sprouts that can transport blood but are initially very leaky. IV. Only after the formation of a new extracellular matrix and basement membrane, a new blood vessel is available for oxygenation of the tissue and removal of waste products. New vessels in tumors can be leaky and may allow migration of tumor cells to distant sites.

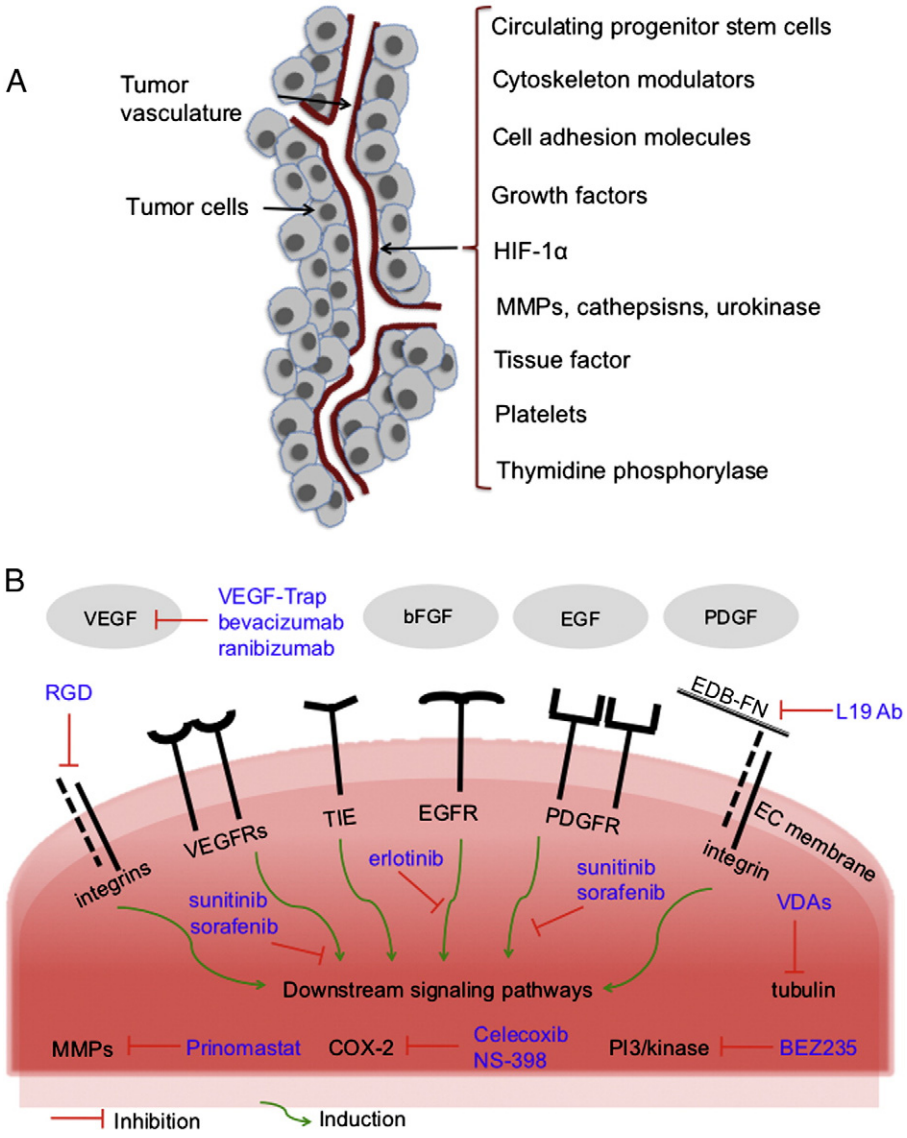

Fig. 3. Main mechanisms involved in tumor angiogenesis on a vascular and cellular level. (A) Overview of tumor blood vessel including different components of its structure and factors that can influence angiogenesis. (B) A simplified diagram of the angiogenesis targets and inhibitors discussed in this review. Green arrows represent induction and red inhibition of signaling. RGD, the tripeptide sequence ligand of (αvβ3-)integrin; EDB-FN, extra domain B containing splice variant of fibronectin associated to tumor blood vessels; L19 Ab, the anti-EDB-FN antibody; MMPs, matrix metalloproteinases; COX-2, cyclooxygenase-2.

either monotherapy. Visualization of blood vessels also showed a decrease in tumor vessel density in the bevacizumab monotherapy group, compared to the control, with the remaining vasculature appearing morphologically normalized. On the other hand, blood vessels in the tumors treated with the combination therapy were leaky, showed loss of function and revealed the greatest reduction in mean vessel area. Complete cures were observed in the treatment group receiving PDT and continued bevacizumab therapy. Immunohistochemistry and immunofluorescence studies confirmed these observations, showing a significant downregulation of VEGF expression in tumors treated with the combination therapy.

An entirely different approach to inhibiting the VEGF signaling axis was undertaken in the study by Jiang et al. [62]. Here, the use of a mix of two antibodies directed to the VEGF-receptor-1 (MF1) and -2 (DC101) were used as monotherapies or in combination with Photofrin®-PDT to treat intracerebral U87 glioblastoma xenografts in nude mice. In this study, the angiogenesis inhibiting drugs were administered through i.p. injection every other day from days 8 through 14 after tumor implantation, which corresponded to 24 h following PDT performed on day 7 after implantation. Monotherapies of PDT or MF1 + DC101 significantly reduced the tumor volume and prolonged survival of tumor bearing mice. Anti-angiogenic therapy was observed to decrease proliferation and increase apoptosis of tumor cells. Combination therapy also increased survival time, while decreasing tumor volume, showing significantly better outcomes than either monotherapy. The benefit of the combination, however, was reported to be additive and not synergistic. Again, a decrease in the expression of the angiogenic growth factors VEGF and von Willebrand factor (vWF) was seen in the combination therapy group as compared to the PDT-only treated group.

Anti-VEGF therapy combined with verteporfin-PDT has been successfully used in the treatment of ocular tumors. Circumscribed choroidal haemangioma (CCH) is an uncommon, benign vascular tumor manifesting as a discrete smooth, round, orange-red mass located posterior to the equator, normally in the macular and peripapillary region. Sagong et al. discussed two patients with CCH who were treated with intravitreal injection of Avastin® and subsequent verteporfin-PDT [63]. The result of this clinical application on a

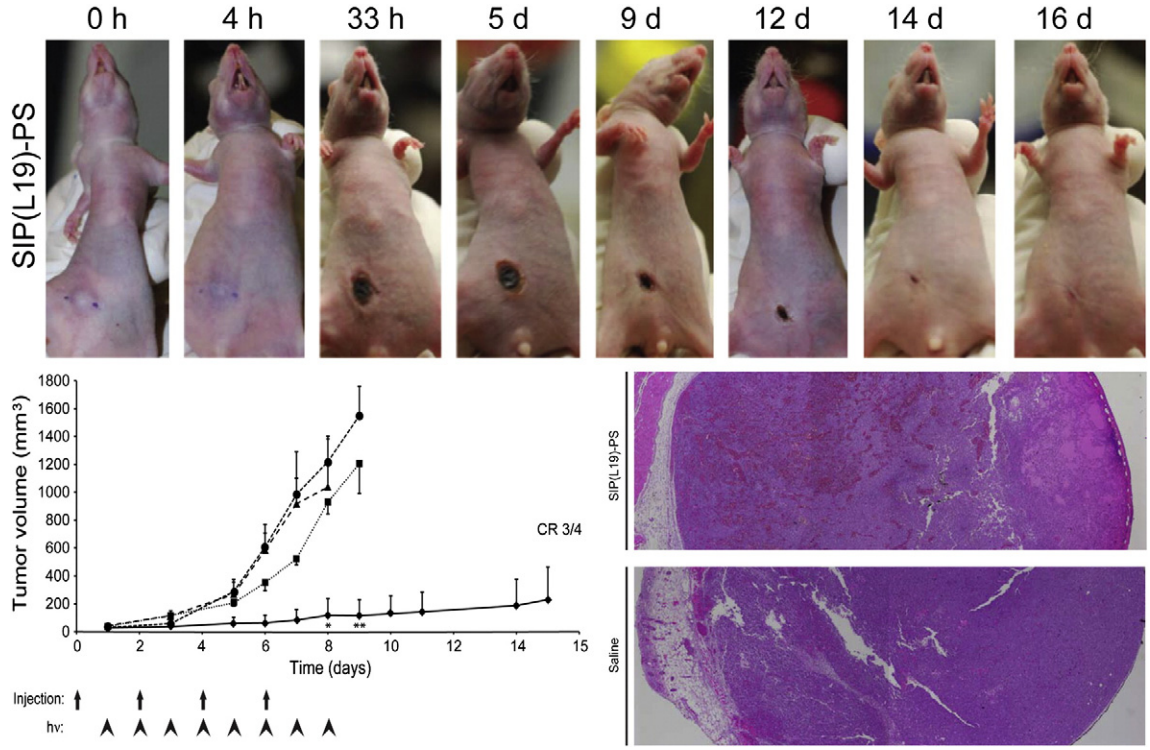

Fig. 4. Improvement of PDT by targeted delivery of photosensitizers. (A) Subcutaneous xenografts of human A431 epidermoid carcinoma in nude mice are ablated by PDT after injection of a single dose of SIP(L19)–PS. Photographs are taken at several points in time after a single dose of irradiation with light. (B) Tumor responses in F9 teratocarcinomas after i.v. injections with SIP(L19)–PS (diamonds and circles), SIP(F16)–PS (triangles) or saline (squares) on days 0, 2, 4 and 6 of the treatment schedule. Irradiation was given daily from days 1 to 8 (in diamonds-, triangles-, and squares graphs). *P<0.01 vs not irradiated, **P<0.01 vs saline. (C) Effect of PDT with SIP(L19)-PS on tumor histology. Sections of F9 tumors excised 1 h after PDT, stained with haematoxylin/eosin and imaged at ×2.5 magnification. (Adapted with permission from Macmillan Publishers Ltd on behalf of Cancer Research UK: Palumbo et al., Br. J. Cancer, copyright 2011).

very limited number of patients turned out to be very successful, as complete regression of lesions was observed for 6–9 months. Furthermore, improved visual acuity was accompanied by complete resorption of subretinal fluid due to partial vessel normalization. PDT alone, however, has also been used in case studies to successfully treat patients with CCH resulting in minimal side-effects [64]. Randomized controlled trials are therefore warranted to confirm the efficacy of both verteporfin-PDT and combination therapy in circumscribed choroidal haemangioma, and to further investigate the potential of combination therapies utilizing verteporfin-PDT with anti-VEFG or intravitreal triamcinolone, for which there is little but encouraging data.

In another case study, verteporfin-PDT in combination with bevacizumab was used as an effective and well-tolerated option for juxtapapillary retinal capillary haemangioma (RCH), another form of vascular tumor located at the border of the optic nerve head and frequently associated with von Hippel–Lindau syndrome [65]. PDT is considered the treatment of choice for juxtapapillary RCH, however the need for multiple treatments can result in damage to surrounding tissue and poor overall results for patients [66]. The anti-exudative effect of bevacizumab promises to provide better delineation of RCH and, consequently, allows for more precise adjustment of PDT irradiation to minimize collateral damage. In this study, a single combination therapy of bevacizumab and verteporfin-PDT showed long-lasting effects and, thus, eliminated the necessity of further destruction by repeated PDT treatment. Long-term follow-up of other cases is necessary to make a conclusive statement about the efficacy of combination therapy in the treatment of RCH.

Taken together, these results suggest that the inhibition of VEGF signaling, through bevacizumab or other molecules, can provide a viable means to combat PDT-induced angiogenesis, and more importantly, that these combination therapies can help to prevent tumor recurrence, thereby increasing the overall efficacy of PDT treatment. The study of bevacizumab in preclinical models, however, is not entirely straight forward, as controversy exists regarding the binding affinity of bevacizumab to non-human VEGF. Bevacizumab is a humanized (93% human) antibody, derived from the murine VEGF monoclonal antibody A4.6.1 [67,68]. A key difference between human and mouse VEGF-A (mVEGF-A) is that the Gly-88 in human VEGF-A corresponds to Ser-87 in mVEGF-A, a difference that is located at the core of the protein-antibody interface [69]. Crystal structure analysis of hVEGF-A when it is bound to bevacizumab, shows a tightly packed interface between the protein and antibody. In the mVEGF-A–antibody complex, where Ser-87 is present, the addition of two non-hydrogen atoms at the interface prevents a strong interaction. This indicates that bevacizumab would have a weak affinity for mVEGF-A compared to hVEGF-A. The study by Bock et al. [70], however, reported strong anti-angiogenic effects of systemically administered bevacizumab on corneal angiogenesis in a murine model, spurring the group of Ferrara to reinvestigate the activity of bevacizumab in murine models in a study published in 2008 [71]. They reported a very weak interaction between bevacizumab and mVEGF-A in the western blot analysis, and no inhibitory effect on mVEGF-stimulated endothelial cell proliferation or inhibitory action in *in vivo* models. The authors of this paper concluded that it is "unlikely that the *in vivo* findings described by Bock et al. resulted from a specific immunoneutralization of mVEGF-A by bevacizumab" [71].

3.1.2. Other growth factor targeted agents

The epidermal growth factor (EGF) pathway also provides an interesting target for anti-angiogenic and anti-cancer therapies, as it is known to be involved in the regulation of normal cellular processes and its overexpression is frequently correlated with the development

of malignancy [72]. EGF promotes cell cycle progression from G1 to S phase, thus promoting an increase in the number of proliferating cells [73]. The overexpression of EGF has been shown to be associated with a wide array of cancers, including head and neck-, breast-, colon-, lung-, prostate-, kidney- and bladder carcinomas [72,74]. The use of EGF inhibitors in combination with PDT has also become of particular interest, as it has been shown that PDT can result in increased phosphorylation of the EGF receptor (EGFR) and increased activity of downstream signals, such as ERK1 and ERK2, protecting cells from PDT-induced damage. Recently, a review was published on studies examining the activity of EGFR pathways in PDT induced cell death [75]. The studies indicated that the inhibition of EGFR plays an important role in post-PDT effect and, that following PDT, both normal and cancerous cells become less sensitive to EGF signaling. Additionally, the activation of ERK 1/2 and EGFR-PI3K-Akt pathways following PDT appeared to aid in cell survival. These results indicate that the inhibition of EGFR in combination with PDT may provide potential for clinical applications. The combination of PDT with EGF inhibitors, particularly cetuximab (Erbitux®), an antibody which binds competitively with the EGFR, has shown good potential to increase the efficacy of therapy, not only through the inhibition of PDT-induced angiogenesis, but also through the inhibition of other PDT-activated pathways in tumor cells.

An early study examining the synergistic effects of EGFR inhibition and verteporfin-PDT was performed by Del Carmen et al. [73] on an ovarian carcinoma model in nude mice. Treatment of tumor bearing mice with PDT followed by cetuximab resulted in synergistic inhibition of tumor growth and an overall reduction in tumor size (9.8% of the control) as compared to verteporfin-PDT (38.2%) or cetuximab alone (66.6%). The overall survival rate in this group was found to be three times higher than non-treated mice, i.e. at day 180, 3/9 mice in the combination group were alive compared to 0/12 in the drug only group and 1/10 in the PDT-only group, while all non-treated mice died by day 40. The *in vivo* effects of administering hypercin-PDT followed by cetuximab in human bladder carcinoma-bearing nude mice have also been reported [76]. The results of this study showed strong inhibition of tumor growth in the combination therapy group, with a relative tumor inhibition of 93% compared to control mice, while monotherapies of PDT and cetuximab showed only 57.8% and 74.8% inhibition of tumor growth, respectively. Although an initial acceleration in tumor growth was seen one week following treatment, this was followed by a decrease in tumor size, eventually leading to complete tumor regression. The combination therapy group showed an increase in apoptosis and downregulation of EGFR expression, as well as inhibition of phosphorylation at most of the EGFR phosphorylation sites and downregulation of EGFR target genes, such as cyclin D1 and c-myc. These researchers also performed an additional study, applying hypericin-PDT followed by the administration of the EGFR inhibitor cetuximab, the VEGF inhibitor bevacizumab, or both drugs, *in vitro* on bladder cancer and HUVEC cells and *in vivo* on a murine bladder tumor model [58]. Although both drugs showed efficient inhibition of cell migration when administered alone, the combination of cetuximab and bevacizumab did not show a significant increase in the inhibition of cell migration. Cell invasion and tube formation, however, were suppressed by both drugs individually, while being significantly more suppressed by the combination of both drugs, even resulting in complete prevention of tube formation. Assessment of tumor volume in treated mice revealed that mice receiving combination therapies (PDT + bevacizumab, PDT + cetuximab, or PDT + bevacizumab + cetuximab) showed significantly greater treatment responses when compared to control and PDT-only treated groups. Mice treated with bevacizumab and cetuximab only exhibited tumor regression, but no complete cures, indicating that while anti-angiogenic drug therapy is somewhat effective alone, its combination with hypericin-PDT results in a much greater tumor response (see Fig. 5). These results support the hypothesis that the combination of hypericin-PDT with the angiogenesis inhibitors bevacizumab and/or cetuximab can help to prevent the PDT-induced angiogenic process, improving treatment efficiency and resulting in complete cures in tumor bearing mice.

Yip et al. used photochemical internalization (PCI) in order to achieve drug delivery and PDT [77]. PCI is a drug delivery method where endocytosed macromolecules can be directly delivered into the cytosol of cells and PDT can be performed upon the activation of PSs by light [78]. In this study, cetuximab was linked to a type I ribosome-inactivating protein called saporin (cetuximab-saporin), thereby targeting the immunotoxin saporin to EGFR-expressing cells. Results showed the selective binding and uptake of the drug in EGFR-positive cells, as well as reduced levels of unspecific cetuximab uptake. In addition, non-conjugated cetuximab therapy alone only decreased cell viability by 10% at its highest concentration and showed no enhancement of cytotoxicity with increased doses of non-conjugated cetuximab when used in combination $TPPS_{2a}$-PDT, mimicking the PCI protocol. Combination of cetuximab and saporin with $TPPS_{2a}$-PDT resulted in a triplication of the cytotoxicity, due to the action of cetuximab, PDT and PCI of saporin.

These studies have shown that the combination of PDT with EGF inhibition has great potential in the treatment of certain types of cancer. This is of particular interest as the overexpression of EGFR in tumor cells has been shown to be an indicator of aggressive and chemotherapy resistant tumors [73]. In addition, PDT has been shown to be effective against epithelial ovarian carcinoma that is refractory to

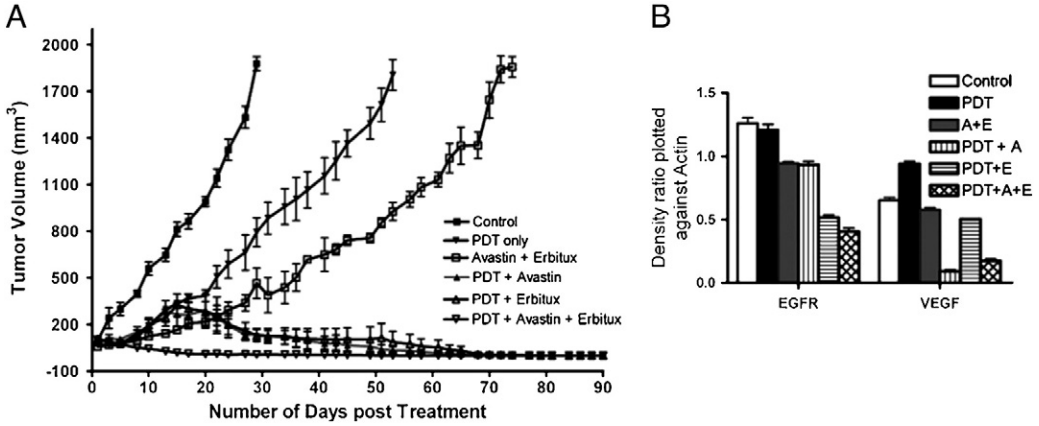

Fig. 5. (A) Tumor growth inhibition by PDT, anti-angiogenesis or the combination. The combination therapy groups of PDT + Avastin®, PDT + Erbitux®, and PDT + Avastin® + Erbitux® exhibited greater tumor response in comparison with the other groups. (B) Ratio of EGFR and VEGF density plotted against actin for all treatment groups (reprinted from Bhuvaneswari et al., Lasers in Surgery and Medicine, 2011, after receiving permission from the publisher).

chemotherapy and radiation therapy [79,80] and is known to overexpress EGFR. Overall, these studies indicate that synergistic effects may be seen when combining individual monotherapies which target and affect non-overlapping pathways [73], resulting in maximal treatment benefits with minimal additional side effects.

3.1.3. Tyrosine kinase inhibitors

Complex signaling in malignant cells and cellular heterogeneity within each tumor dictates the necessity of targeting multiple signaling pathways [81]. The above-mentioned strategies target only single growth factor receptors. There is not only a theoretical advantage of targeting multiple pathways, as for example, it has been demonstrated for non-small-cell lung cancer (NSCLC) that there is a strong relationship between the EGFR and VEGFR pathways [82]. It is thus reasonable to hypothesize that targeting both pathways with separate drugs would have an additive or even a synergistic inhibitory effect on tumor growth. With the development of small molecule tyrosine kinase inhibitors, it is possible to target multiple growth factor receptors by blocking their intracellular phosphorylation sites and, thereby, their signaling capacity. Several of these drugs have received FDA approval in the treatment of solid tumors, such as sunitinib (Sutent®) for the treatment of renal cell carcinoma. Sunitinib targets and inhibits multiple kinase pathways, among which are VEGFR2, platelet derived growth factor receptor (PDGFR), c-kit, FLT3 and RET. Sorafenib (Nexavar®) targets VEGFR2 and -3, PDGFR and FLT-3 and is now registered for treatment of advanced renal cell carcinoma and hepatocellular carcinoma. Some of these compounds have a more narrow spectrum of action, such as erlotinib (Tarceva®), which more selectively inhibits epidermal growth factor receptor (EGFR) and is approved for NSCLC and pancreatic cancer. These compounds have been tested in combination with PDT to combat the secondary angiogenesis response in the expectation that broader receptor targeting may result in more robust inhibition of angiogenesis, further enhancing the therapeutic benefits of PDT.

A recent study was performed by us aimed to compare the ability of different anti-angiogenic compounds to prolong verteporfin-PDT-induced vascular occlusion in the chicken chorioallantoic membrane (CAM) model [23]. In this study, the action of bevacizumab was compared with various clinically approved TKIs with varying spectrums of action, including sorafenib [83], erlotinib [84] and sunitinib [85]. The results of this study showed that all compounds tested were capable of inhibiting both physiological angiogenesis in the CAM, as well as verteporfin-PDT-induced angiogenesis, resulting in prolonged vascular occlusion in the treated areas. Image processing analysis of the treated blood vessels revealed that sorafenib induced the strongest anti-angiogenic effect, outperforming the other drugs at improving verteporfin-PDT. Interestingly, the morphology of regrown blood vessels in the PDT treated areas differed from untreated blood vessels [32]. In addition, there was also a clear difference in the morphology of blood vessels growing after treatment with the different anti-angiogenic drugs (see Fig. 6). The results of this study showed that the inhibitor with the broadest spectrum of action resulted in the strongest inhibition of PDT-induced angiogenesis in the CAM.

A study by Dimitroff et al. [86] verified the action of a broad (PD166285, inhibiting c-src, FGFR-1, PDGFR-β and EGFR) and a narrow (PD173074, inhibiting selectively FGFR-1) spectrum experimental TKI in combination with Photofrin®-PDT, *in vivo* in the murine 16c breast carcinoma model. Independent of their spectrums of action, oral administration of either PD166285 (1–25 mg/kg) or PD173074 (25–100 mg/kg) generated dose-dependent inhibition of angiogenesis. Additionally, significantly prolonged tumor regression was achieved with daily doses of PD166285 (5–10 mg/kg) or PD173074 (30–60 mg/kg) following Photofrin®-PDT, as compared to Photofrin®-PDT alone.

The use of the TKIs SU5416 and SU6668 in combination with hypericin-PDT was investigated by Zhou et al. in human nasopharyngeal carcinoma (NPC) bearing BALB/c athymic mice [87]. Single hypericin-PDT alone resulted in the increased expression of angiogenic factors, including VEGF, HIF-1α, COX-2 and bFGF, when compared to control mice. The combination of single hypericin-PDT with subsequent administration of SU6668 resulted in the best therapeutic response. It increased the survival rate of treated mice to 100%,

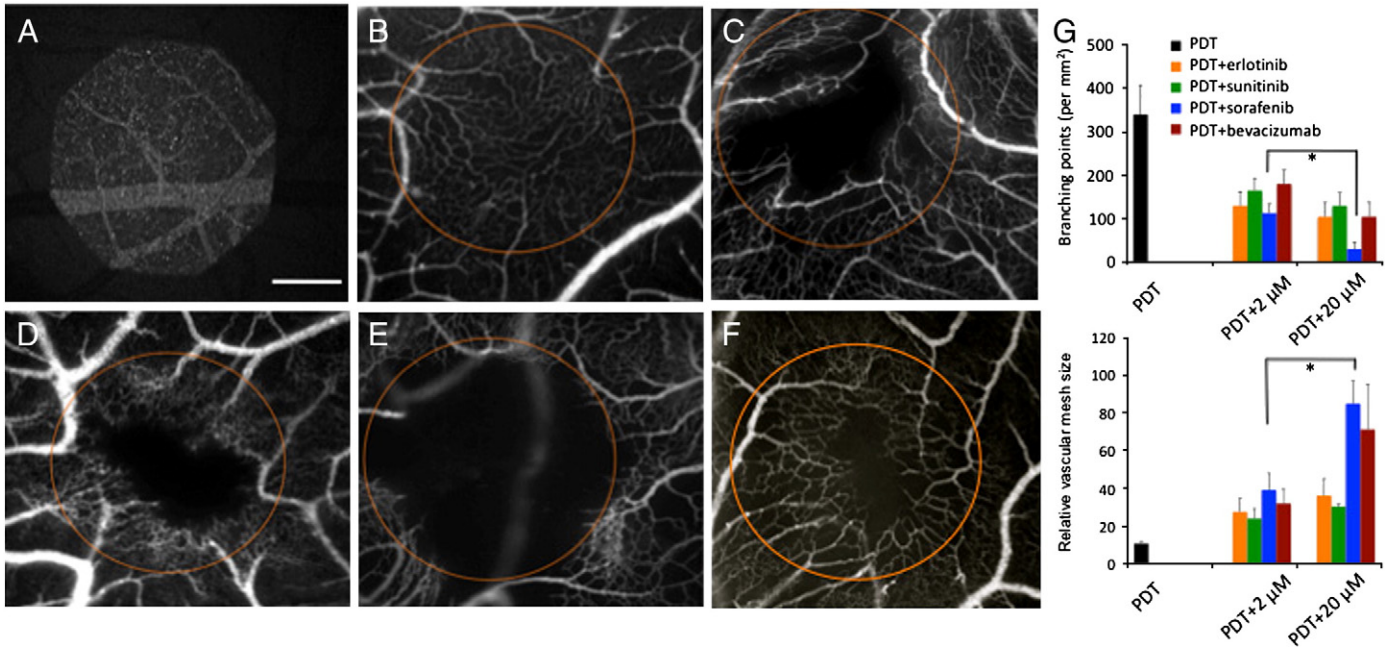

Fig. 6. Sustained photodynamic vaso-occlusion by angiogenesis inhibitors. (A) Fluorescence angiograms taken before (A), 48 h after Visudyne®-PDT (0.20 mg/kg embryo weight, $\lambda_{ex} = 420$ nm, $\lambda_{em} > 470$ nm; light dose of 20 J/cm^2 and an irradiance of 50 mW/cm^2, drug-light interval 1 min) alone (B), and combination therapy of PDT with topically administered angiogenesis inhibitors erlotinib (C) sunitinib (D), sorafenib (E), and bevacizumab (F). All agents were applied twice (immediately and 24 h post PDT). The vasculature is visualized by FITC-dextran fluorescence angiography (25 mg/kg, 20 kDa, $\lambda_{ex} = 470$ nm, $\lambda_{em} > 520$ nm). (G) Quantifications of 2 descriptors for two concentrations of the drugs. Mean values are shown, error bars represent standard error of the mean.

as compared to 0%, 33%, 75%, 33%, and 33% in the control, hypericin-PDT alone, SU5416 alone, SU6668 alone and PDT followed by SU5416 treatment groups, respectively. The better therapeutic response of SU6668 with hypericin-PDT was, at least partially, attributed the broader spectrum of action of SU6668, blocking the signaling of receptors for VEGF, PDGF and FGF. An interesting observation, however, was that even though SU6668 was more effective at inhibiting tumor growth in combination with hypericin-PDT, following the arrest of anti-angiogenic treatment, control of tumor volume lasted much longer in the SU5416-treated mice than in SU6668-treated mice. These findings are supported by an *in vitro* study which showed that SU5416 resulted in long-lasting inhibition of VEGFR phosphorylation and function, reducing proliferation of endothelial cells [88], while similar studies did not show the same effect for SU6668. This may also help to explain why SU6668 was less effective than SU5416 as a monotherapy to increase the overall survival of treated mice.

Summarizing, angiogenesis is an intricately regulated biological process, which may be more effectively inhibited through novel drug strategies, such as with the use TKIs, which target multiple different pathways simultaneously. Even though many of these studies seem to suggest that the inhibition of more than one angiogenesis signal pathway could result in more efficacious inhibition of PDT-induced angiogenesis, it is clear that more knowledge is needed to substantiate this claim. This advantage, however, may in fact be a double-edged sword, as kinase inhibitors frequently have off-target interactions, which can result in drug related adverse side-effects and toxicities. For this reason, the spectrum of the drug in question must be carefully taken into account in order to maximize its therapeutic effects, while maintaining tolerable side effects.

3.2. Non-growth factor targeted agents

3.2.1. Matrix metalloproteinases (MMPs)

In addition to growth factor mediated signals stimulating angiogenesis, there are many other, later steps in the angiogenic cascade that are amenable for intervention to improve the effects of PDT. First of all, the release of proteases, particularly matrix metalloproteinases (MMPs), can be targeted. MMPs are produced by endothelial cells in order to prepare the extra-cellular matrix for invasion by vascular sprouts. In addition, specifically targeting the processes of endothelial cell migration and proliferation provide attractive approaches for angiogenesis inhibition, as well as interfering with vascular maturation steps, such as the formation of the basal membrane and the attraction of pericytes. MMPs are proteolytic enzymes, which are known to aid in the degradation of components of the extracellular matrix. They play a critical role in cancer cell invasion and metastasis, as well as in angiogenesis [89,90].

A variety of studies have examined the effects of PDT on MMP expression in various tumor cell lines and tumor models. In one such study, Du et al. [91] performed hypericin-PDT on two nasopharyngeal cancer (NPC) cell lines, well differentiated HK1 cells and poorly differentiated CNE-2 cells, and *in vivo* on HK1-NPC tumors in mice. It was found that PDT increased MMP-1 protein and mRNA expression, both in cell lines and *in vivo* in tumor models. A subsequent study by the same group [92] reported on the effects of hypericin-PDT on MMP-9 expression in HK1 NPC *in vitro* and *in vivo*, reporting a downregulation in the expression of MMP-9. This study also reported that PDT inhibited the secretion of granulocyte–macrophage colony stimulating factor (GM-CSF), resulting in decreased transcriptional activity in two of its downstream proteins, activator protein-1 (AP-1) and nuclear factor (NF)–κB. Additionally, incubating cells in exogenous GM-CSF prior to PDT treatment resulted in an additional decrease in MMP-9 production. This finding indicate that PDT-induced downregulation of MMP-9 is mediated by the inhibition of GM-CSF and leads to the modulation of AP-1 and NF-kB. It is interesting to note here that decreased activity in NF-κB has been associated with increased angiogenesis, as angiogenesis inhibition involves NF-κB activity [93,94]. The decreased expression of MMP-9 following PDT has also been noted in two other studies. One study by Au et al. [95] showed that the treatment of glioma cells with ALA-, Photofrin®- and calphostin C-PDT resulted in a reduction in cell migration, which was partially attributed to a reduction in MMP-9 production. The other study, performed by Sharwani et al. [96], showed that PDT suppressed the production of MMP-9 in keratinocyte cell lines from human oral squamous-cell carcinoma (SCC). A study by Chu et al. [97] examined the effects of Hexyl-ALA (ALA-H)-PDT and 5-ALA-PDT on MMP expression in the medulloblastoma cell line TE-671, showing a small, yet significant, decrease in MMP-2 expression and an inhibition of cell migration 24 h post PDT, which was believed to be, at least partially, attributed to MMP-2 downregulation. Interestingly, this study did not report a change in the expression of MMP-9 in PDT treated cells.

Although changes in MMP expression after PDT are not unidirectional in the sense of upregulation, combinations with MMP inhibitors have been performed. Ferrario et al. [98] studied the effects of combining Photofrin®-PDT with Prinomastat®, an inhibitor of MMP-2 and -9, on mouse mammary carcinoma, mouse brain endothelial cells, mouse macrophages and human fibrosarcoma cells. This study showed that PDT alone increased the expression and activity of MMP-9 (as well as MMP-1, -3, and -8) 24 h following therapy. In this study, the expression and activity of MMP-2, however, was not affected by PDT. In addition, PDT resulted in endothelial cell expression of MMP-9, as well as an influx of MMP-9 expressing inflammatory host cells. Administration of Prinomastat® significantly improved PDT-mediated tumor response without affecting normal skin photosensitization. While these results may seem contradictory to the study by Du et al. in 2007, which showed a decrease in MMP-9 expression following PDT, it should be stressed that the BA mammary cells in the study of Ferrario et al. did not secrete any detectable levels of MMP-9, nor were they induced to do so by PDT. The increase in MMP-9 seen in this study can be attributed to the induction of MMP-9 expression in endothelial cells and the influx of MMP-9 expressing inflammatory host cells [92]. Treatment with the MMP inhibitor Prinomastat® alone reduced the rate of tumor growth, but did not result in a significant decrease in tumor size or long-term remissions. The combination of PDT with Prinomastat® therapy, however, resulted in increased cure rates of 46%, compared to only 20% in PDT-only treated mice, indicating the therapeutic potential of combining PDT with MMP inhibiting drugs.

Sufficient evidence has been acquired to indicate a pivotal role of MMPs, not only in the progression of many forms of cancer, but also in the initiation of the angiogenic process. However, the role of MMPs in the response to PDT is still not completely clarified. In fact, the regulation of MMPs after PDT has been controversial in literature; however several studies have reported a potential benefit for their combination with PDT. Additionally, the fact that many MMP inhibitors have failed for cancer treatment in translational development during clinical studies is mainly due to unacceptable adverse side effects. Therefore, MMP inhibitors should not be select as the first choice of adjuvant therapy to PDT.

3.2.2. Cyclooxygenase-2 (COX-2)

Cyclooxygenase-2 (COX-2) is another known inducer of angiogenesis, acting through the production of VEGF and resulting in increased vascular sprouting, migration and tube formation [99,100]. COX-2 functions by performing the rate limiting reaction in the conversion of arachidonic acid to prostaglandins (PGs). PGs are involved in the regulation of biological processes ranging from immune function and kidney development, to modulating platelet aggregation, inflammation [101], and the induction of VEGF expression. Through these functions, it aids in the processes of tumor progression and

metastasis [102]. More importantly, COX-2 plays a role in tumor growth and angiogenesis. It has been shown to be over-expressed in many cancer types including colon [103], breast [104] and lung [105] cancers, and studies have shown that overexpression of COX-2 is sufficient to induce tumorigenesis in animal models [106]. The combination of PDT with COX-2 inhibitors has also been examined in a variety of studies and has shown good therapeutic potential for increasing the efficacy of PDT treatment.

Ferrario et al. [107] examined the *in vivo* and *in vitro* effects of Photofrin®- and chlorine(NPe6)-based PDT, alone and in combination with a selective COX-2 inhibitor, NS-398 (*N*-(2-cyclohexyloxy-4-nitrophenyl)-methanesulfonamide), on radiation-induced fibrosarcoma (RIF) tumors. Results of *in vitro* assays showed that PDT can effectively activate COX-2 expression in multiple cell lines. In addition, PDT resulted in increased levels of PGs (particularly PGE_2), which were directly related to COX-2 activation, as selective inhibition of COX-2 with NS-398 resulted in a reduction in the synthesis of PGs. *In vivo* assays confirmed these findings showing increased activity of COX-2 in PDT treated mice, as measured through PGE_2 levels in tumor lysates. Inhibition of COX-2 by NS-398 resulted in the reduction of the PDT-induced increase in PGE_2 production in treated tumors. In addition, PDT induced an increase in VEGF expression, which was inhibited in mice treated in combination with NS-398, indicating that COX-2 is indeed involved in the PDT-induced expression of pro-angiogenic molecules. Finally, PDT in combination with NS-398 resulted in a significant increase in tumor cures when compared to PDT alone (NS-398 alone did not affect tumor response), while having no significant effect on normal tissue, indicating its potential for enhancing the efficacy of PDT treatment with minimal added side-effects. Harvey et al. also noted decreased tumor weights after treatment with NPe6-PDT in single dose when combined with the COX-2 inhibitor NS-398 as compared to PDT alone on colon-38 tumors in mice [108].

A study by Makowski et al. [109] examined the effects of Photofrin®-PDT on C-26 cells alone and in combination with COX-2 inhibitors NS-398, rofecoxib or nimesulide. Interestingly, treatment of cells or C-26 tumor bearing mice with COX-2 inhibitors prior to PDT treatment did not sensitize cells or tumors to PDT-induced damage. In contrast, administration of COX-2 inhibitors following PDT increased the antitumor effects of PDT significantly, resulting in complete cures in 6 out of 8 mice. These results suggest that the antitumor activities of COX-2 inhibitors in combination with PDT are indirect, likely acting through the inhibition of angiogenesis. Ferrario et al. [110] also combined Photofrin®-PDT with the COX-2 inhibitors celecoxib or NS-398 in the mouse BA mammary carcinoma model. *In vitro* experiments showed that combination therapy with celecoxib or NS-398 increased the cyctotoxic effect of PDT and increased apoptosis in the mouse mammary carcinoma cell line. Reduced expression of pro-inflammatory molecules interleukin-1β and TNF-α and increased expression of the anti-inflammatory cytokine interleukin-10, were also noted in the combination treatment group.

Yee et al. studied the *in vivo* effects of non-curative doses of hypericin-PDT in combination with the COX-2 inhibitor celecoxib on NPC *in vivo* [111]. PDT monotherapy resulted in the hypoxia-induced upregulation of COX-2 and HIF-1α genes and a reduction in tumor size. Tumor inhibition, however, was followed by tumor regrowth by 24–48 days after PDT treatment, which could likely be attributed to COX-2 and HIF-1α upregulation. Tumors treated with PDT and celecoxib showed a downregulation of COX-2, HIF-1α, and VEGF-A isoforms 165 and 121 genes, compared to PDT-only treated tumors. Interestingly, tumors first treated with celecoxib 6 h after PDT showed the best control of tumor regrowth, while tumors first treated with celecoxib 24 h after PDT showed no control of tumor growth, indicating that the time of celecoxib administration is an important factor affecting tumor response. A similar and confirming study was reported by Akita et al. [112].

The role of COX-2 suppression in PDT-induced angiogenesis, however, is still not completely understood and has been specifically investigated by many different groups. The group of Hendrickx investigated the pathways involved in the PDT-induced expression of COX-2, reporting that COX-2 regulation in tumor cells is achieved through p38 MAPK and phospholipase A2 [113,114]. Additionally, these studies indicated that the inhibition of p38α MAPK blocked the release of VEGF and inhibited tumor-promoted endothelial cell migration. The combination of PDT with PD169316, a selective P38 MAPK inhibitor, resulted in more effective inhibition of VEGF synthesis than PDT in combination with NS398, a COX-2 inhibitor. Moreover, a genetic deficiency of p38α MAPK shifted the balance towards cell death in a manner which could not be reproduced by COX-2 inhibition, indicating that the targeting of p38α MAPK could surpass the ability of COX-2 inhibition, as it most likely also targets additional angiogenic and cell survival signaling pathways.

The mechanisms of PDT-induced COX-2 expression, as well as the effects of COX-2 expression on the activation of angiogenesis and cell survival pathways, have been the topic of many studies over the past decades. The combination of PDT with COX-2 inhibition appears to result in increased efficacy through the inhibition of angiogenesis [107].

3.2.3. Kinase inhibitor p21

The studies presented above describe some of the endogenous non-growth factor molecules, such as MMPs and COX-2, which are involved in the activation of angiogenesis following PDT and can be used as targets in the inhibition of PDT-induced angiogenesis. TNP-470 is one of the first identified non-endogenous angiogenesis inhibitors. It is a synthetic analogue of fumagillin, which acts as a potent angiogenesis inhibitor through strong inhibition of endothelial cell proliferation and migration [115]. TNP-470 has been shown to arrest endothelial cell growth by activating P53 through a unique mechanism in endothelial cells [116,117]. Yeh et al. [117] found that treatment of endothelial cells with nanomolar concentrations of TNP-470 resulted in the accumulation of $p21^{CIP/WAF}$ proteins, correlating to dose-specific inhibition of endothelial cell growth. It was shown that TNP-470 engaged the p53 pathway to exert $p21^{CIP/WAF}$-dependent G1 checkpoint control in endothelial cells. These findings were extended *in vivo* by showing that $p21^{CIP/WAF-/-}$ mice were unresponsive to TNP-470 in the corneal micropocket angiogenesis assay. Due to its potent anti-angiogenic activity, the use of TNP-470 in combination with PDT has also been briefly investigated.

Solban et al. reported subcurative verteporfin-PDT-induced expression of VEGF in an orthotopic model of LNCaP prostate cancer [118]. It was shown that verteporfin-PDT followed by the administration of TNP-470 not only inhibited the increase in VEGF secretion, which was seen in PDT-only treated animals, but also reduced local tumor growth rate, tumor volume, and the fraction of animals with lymph node metastases [119]. Interestingly, administration of TNP-470 prior to PDT resulted in less effective control of tumor growth.

It should be mentioned that certain anti-proliferative and cytostatic agents, such as chemotherapeutics, have been shown to exhibit intrinsic anti-angiogenic activity when administered over a long time period at low dose [120]. These so called metronomic dosing schedules have been tested in clinical trials. Additionally, other experimental approaches have been tested in the context of PDT [121–124].

4. PDT in combination with vascular normalization through anti-VEGF therapy

There is a large body of research in the literature in regards to anti-VEGF therapy in combination with conventional treatment regimens, such as chemo- and radiation therapies. Tumor blood vessels are known to be irregularly shaped, creating uneven blood flow to different parts of the tumor. In addition, the capillary endothelial

cells lining the inner surface of tumor capillaries are often discontinuous, resulting in vessel leakiness. These structural characteristics contribute to tumor vascular hyper-permeability, non-uniform blood flow and distribution, and high interstitial pressure, which together limit the effectiveness of anti-cancer chemo- and radiotherapies by impairing the delivery of drugs and oxygen to the tumor site. Interestingly, anti-angiogenic treatments have been shown to have a temporary normalization effect on tumor vasculature [54]. This subsequently leads to normalization of the interstitial tissue pressure and increased oxygenation. Therefore, it has been hypothesized that the use of anti-angiogenic therapy prior to the application of these conventional anti-cancer strategies may result in synergistic anti-tumor activity. This may be a result of improved drug delivery of chemotherapeutic agents, which can penetrate deeper into the tumor tissue due to the lower tissue pressure. For radiotherapy, which is known to be more efficacious in well-oxygenized tissues, activity will increase due to normalized circulation in the tumor tissue [125]. PDT is also a strategy that is dependent on oxygenation of the target tissue. Therefore, it has been hypothesized that PDT can be more efficacious when applied after angiostatic therapy [22], as it is the case for other treatment regimens [63,125,128]. Nevertheless, the overall and eventual effect of anti-angiogenic therapy is to decrease microvasculature, leading to a decrease in circulation and tissue oxygenation [126,127]. It is clear that a deeper understanding of the mechanisms responsible for the formation of abnormal tumor vasculature is required to further improve future therapeutic success [22].

5. PDT in combination with vascular disrupting agents (VDA)

Vascular disrupting agents (VDA) are a separate class of compounds, which are comprised of agents that target and disrupt already matured and established tumor vasculature. These agents cause an instantaneous vascular shutdown, leading to extensive necrosis at the tumor core [129,130]. It is known that VDAs act well in the tumor core, but leave cells at the rim of the tumor alive, probably as a result of the continuous diffusion of nutrients and oxygen from the surrounding tissues [131]. It has even been demonstrated that such compounds may work better on larger tumors than on smaller ones [132].

Siemann and Shi evaluated the antitumor efficacy of combining bevacizumab with VDAs, combretastatin (CA4P) or OXi4503, in a tumor model of human clear cell renal cell carcinoma, Caki-1[133]. This study demonstrated marked enhancement of tumor response in mice treated with bevacizumab in combination with either CA4P or OXi4503 in a preclinical setting, and provided an experimental basis for the consideration of such a treatment strategy in the clinics.

VDAs have previously been combined with other treatment modalities, like chemotherapy, giving better results than the respective single agents without increased host toxicity [134]. Mechanistically, vascular disruption would lead to decreased blood flow and prolonged entrapment of chemotherapeutic agents within the tumor mass. Unfortunately, a recently published randomized phase III placebo-controlled trial of carboplatin and paclitaxel with or without the vadimezan (5,6-dimethylxanthenone-4-acetic acid; ASA404 or DMXAA), although well tolerated, failed to improve frontline efficacy in advanced NSCLC [135]. However, the combination of vadimezan with radiotherapy gave promising results in a preclinical model [136].

There are only a few combination studies reported on VDAs and photodynamic therapy in preclinical models. Treatment with low dose DMXAA two hours prior to short-duration/ high irradiance photochlor-PDT in CT-26 colon carcinoma-bearing mice showed a synergistic interaction, resulting in long-term cures in 60% of treated animals [137]. Moreover, the combination therapy resulted in significantly less peritumoral edema than the dose of PDT monotherapy required for an equivalent cure rate. These results indicate that this combination has the potential to improve treatment efficacy and selectivity.

He et al. tested the combination of verteporfin-PDT with combretastatin in SVEC4-10 mouse endothelial cells and found it significantly increased endothelial cell apoptosis as compared to the single therapy [138]. In a study on the PC-3 prostate tumor model, it was found that combretastatin highly enhanced tumor response to verteporfin-PDT, when both treatments were used in sub-lethal regimens. Pretreatment with the VDA decreased the rate of blood flow in tumors, while making them more sensitive to verteporfin-PDT. This observation is rather counterintuitive and the mechanisms underlying this phenomenon remain unknown.

Promising effects were obtained for the combination of verteporfin-PDT and the PI3 kinase inhibitor BEZ235 in SVEC4-10 mouse endothelial cells and PC-3 prostate tumor cells [139]. PI3K is an important enzyme involved in extracellular signal transduction. The study showed an increased extent of commitment to apoptotic cell death in SVEC, and slower, but increased, commitment to autophagy in PC3 cells following verteporfin-PDT with PI3 kinase inhibitors. This increase in endothelial cell death would be of interest in the context of vascular-PDT aimed at faster vessel ablation. A similar principle was used by Morrero et al. who combined topically applied vadimezan with aminolevulinic acid (ALA)-PDT [140] in Colon26 murine colon adenocarcinoma bearing mice. The onset of blood flow reduction was rapid in tumors treated with both ALA-PDT and vadimezan. CD31-immunostaining of tumor sections confirmed vascular damage following the topical application of vadimezan. Tumor weight measurements revealed enhanced tumor growth inhibition with combination treatment, as compared with ALA-based PDT or vadimezan treatment alone. In conclusion, vadimezan as a topical agent enhances treatment efficacy when combined with ALA-based PDT. The use of VDAs in combination with PDT provides a promising therapeutic strategy, which is even more supported by the finding that some of these compounds have intrinsic anti-angiogenic activity [141].

6. PDT with photosensitizers targeted specifically to the tumor endothelium

Tumor angiogenesis does not only provide a target for combination therapy, but also provides an altered vasculature with endothelial cells that have adapted to the increased metabolic needs of the tumor cells. This activated tumor endothelium has an altered molecular make-up [142,143] providing targets for selective delivery of drugs, including PSs [144]. Recently, the idea of selectively targeting the tumor vasculature, through the conjugation of PSs to antibodies or molecules which bind to markers of an angiogenic endothelium, was challenged. Such strategies aim to increase the efficacy of PDT, while reducing normal tissue toxicity [145].

6.1. ED-B domain of fibronectin

During certain stages of embryonic development, fibronectin can be alternatively spliced to generate a variant that has an extra domain B, which later in life is rarely expressed and confined to pathological conditions, including cancer. This extra domain B (ED-B domain) of fibronectin is also a marker of angiogenic vasculature in tumors [146]. Antibodies targeting ED-B-fibronectin, such as the scFv (L19) antibody fragment, therefore can be used to selectively deliver therapeutic agents to angiogenic tumor endothelium. Such targeting has been confirmed by the accumulation of radio-labeled L19 antibody ($L19(scFv)_2$) in glioblastoma and lung carcinomas [146]. These findings suggest the potential for increasing the selectivity and efficacy of PDT through direct targeting of PSs to markers of angiogenic tumor endothelium.

This concept was tested by Birchler et al. [147] using PSs which were chemically bound to L19 antibodies. These conjugates were

shown to selectively target neovessels in a rabbit model of ocular angiogenesis. In this study, ED-B-fibronectin targeted PDT resulted in complete and selective occlusion of neovessels, which corresponded with endothelial cell apoptosis in targeted vessels, while blood vessels of the conjunctiva and other ocular structures were left unaffected [147]. The same group also conjugated porphyrin-based PSs to the L19 antibody in its small immune protein (SIP) format, SIP (L19)-PS, which is believed to have better accumulation in tumor tissue. These SIP(L19)-PS were used in the PDT treatment of aggressive tumors, including an SCC model (A431) implanted in the skin of nude mice. The results of this study showed that PDT using these targeted PSs had a strong anti-cancer effect, resulting in complete, long-term (100 days following treatment) tumor eradication [148] (see also Fig. 4). The treatment induced extensive hemorrhage and edema. Additionally, it was observed that natural killer cells were required in order to achieve complete tumor ablation, as removal of these cells resulted in a transient inhibition of tumor growth followed by tumor progression.

Fabbrini et al. showed that intravenous injection and subsequent irradiation of the porphyrin based PS SnChe6 conjugated to L19 antibodies resulted in the arrest of tumor growth in mice with subcutaneous tumors (FE8 sarcoma, F9 teratocarcinoma and C51 colon adenocarcinoma) [149]. This study showed that the SIP form of the L19 antibody was more effective at targeting tumor vasculature than the scFv format, inducing a significant reduction in tumor mass 6 days after irradiation. Treating mice repetitively resulted in the stasis of tumor growth until day 20. Additionally, the average tumor weight in these mice was significantly lower than in mice treated with PSs conjugated to an antibody lacking ED-B specificity (0.04 vs 1.10 g) [149]. These studies indicate that the ED-B domain of fibronectin can be used to selectively target the angiogenic endothelium of tumor vasculature and, more importantly, that targeted PSs can indeed increase the anti-tumor activity of PDT.

6.2. $\alpha_v\beta_3$ integrin

Integrins are a group of adhesion molecules, which are involved in a wide array of biological functions. Endothelial cells have integrins to aid in their adherence to each other and to the extracellular matrix. These integrins consist of an α and a β subunit, for which several different genes are available. Generally, integrins which contain a β_1-subunit are involved in matrix interactions. A specific integrin, the $\alpha_v\beta_3$ integrin, which binds to tri-peptide Arg-Gly-Asp (RGD) containing proteins, was found to be overexpressed in angiogenically stimulated endothelial cells of tumors [150]. The $\alpha_v\beta_3$ integrin is believed to play a critical role in angiogenesis by mediating the adhesion of endothelial cells to fibronectin, fibrinogen, laminin, collagen, vWF and osteopontin, through their RGD-moiety [151]. Additionally, the inhibition or blocking of $\alpha_v\beta_3$ integrin, by RGD peptides or antibodies, results in endothelial cell apoptosis, indicating that this integrin also plays a role in endothelial cell survival [152,153]. Therefore, the targeted delivery of PSs using RGD peptides has become a topic of interest, as it may result in the increased selectivity of PDT, with the possibility of additive anti-angiogenic effects due to the RGD peptide.

Chen et al. [154] investigated RGD targeted Photofrin® encapsulated in a surfactant-like tetra-tail amphiphilic nanoparticle. The effects of the Photofrin®-loaded micelles were tested *in vitro* on two different cell lines, human cervix carcinoma (HeLa) and human embryonic kidney transformed 239 (293 T), the first of which is known to over-express $\alpha_v\beta_3$ integrin. The porphyrin-loaded micelles showed no apparent dark toxicity in HeLa cells, but showed significant phototoxicity upon irradiation with light. The amount of Photofrin® internalized by the HeLa cells was shown to be much greater than that internalized by the 293 T cells, suggesting the success of the RGD targeting ligand. Furthermore, it was shown that cell death was due to the presence of reactive oxygen species, and that Photofrin® had accumulated in the nucleus of these cells, indicating successful targeted drug delivery.

Integrins can also be targeted using adenovirus type 2 structural proteins, such as hexon and penton bases and fiber antigens, as these are known to contain the RGD peptide sequence [155]. Allen et al. [156] covalently coupled such adenoviral proteins to tetrasulfonated aluminum phthalocyanine ($AlPcS_4$) PSs and tested their effects *in vitro* and *in vivo*. All of the $AlPcS_4$- protein complexes induced greater cytotoxicity than the unconjugated $AlPcS_4$ PSs. Additionally, *in vivo* experiments in nude mice with EMT-6 tumors showed enhanced anti-tumor activity with the targeted PSs.

Angiogenic blood vessels can also be targeted using the 5-mer peptide Ala-Pro-Arg-Pro-Gly (APRPG), which has been shown to specifically bind to angiogenic tumor blood vessels [157]. Ichikawa et al. [158] reported the use of polyethylene glycol (PEG)-modified liposomes to encapsulate verteporfin, which were then functionalized using the APRPG pentapeptide (APRPG-PEG-lip verteporfin). The APRPG-PEG-lip verteporfin was shown to selectively accumulate *in vivo* in the tumors of Meth-A sarcoma bearing mice to a degree which was approximately 4-fold higher than verteporfin delivered in non-modified liposomes. In addition, PDT using the APRPG-PEG-lip verteporfin resulted in strong suppression of tumor growth and prolonged life in treated mice compared to the untargeted PEG-lip verteporfin, which resulted in a small amount of tumor growth suppression but no increase in the survival time of treated mice. More recently, Oku et al. performed a confirmatory study using these targeted nanoparticles. The results of this study demonstrated that enhanced efficacy can be attained using liposomes which are targeted to angiogenic endothelial cells for the selective delivery of PSs [159].

7. Translation to the clinic

Most of the studies discussed above describe new experimental approaches to combination therapies tested *in vitro* on cell cultures or *in vivo* in different animal models. The ultimate goal in this field of research, however, is to identify viable new treatments that can be translated and applied in clinical settings. As past experience in the field of angiogenesis research has shown, promising pre-clinical results in mice do not always lead to similar effects in clinical trials [160]. This leaves open the question of how one should go about translating pre-clinical results into clinical applications. Recent advances have allowed for the development of genetically engineered mouse models, which can be made to mimic both the pathophysiological and molecular characteristics of human tumors [161]. These models, which more accurately reflect human disease, will hopefully provide results that more precisely predict success in clinical application.

Recently, two different inhibitors of angiogenesis, sunitinib and everolimus, have shown potential for the treatment of human pancreatic neuroectodermal tumors (PNET) in phase II clinical trials [162,163]. The potential of both of these drugs for this indication was initially predicted by promising results of preclinical studies [164] performed in a genetically engineered mouse model, the RIP-TAG2 model, which has similar features as human PNET tumors [162,163,165]. In this case, the preclinical model helped to identify not only a potential new therapy, but also the possible limitations of this therapy. The phase II clinical trial revealed a significant increase in the progression free survival of treated patients, however, a lack of increase in overall survival (seen thus far in the study as it is still ongoing), a limitation which was predicted by tumor shrinkage and long-term survival in the mouse model [163,166]. Additionally, preclinical models have also predicted the eventual failure of sunitinib therapy alone [167], due the development of drug resistance, which is now also being seen in clinical trials [168,169].

Table 1
Brief description of selected studies examining the effects of PDT in combination with various angiogenesis and vascular targeted agents.

Target	Photosensitizer	Angiostatic agent	Sequence	Model	Observations	Reference
VEGF	Hypericin	Avastin®	PDT + AI	Human bladder carcinoma in nude mice	Leaky vasculature with functional alteration in combination PDT and Avastin® and greater reduction in vessel area than PDT-only; decreased vessel area and intact vasculature in Avastin® only group.	[61]
	Photofrin®	Avastin®	PDT + AI	Human Kaposi's sarcoma in nude mice	Significant increase in long-term tumor response in mice treated with combination therapy without increase in normal tissue toxicity.	[59]
	Hypericin	Avastin®	PDT + AI	Bladder carcinoma in nude mice	Significant improvement of tumor response in combination group. Relationship to downregulated expression of angiogenic molecules (VEGF, angiogen, bFGF, EGF and IL-6 and −8).	[60]
	Visudyne®	Avastin®	AI + PDT	Circumscribed choroidal haemangioma (in patients)	Complete regression of the lesions for 6–9 month.	[63]
	Visudyne®	Avastin®	AI + PDT	Juxtapapillary retinal capillary haemangioma (in patients)	The single combination therapy of Avastin® and PDT had a long-lasting effect and thus eliminated the necessity of further destruction by repeated PDT.	[66]
VEGFR-1 *VEGFR-2*	Photofrin®	MF1 DC101	PDT + AI	Intracerebral U87 glioblastoma	Significant inhibition of tumor growth and increased long-term survival in combination group associated with decreased expression of angiogenic factors (VEGF and VWF).	[62]
EGF	Hypericin	Erbitux®	PDT + AI	Ovarian carcinoma in nude mice	Synergistic effect of combination PDT + Erbitux® therapy.	[73]
	Hypericin	Erbitux®	PDT + AI	Human bladder carcinoma in nude mice	Increased tumor response in combination group and increased apoptosis and down-regulation of EGFR expression in combination group.	[76]
EGF *VEGF*	Hypericin	Erbitux® Avastin®	PDT + AI	Bladder carcinoma in nude mice	Significant difference between control (no therapy), PDT-only and PDT combinations (PDT + Avastin®, PDT + Erbitux®, PDT + Avastin® + Erbitux®).	[58]
Protein Kinase p21	Visudyne®	NP-470	PDT + AI AI + PDT	Prostate cancer (LNCaP)	Combination therapy inhibited PDT-induced increase of VEGF and significantly reduced tumor weight and volume.	[119]
VEGFRs *PDGFR* *FGFR*	Hypericin	SU5416 SU6668	PDT + AI	Nasopharyngeal carcinoma In BALB/c athymic mice	Increased therapeutic response in combination therapy treated mice (SU6668 + PDT had better response than SU5416 + PDT).	[87]
c-src *FGFR-1* *PDGFR-β* *EGFR*	Hexylether Pyropheophorbide	PD166285 PD173074	PDT + AI	Murine mammary 16c tumors	Improved overall survival outcome and inhibition of PDT-induced angiogenesis with both inhibitors.	[86]
COX-2	Photofrin®	Rofecoxib NS-398 Nimesulide	PDT + AI AI + PDT	Colon adenocarcinoma (C-26)	Combination with COX-2 inhibitors following PDT, but not prior to PDT, increased antitumor activity.	[109]
	ALA	Nimesulide	PDT + AI	Oral squamous cell carcinoma	Combination of PDT with COX-2 inhibitors significantly reduced tumor growth in cell line over-expressing COX-2.	[112]
COX-2 *VEGF* *HIF-α*	hypericin	Celecoxib	PDT + AI	Nasopharyngeal carcinoma	Combination of PDT and COX-2 inhibitors resulted in increased tumor growth inhibition due to down-regulation of COX-2, HIF-1 and VEGF-A.	[111]
MMP-2 *MMP-9*	Hypericin	Prinomastat	PDT + AI	Mouse mammary carcinoma (BA) in mice	Significant increase in long-term cures in combination group.	[98]
Endothelium	Photochlor	Vadimezan	PDT + VDA	CT colon carcinoma in BALB/c mice	Improved efficacy and selectivity, and decreased phototoxicity over PDT alone.	[137]
Pl3/mTOR kinase	Visudyne®	Pl3/mTOR kinase inhibitor	PDT + VDA	SVEC4-10 endothelial cells, PC-3 prostate tumor cells	Increased apoptotic endothelial cell death rate in combination group.	[136]
Endothelium	ALA	Vadimezan	n.a.	Colon26 murine colon adenocarcinoma	Increased tumor growth inhibition.	[140]
ED-B fibronectin	SnChe6	Human antibody L19	n.a.	FE8 sarcoma bearing CD-1 nude mice	Average tumor weight in treated mice was significantly lower than in mice treated with PS conjugated to an antibody lacking ED-B specificity.	[149]
$\alpha_v\beta_3$ *integrin*	Photofrin®	Surfactant-like tetra-tail amphiphilic with an RGD ligand	n.a.	HeLa human embryonic kidney transformed (293 T)	The amount of Photofrin® internalized by the HeLa cells was shown to be much greater than that internalized by the 293 T cells, indicating the success of the RGD targeting ligand.	[154]

(continued on next page)

Table 1 (*continued*)

Target	Photosensitizer	Angiostatic agent	Sequence	Model	Observations	Reference
	$AlPcS_4$	n.a.	n.a.	Nude mice with EMT-6 tumors	Enhanced PS localization and accumulation in the tumor tissue as a result of being targeted to the $\alpha_v\beta_3$ integrin.	[156]
	(PEG)-modified liposomes of verteporfin	n.a.	n.a.	Meth-A sarcoma bearing mice	PDT with the APRPG-PEG-lip verteporfin resulted in strong suppression of tumor growth resulting in prolonged life of treated mice and in vascular damage.	[158]
	APRPG-verteporfin	n.a.	n.a.	n.a.	The APRPG-PEG-modified verteporfin liposomes were shown to selectively accumulate in tumor tissue and strongly inhibit tumor growth upon irradiation.	[159]
	5-[4-(succinimide-*N*-oxycarbonyl) phenyl]-10,15,20-tris-(4-*N*-methylpyridimiumyl)porphyrin trichloride	SIP(L19)	n.a.	F9 murine teratocarcinoma	Selective *in vivo* localization around tumor blood vessels. The conjugate with a photosensitizer allows selective disruption of tumor vasculature upon irradiation, leading to complete and long-lasting cancer eradication.	[148]

AI, angiogenesis inhibitor; RGD, Arg-Gly-Asp tri-peptide; VDA, vascular disrupting agent; n.a., not applicable

8. Back to the drawing board

The concept of PDT, i.e. the treatment of disease through the administration of a photosensitive drug and the application of light, is an old idea, but nowadays is successfully implemented in a limited number of ocular applications. This includes the treatment of exudative age-related macular degeneration (AMD) or polypoidal choroidal vasculopathy (PCV), and for the treatment of certain cancerous and precancerous skin conditions, including basal cell carcinoma and actinic keratosis, as well as rheumatoid arthritis. The limited number of successful applications for the PDT treatment of cancer may, in part, be due to the local nature of the therapy making it an inappropriate form of treatment for disease in advanced stages, which has already progressed to multiple locations in the body. Additionally, treatment of early stage localized disease is limited, as cancer is frequently not detected until its later stages, due to the asymptomatic nature of many early forms of cancer. Adding to complications associated with the local nature of PDT is the difficult, but necessary requirement of achieving optimal light exposure to all parts of the tumor, including deeper tissue areas. This problem tends to limit the application of PDT in oncology to superficially growing tumors in the skin, or in hollow organs. It is now being realized, however, that there may be opportunities to overcome some of these limitations that have precluded the development of efficient PDT-based anti-cancer strategies.

Although PDT is largely regarded as a local treatment, several more recent studies have recognized that localized PDT can result in the activation of a systemic anti-tumor immune response, which will attack distant tumor growth [170–172], as well as inhibit new growth [173,174]. One study showed that the PDT treatment of BCC in humans resulted in the activation of a systemic anti-tumor immunity to a BCC associated tumor antigen, Hip1, which was much greater than in patients whose lesions had been surgically removed [175]. A case study also showed the activation of anti-tumor immunity in the treatment of a patient suffering from a multifocal angiosarcoma of the head and neck, which was associated with the regression of distant untreated tumors [171]. Similarly promising results have also been obtained from the PDT treatment of gliomas in mouse models [176]. Gaining a better understanding of the mechanisms and pathways involved in PDT-induced anti-tumor immunity may allow for the development of new treatment regiments which enhance this effect and may lead to more diverse oncological applications for PDT.

Additionally, the recent development of new light distributing fibers and the availability of improved PSs with long-wavelength absorption have allowed for improved light exposure, particularly in deeper tissues [177]. Furthermore, for some applications, like prostate cancer, the device industry has made great strides to develop methods to achieve localized light delivery deep in the tissue, for example with the use of multifiber technology [178].

As mentioned above, the local nature of light delivery during the administration of PDT is frequently incompatible with systemic disease. However, increasing the selectivity of the photosensitive drug for neoplastic tissues is another viable method to help overcome this limitation. In this sense, some of the advancements in the field of PDT can be attributed to increasing knowledge in the field of angiogenesis research, which is providing the main opportunities for targeted delivery of PSs, i.e. new markers of tumor angiogenesis have been identified, to which the PS can be targeted. Thus, specific targeting of PSs to the tumor endothelium is an attractive approach to enhance the selectivity and specificity of PDT. New targets are continuously being identified, which can be used to selectively deliver PS to the tumor endothelium. This strategy also holds promise for the future, as the field of anti-angiogenesis research has become even more active in recent years. We and others have identified the gene expression profile of tumor endothelial cells. Only a limited number of genes are overexpressed in tumor vessels, as compared to normal vessels and vessels in angiogenically stimulated normal tissue, such as placenta tissue [142,143]. These genes are currently being tested for targeted delivery. We have recently shown that antibody mediated targeting of HMGB1 is an effective strategy to prolong the PDT-induced anti-vascular effect [179]. Future studies will investigate improved selectivity of PDT, when PSs are targeted to HMGB1. Recently obtained knowledge has indicated that dual targeting of particles to more than one marker of the tumor endothelium may result in significantly enhanced targeting capacity. For instance, it was shown by Kluza et al. [180] that nanoparticles directed to galectin-1 by the angiostatic peptide anginex [181,182] and, simultaneously, to $\alpha_v\beta_3$ integrin by a cyclic RGD-peptide showed increased targeting capacity. It is consequently suggested that targeting PSs simultaneously to more than one endothelial cell marker can increase the anti-tumor selectivity and activity of PDT, while simultaneously reducing the dose of PS needed compared to non-targeted PDT [148]. Specific delivery of PSs will lead to better options for selective PDT and salvation of adjacent normal tissues. Still, systemic treatment will be difficult, but it should be realized that many anti-cancer therapies are sufficient when applied locally, for instance to the surface of a given hollow organ.

Finally, and probably most importantly, the secondary induction of angiogenesis by PDT is likely responsible for a significant reduction in the success rates of treatment. It is now realized that this limitation can be overcome by simultaneous treatment of the cancer with

angiogenesis inhibitors, many of which are currently becoming clinically available. We favor the view that such a combination strategy may allow for a revival of the field of PDT, leading to the development of effective new therapies for a number of cancers. In this spirit, we reviewed a series of efforts that were undertaken to give the combination of anti-angiogenesis with PDT a go (see also Table 1). Many of these studies were undertaken using compounds, which are now known to not be the best inhibitors of angiogenesis. This holds an intrinsic promise for further testing of combination therapies using newer and more successful angiostatic compounds. In this respect, it is especially interesting that several tyrosine kinase inhibitors (TKIs) have shown preclinical promise in combination with PDT. Several new TKIs were recently developed, that represent a second generation of angiostatic compounds with better activity and fewer side effects. Examples of such compounds are axitinib and tivozanib, and results obtained with these agents in combination with PDT are eagerly expected.

It should be realized, however, that new combination treatments will only be efficient when designed in an optimal way. In order to do this, the sequencing of both therapies is an important issue. Although it is logical to start anti-angiogenic therapy after the PDT treatment, because of the angiogenesis induction following PDT, it is remarkable that little effort has been undertaken to test the efficacy of scheduling anti-angiogenic therapy before PDT. Here one may note that it has been demonstrated that anti-angiogenic therapy can normalize the vasculature in a tumor, resulting in less permeable vessels, reduction of hypoxia and improved blood flow [54]. This was found to explain the synergism of angiostasis with conventional therapies, such as chemo- and radiotherapies. Similar reasoning is valid for PDT, which depends on both efficient delivery of the PS, as well as the oxygenation of the target tissue [22]. We conclude that it is consequently also of extreme importance to do a systematic search for optimal sequencing of angiogenesis inhibition for improvement of the PDT approach.

Acknowledgements

The authors are grateful for financial support from Dr. J. Jacobi and the Dutch Science Foundation (NWO grant # 040.11.195).

References

[1] P. Ghaliounghui, The Ebers Papyrus: A New English Translation, Commentaries and Glossaries, Egyptian Academy of Scientific Research and Technology, Cairo, 1987.
[2] O. Raab, Ueber die Wirkung Fluorescierenden Stoffe auf Infusorien, Z. Biol. 39 (1904) 524–546.
[3] H. Jesionek, H. von Tappeiner, Zur Behandlung der Hautcarcinome mit fluoreszierenden Stoffen, Dtsch. Arch. Klin. Med 82 (1905) 223–226.
[4] T.J. Dougherty, J.E. Kaufman, A. Goldfarb, K.R. Weishaupt, D. Boyle, A. Mittleman, Photoradiation therapy for the treatment of malignant tumors, Cancer Res. 38 (1978) 2628–2635.
[5] H.B. Van den Bergh, J.-P., Photodynamic therapy: basic principles and mechanisms, Lasers Opthalmol. (2003) 183–195.
[6] T.J. Dougherty, C.J. Gomer, B.W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan, Q. Peng, Photodynamic therapy, J. Natl. Cancer Inst. 90 (1998) 889–905.
[7] P. Nowak-Sliwinska, A. Karocki, M. Elas, A. Pawlak, G. Stochel, K. Urbanska, Verteporfin, photofrin II, and merocyanine 540 as PDT photosensitizers against melanoma cells, Biochem. Biophys. Res. Commun. 349 (2006) 549–555.
[8] B. Chen, B.W. Pogue, J.M. Luna, R.L. Hardman, P.J. Hoopes, T. Hasan, Tumor vascular permeabilization by vascular-targeting photosensitization: effects, mechanism, and therapeutic implications, Clin. Cancer Res. 12 (2006) 917–923.
[9] M. Korbelik, PDT-associated host response and its role in the therapy outcome, Lasers Surg. Med. 38 (2006) 500–508.
[10] F. Fantini, A. Greco, C. Del Giovane, A.M. Cesinaro, M. Venturini, C. Zane, T. Surrenti, K. Peris, P.G. Calzavara-Pinton, Photodynamic therapy for basal cell carcinoma: clinical and pathological determinants of response, J. Eur. Acad. Dermatol. Venereol. 25 (2011) 896–901.
[11] M.A. Biel, Photodynamic therapy treatment of early oral and laryngeal cancers, Photochem. Photobiol. 83 (2007) 1063–1068.
[12] N.R. Rigual, K. Thankappan, M. Cooper, M.A. Sullivan, T. Dougherty, S.R. Popat, T.R. Loree, M.A. Biel, B. Henderson, Photodynamic therapy for head and neck dysplasia and cancer, Arch. Otolaryngol. Head Neck Surg. 135 (2009) 784–788.
[13] A. Klein, P. Babilas, S. Karrer, M. Landthaler, R.M. Szeimies, Photodynamic therapy in dermatology—an update 2008, J. Dtsch. Dermatol. Ges. 6 (2008) 839–845 839–846.
[14] K. Moghissi, K. Dixon, J.A. Thorpe, M. Stringer, C. Oxtoby, Photodynamic therapy (PDT) in early central lung cancer: a treatment option for patients ineligible for surgical resection, Thorax 62 (2007) 391–395.
[15] J. Usuda, H. Kato, T. Okunaka, K. Furukawa, H. Tsutsui, K. Yamada, Y. Suga, H. Honda, Y. Nagatsuka, T. Ohira, M. Tsuboi, T. Hirano, Photodynamic therapy (PDT) for lung cancers, J. Thorac. Oncol. 1 (2006) 489–493.
[16] J. Sun, I. Cecic, C.S. Parkins, M. Korbelik, Neutrophils as inflammatory and immune effectors in photodynamic therapy-treated mouse SCCVII tumours, Photochem. Photobiol. Sci. 1 (2002) 690–695.
[17] S.O. Gollnick, S.S. Evans, H. Baumann, B. Owczarczak, P. Maier, L. Vaughan, W.C. Wang, E. Unger, B.W. Henderson, Role of cytokines in photodynamic therapy-induced local and systemic inflammation, Br. J. Cancer 88 (2003) 1772–1779.
[18] P. Agostinis, K. Berg, K.A. Cengel, T.H. Foster, A.W. Girotti, S.O. Gollnick, S.M. Hahn, M.R. Hamblin, A. Juzeniene, D. Kessel, M. Korbelik, J. Moan, P. Mroz, D. Nowis, J. Piette, B.C. Wilson, J. Golab, Photodynamic therapy of cancer: an update, CA Cancer J. Clin. 61 (2011) 250–281.
[19] I.P. van Geel, H. Oppelaar, P.F. Rijken, H.J. Bernsen, N.E. Hagemeier, A.J. van der Kogel, R.J. Hodgkiss, F.A. Stewart, Vascular perfusion and hypoxic areas in RIF-1 tumours after photodynamic therapy, Br. J. Cancer 73 (1996) 288–293.
[20] B.W. Henderson, T.J. Dougherty, How does photodynamic therapy work? Photochem. Photobiol. 55 (1992) 145–157.
[21] C. Hopper, Photodynamic therapy: a clinical reality in the treatment of cancer, Lancet Oncol. 1 (2000) 212–219.
[22] P. Nowak-Sliwinska, G. Wagnieres, H. van den Bergh, A.W. Griffioen, Angiostasis-induced vascular normalization can improve photodynamic therapy, Cell. Mol. Life Sci. 67 (2010) 1559–1560.
[23] P. Nowak-Sliwinska, A. Weiss, J.R. Beijnum, T.J. Wong, J.P. Ballini, B. Lovisa, H.V. Bergh, A.W. Griffioen, Angiostatic kinase inhibitors to sustain photodynamic angio-occlusion, J. Cell. Mol. Med., in press, PMID: 21880113.
[24] A.C. Moor, Signaling pathways in cell death and survival after photodynamic therapy, J. Photochem. Photobiol. B 57 (2000) 1–13.
[25] A. Ferrario, K.F. von Tiehl, N. Rucker, M.A. Schwarz, P.S. Gill, C.J. Gomer, Antiangiogenic treatment enhances photodynamic therapy responsiveness in a mouse mammary carcinoma, Cancer Res. 60 (2000) 4066–4069.
[26] M.Y. Nahabedian, R.A. Cohen, M.F. Contino, T.M. Terem, W.H. Wright, M.W. Berns, A.G. Wile, Combination cytotoxic chemotherapy with cisplatin or doxorubicin and photodynamic therapy in murine tumors, J. Natl. Cancer Inst. 80 (1988) 739–743.
[27] J. Golab, D. Olszewska, P. Mroz, K. Kozar, R. Kaminski, A. Jalili, M. Jakobisiak, Erythropoietin restores the antitumor effectiveness of photodynamic therapy in mice with chemotherapy-induced anemia, Clin. Cancer Res. 8 (2002) 1265–1270.
[28] Z. Luksiene, A. Kalvelyte, R. Supino, On the combination of photodynamic therapy with ionizing radiation, J. Photochem. Photobiol. B 52 (1999) 35–42.
[29] R. Bhuvaneswari, Y.Y. Gan, K.C. Soo, M. Olivo, The effect of photodynamic therapy on tumor angiogenesis, Cell. Mol. Life Sci. 66 (2009) 2275–2283.
[30] V.H. Fingar, P.K. Kik, P.S. Haydon, P.B. Cerrito, M. Tseng, E. Abang, T.J. Wieman, Analysis of acute vascular damage after photodynamic therapy using benzoporphyrin derivative (BPD), Br. J. Cancer 79 (1999) 1702–1708.
[31] B.W. Henderson, S.M. Waldow, T.S. Mang, W.R. Potter, P.B. Malone, T.J. Dougherty, Tumor destruction and kinetics of tumor cell death in two experimental mouse tumors following photodynamic therapy, Cancer Res. 45 (1985) 572–576.
[32] P. Nowak-Sliwinska, J.R. van Beijnum, M. van Berkel, H. van den Bergh, A.W. Griffioen, Vascular regrowth following photodynamic therapy in the chicken embryo chorioallantoic membrane, Angiogenesis 13 (2010) 281–292.
[33] W.M. Star, H.P. Marijnissen, A.E. van den Berg-Blok, J.A. Versteeg, K.A. Franken, H.S. Reinhold, Destruction of rat mammary tumor and normal tissue microcirculation by hematoporphyrin derivative photoradiation observed *in vivo* in sandwich observation chambers, Cancer Res. 46 (1986) 2532–2540.
[34] H. van den Bergh, J.P. Ballini, Photodynamic therapy: basic principle, in: S. F.F.a.K. (Ed.), Lasers in Ophthalmology—Basic, diagnostic and Surgical Aspects, Kugler Publications, The Hague, 2003, pp. 183–195.
[35] T.H. Foster, M.C. Primavera, V.J. Marder, R. Hilf, L.A. Sporn, Photosensitized release of von Willebrand factor from cultured human endothelial cells, Cancer Res. 51 (1991) 3261–3266.
[36] J.S. Nelson, L.H. Liaw, M.W. Berns, Tumor destruction in photodynamic therapy, Photochem. Photobiol. 46 (1987) 829–835.
[37] U. Schmidt-Erfurth, T. Hasan, E. Gragoudas, N. Michaud, T.J. Flotte, R. Birngruber, Vascular targeting in photodynamic occlusion of subretinal vessels, Ophthalmology 101 (1994) 1953–1961.
[38] H.M. Solomon, P.D. Zieve, J.R. Krevans, The effect of hematoporphyrin and light on human platelets. II. Uptake of hematoporphyrin, J. Cell. Physiol. 67 (1966) 281–284.
[39] P.D. Zieve, H.M. Solomon, J.R. Krevans, The effect of hematoporphyrin and light on human platelets. I. Morphologic, functional, and biochemical changes, J. Cell. Physiol. 67 (1966) 271–279.
[40] B.W. Henderson, B. Owczarczak, J. Sweeney, T. Gessner, Effects of photodynamic treatment of platelets or endothelial cells *in vitro* on platelet aggregation, Photochem. Photobiol. 56 (1992) 513–521.
[41] M.W. Reed, F.N. Miller, T.J. Wieman, M.T. Tseng, C.G. Pietsch, The effect of photodynamic therapy on the microcirculation, J. Surg. Res. 45 (1988) 452–459.
[42] V.H. Fingar, Vascular effects of photodynamic therapy, J. Clin. Laser Med. Surg. 14 (1996) 323–328.

[43] J.M. Klausner, I.S. Paterson, G. Goldman, L. Kobzik, C. Rodzen, R. Lawrence, C.R. Valeri, D. Shepro, H.B. Hechtman, Postischemic renal injury is mediated by neutrophils and leukotrienes, Am. J. Physiol. 256 (1989) F794–F802.
[44] J. Doukas, H.B. Hechtman, D. Shepro, Vasoactive amines and eicosanoids interactively regulate both polymorphonuclear leukocyte diapedesis and albumin permeability *in vitro*, Microvasc. Res. 37 (1989) 125–137.
[45] V.H. Fingar, T.J. Wieman, S.A. Wiehle, P.B. Cerrito, The role of microvascular damage in photodynamic therapy: the effect of treatment on vessel constriction, permeability, and leukocyte adhesion, Cancer Res. 52 (1992) 4914–4921.
[46] M. Dellian, C. Abels, G.E. Kuhnle, A.E. Goetz, Effects of photodynamic therapy on leucocyte–endothelium interaction: differences between normal and tumour tissue, Br. J. Cancer 72 (1995) 1125–1130.
[47] A.W. Griffioen, C.A. Damen, S. Martinotti, G.H. Blijham, G. Groenewegen, Endothelial intercellular adhesion molecule-1 expression is suppressed in human malignancies: the role of angiogenic factors, Cancer Res. 56 (1996) 1111–1117.
[48] A.E. Dirkx, M.G. Oude Egbrink, M.J. Kuijpers, S.T. van der Niet, V.V. Heijnen, J.C. Bouma-ter Steege, J. Wagstaff, A.W. Griffioen, Tumor angiogenesis modulates leukocyte-vessel wall interactions *in vivo* by reducing endothelial adhesion molecule expression, Cancer Res. 63 (2003) 2322–2329.
[49] E. Ben-Hur, E. Heldman, S.W. Crane, I. Rosenthal, Release of clotting factors from photosensitized endothelial cells: a possible trigger for blood vessel occlusion by photodynamic therapy, FEBS Lett. 236 (1988) 105–108.
[50] W.H. Chang, J.J. Petry, Platelets, prostaglandins, and patency in microvascular surgery, J. Microsurg. 2 (1980) 27–35.
[51] M.L. Ogletree, Overview of physiological and pathophysiological effects of thromboxane A2, Fed. Proc. 46 (1987) 133–138.
[52] M.J. Gilissen, L.E. van de Merbel-de Wit, W.M. Star, J.F. Koster, W. Sluiter, Effect of photodynamic therapy on the endothelium-dependent relaxation of isolated rat aortas, Cancer Res. 53 (1993) 2548–2552.
[53] V.H. Fingar, T.J. Wieman, K.W. Doak, Changes in tumor interstitial pressure induced by photodynamic therapy, Photochem. Photobiol. 53 (1991) 763–768.
[54] R.K. Jain, Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy, Science (New York, N.Y.) 307 (2005) 58–62.
[55] D. Cao, M. Hou, Y.S. Guan, M. Jiang, Y. Yang, H.F. Gou, Expression of HIF-1alpha and VEGF in colorectal cancer: association with clinical outcomes and prognostic implications, BMC Cancer 9 (2009) 432.
[56] N.H. Fernando, H.I. Hurwitz, Targeted therapy of colorectal cancer: clinical experience with bevacizumab, Oncologist 9 (Suppl. 1) (2004) 11–18.
[57] S.M. Couch, S.J. Bakri, Review of combination therapies for neovascular age-related macular degeneration, Semin. Ophthalmol. 26 (2011) 114–120.
[58] G.Y. R.Y. Bhuvaneswari, S.K. Chee, M. Olivo, Antiangiogenesis agents Avastin and Erbitux enhance the efficacy of photodynamic therapy in a murine bladder tumor model, Lasers Surg. Med. 43 (2011) 651–662.
[59] A. Ferrario, C.J. Gomer, Avastin enhances photodynamic therapy treatment of Kaposi's sarcoma in a mouse tumor model, J. Environ. Pathol. Toxicol. Oncol. 25 (2006) 251–259.
[60] R. Bhuvaneswari, G.Y. Yuen, S.K. Chee, M. Olivo, Hypericin-mediated photodynamic therapy in combination with Avastin (bevacizumab) improves tumor response by downregulating angiogenic proteins, Photochem. Photobiol. Sci. 6 (2007) 1275–1283.
[61] R. Bhuvaneswari, P.S. Thong, Y.Y. Gan, K.C. Soo, M. Olivo, Evaluation of hypericin-mediated photodynamic therapy in combination with angiogenesis inhibitor bevacizumab using *in vivo* fluorescence confocal endomicroscopy, J. Biomed. Opt. 15 (2010) 011114.
[62] F. Jiang, X. Zhang, S.N. Kalkanis, Z. Zhang, H. Yang, M. Katakowski, X. Hong, X. Zheng, Z. Zhu, M. Chopp, Combination therapy with antiangiogenic treatment and photodynamic therapy for the nude mouse bearing U87 glioblastoma, Photochem. Photobiol. 84 (2008) 128–137.
[63] M. Sagong, J. Lee, W. Chang, Application of intravitreal bevacizumab for circumscribed choroidal hemangioma, Korean J. Ophthalmol. 23 (2009) 127–131.
[64] F.D. Verbraak, R.O. Schlingemann, M.D. de Smet, J.E. Keunen, Single spot PDT in patients with circumscribed choroidal haemangioma and near normal visual acuity, Graefes Arch. Clin. Exp. Ophthalmol. 244 (2006) 1178–1182.
[65] T. Baba, M. Kitahashi, M. Kubota-Taniai, T. Oshitari, S. Yamamoto, Subretinal hemorrhage after photodynamic therapy for juxtapapillary retinal capillary hemangioma, Case Rep. Ophthalmol. 2 (2011) 134–139.
[66] F. Ziemssen, M. Voelker, W. Inhoffen, K.U. Bartz-Schmidt, F. Gelisken, Combined treatment of a juxtapapillary retinal capillary haemangioma with intravitreal bevacizumab and photodynamic therapy, Eye (Lond.) 21 (2007) 1125–1126.
[67] K.J. Kim, B. Li, K. Houck, J. Winer, N. Ferrara, The vascular endothelial growth factor proteins: identification of biologically relevant regions by neutralizing monoclonal antibodies, Growth Factors 7 (1992) 53–64.
[68] N. Ferrara, K.J. Hillan, H.P. Gerber, W. Novotny, Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer, Nat. Rev. Drug Discov. 3 (2004) 391–400.
[69] H.P. Gerber, X. Wu, L. Yu, C. Wiesmann, X.H. Liang, C.V. Lee, G. Fuh, C. Olsson, L. Damico, D. Xie, Y.G. Meng, J. Gutierrez, R. Corpuz, B. Li, L. Hall, L. Rangell, R. Ferrando, H. Lowman, F. Peale, N. Ferrara, Mice expressing a humanized form of VEGF-A may provide insights into the safety and efficacy of anti-VEGF antibodies, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 3478–3483.
[70] F. Bock, J. Onderka, T. Dietrich, B. Bachmann, F.E. Kruse, M. Paschke, G. Zahn, C. Cursiefen, Bevacizumab as a potent inhibitor of inflammatory corneal angiogenesis and lymphangiogenesis, Investig. Ophthalmol. Vis. Sci. 48 (2007) 2545–2552.
[71] L. Yu, X. Wu, Z. Cheng, C.V. Lee, J. LeCouter, C. Campa, G. Fuh, H. Lowman, N. Ferrara, Interaction between bevacizumab and murine VEGF-A: a reassessment, Investig. Ophthalmol. Vis. Sci. 49 (2008) 522–527.
[72] R.S. Herbst, D.M. Shin, Monoclonal antibodies to target epidermal growth factor receptor-positive tumors: a new paradigm for cancer therapy, Cancer 94 (2002) 1593–1611.
[73] M.G. del Carmen, I. Rizvi, Y. Chang, A.C. Moor, E. Oliva, M. Sherwood, B. Pogue, T. Hasan, Synergism of epidermal growth factor receptor-targeted immunotherapy with photodynamic treatment of ovarian cancer *in vivo*, J. Natl. Cancer Inst. 97 (2005) 1516–1524.
[74] E.S. Kim, F.R. Khuri, R.S. Herbst, Epidermal growth factor receptor biology (IMC-C225), Curr. Opin. Oncol. 13 (2001) 506–513.
[75] P.A. Martinez-Carpio, M.A. Trelles, The role of epidermal growth factor receptor in photodynamic therapy: a review of the literature and proposal for future investigation, Lasers Med. Sci. 25 (2010) 767–771.
[76] R. Bhuvaneswari, Y.Y. Gan, K.C. Soo, M. Olivo, Targeting EGFR with photodynamic therapy in combination with Erbitux enhances *in vivo* bladder tumor response, Mol. Cancer 8 (2009) 94.
[77] W.L. Yip, A. Weyergang, K. Berg, H.H. Tonnesen, P.K. Selbo, Targeted delivery and enhanced cytotoxicity of cetuximab-saporin by photochemical internalization in EGFR-positive cancer cells, Mol. Pharm. 4 (2007) 241–251.
[78] K. Berg, P.K. Selbo, L. Prasmickaite, T.E. Tjelle, K. Sandvig, J. Moan, G. Gaudernack, O. Fodstad, S. Kjolsrud, H. Anholt, G.H. Rodal, S.K. Rodal, A. Hogset, Photochemical internalization: a novel technology for delivery of macromolecules into cytosol, Cancer Res. 59 (1999) 1180–1183.
[79] R.A. Lustig, T.J. Vogl, D. Fromm, R. Cuenca, R. Alex Hsi, A.K. D'Cruz, Z. Krajina, M. Turic, A. Singhal, J.C. Chen, A multicenter Phase I safety study of intratumoral photoactivation of talaporfin sodium in patients with refractory solid tumors, Cancer 98 (2003) 1767–1771.
[80] M.J. Manyak, K. Ogan, Photodynamic therapy for refractory superficial bladder cancer: long-term clinical outcomes of single treatment using intravesical diffusion medium, J. Endourol. 17 (2003) 633–639.
[81] Y. Cao, Opinion: emerging mechanisms of tumour lymphangiogenesis and lymphatic metastasis, Nat. Rev. Cancer 5 (2005) 735–743.
[82] R.S. Herbst, D.H. Johnson, E. Mininberg, D.P. Carbone, T. Henderson, E.S. Kim, G. Blumenschein Jr., J.J. Lee, D.D. Liu, M.T. Truong, W.K. Hong, H. Tran, A. Tsao, D. Xie, D.A. Ramies, R. Mass, S. Seshagiri, D.A. Eberhard, S.K. Kelley, A. Sandler, Phase I/II trial evaluating the anti-vascular endothelial growth factor monoclonal antibody bevacizumab in combination with the HER-1/epidermal growth factor receptor tyrosine kinase inhibitor erlotinib for patients with recurrent non-small-cell lung cancer, J. Clin. Oncol. 23 (2005) 2544–2555.
[83] R. Iyer, G. Fetterly, A. Lugade, Y. Thanavala, Sorafenib: a clinical and pharmacologic review, Expert. Opin. Pharmacother. 11 (2010) 1943–1955.
[84] M. Van den Eynde, J.F. Baurain, F. Mazzeo, J.P. Machiels, Epidermal growth factor receptor targeted therapies for solid tumours, Acta Clin. Belg. 66 (2011) 10–17.
[85] T. Powles, S. Chowdhury, R. Jones, M. Mantle, P. Nathan, A. Bex, L. Lim, T. Hutson, Sunitinib and other targeted therapies for renal cell carcinoma, Br. J. Cancer 104 (2011) 741–745.
[86] C.J. Dimitroff, W. Klohs, A. Sharma, P. Pera, D. Driscoll, J. Veith, R. Steinkampf, M. Schroeder, S. Klutchko, A. Sumlin, B. Henderson, T.J. Dougherty, R.J. Bernacki, Anti-angiogenic activity of selected receptor tyrosine kinase inhibitors, PD166285 and PD173074: implications for combination treatment with photodynamic therapy, Invest. New Drugs 17 (1999) 121–135.
[87] Q. Zhou, M. Olivo, K.Y. Lye, S. Moore, A. Sharma, B. Chowbay, Enhancing the therapeutic responsiveness of photodynamic therapy with the antiangiogenic agents SU5416 and SU6668 in murine nasopharyngeal carcinoma models, Cancer Chemother. Pharmacol. 56 (2005) 569–577.
[88] D.B. Mendel, R.E. Schreck, D.C. West, G. Li, L.M. Strawn, S.S. Tanciongco, S. Vasile, L.K. Shawver, J.M. Cherrington, The angiogenesis inhibitor SU5416 has long-lasting effects on vascular endothelial growth factor receptor phosphorylation and function, Clin. Cancer Res. 6 (2000) 4848–4858.
[89] S. Curran, G.I. Murray, Matrix metalloproteinases in tumour invasion and metastasis, J. Pathol. 189 (1999) 300–308.
[90] W.G. Stetler-Stevenson, Matrix metalloproteinases in angiogenesis: a moving target for therapeutic intervention, J. Clin. Invest. 103 (1999) 1237–1241.
[91] H. Du, M. Olivo, R. Mahendran, B.H. Bay, Modulation of Matrix metalloproteinase-1 in nasopharyngeal cancer cells by photoactivation of hypericin, Int. J. Oncol. 24 (2004) 657–662.
[92] H.Y. Du, M. Olivo, R. Mahendran, Q. Huang, H.M. Shen, C.N. Ong, B.H. Bay, Hypericin photoactivation triggers down-regulation of matrix metalloproteinase-9 expression in well-differentiated human nasopharyngeal cancer cells, Cell. Mol. Life Sci. 64 (2007) 979–988.
[93] S.P. Tabruyn, S. Memet, P. Ave, C. Verhaeghe, K.H. Mayo, I. Struman, J.A. Martial, A.W. Griffioen, NF-kappaB activation in endothelial cells is critical for the activity of angiostatic agents, Mol. Cancer Ther. 8 (2009) 2645–2654.
[94] S.P. Tabruyn, A.W. Griffioen, NF-kappa B: a new player in angiostatic therapy, Angiogenesis 11 (2008) 101–106.
[95] C.M. Au, S.K. Luk, C.J. Jackson, H.K. Ng, C.M. Yow, S.S. To, Differential effects of photofrin, 5-aminolevulinic acid and calphostin C on glioma cells, J. Photochem. Photobiol. B 85 (2006) 92–101.
[96] A. Sharwani, W. Jerjes, C. Hopper, M.P. Lewis, M. El-Maaytah, H.S. Khalil, A.J. Macrobert, T. Upile, V. Salih, Photodynamic therapy down-regulates the invasion promoting factors in human oral cancer, Arch. Oral Biol. 51 (2006) 1104–1111.
[97] E.S. Chu, T.K. Wong, C.M. Yow, Photodynamic effect in medulloblastoma: down-regulation of matrix metalloproteinases and human telomerase reverse transcriptase expressions, Photochem. Photobiol. Sci. 7 (2008) 76–83.

[98] A. Ferrario, C.F. Chantrain, K. von Tiehl, S. Buckley, N. Rucker, D.R. Shalinsky, H. Shimada, Y.A. DeClerck, C.J. Gomer, The matrix metalloproteinase inhibitor prinomastat enhances photodynamic therapy responsiveness in a mouse tumor model, Cancer Res. 64 (2004) 2328–2332.
[99] S. Gately, The contributions of cyclooxygenase-2 to tumor angiogenesis, Cancer Metastasis Rev. 19 (2000) 19–27.
[100] S. Gately, W.W. Li, Multiple roles of COX-2 in tumor angiogenesis: a target for antiangiogenic therapy, Semin. Oncol. 31 (2004) 2–11.
[101] M.T. Wang, K.V. Honn, D. Nie, Cyclooxygenases, prostanoids, and tumor progression, Cancer Metastasis Rev. 26 (2007) 525–534.
[102] M. Romano, J. Claria, Cyclooxygenase-2 and 5-lipoxygenase converging functions on cell proliferation and tumor angiogenesis: implications for cancer therapy, FASEB J. 17 (2003) 1986–1995.
[103] T. Kinoshita, Y. Takahashi, T. Sakashita, H. Inoue, T. Tanabe, T. Yoshimoto, Growth stimulation and induction of epidermal growth factor receptor by overexpression of cyclooxygenases 1 and 2 in human colon carcinoma cells, Biochim. Biophys. Acta 1438 (1999) 120–130.
[104] B. Singh, J.A. Berry, A. Shoher, V. Ramakrishnan, A. Lucci, COX-2 overexpression increases motility and invasion of breast cancer cells, Int. J. Oncol. 26 (2005) 1393–1399.
[105] D.K. Petkova, C. Clelland, J. Ronan, L. Pang, J.M. Coulson, S. Lewis, A.J. Knox, Overexpression of cyclooxygenase-2 in non-small cell lung cancer, Respir. Med. 98 (2004) 164–172.
[106] Y. Cao, S.M. Prescott, Many actions of cyclooxygenase-2 in cellular dynamics and in cancer, J. Cell. Physiol. 190 (2002) 279–286.
[107] A. Ferrario, K. Von Tiehl, S. Wong, M. Luna, C.J. Gomer, Cyclooxygenase-2 inhibitor treatment enhances photodynamic therapy-mediated tumor response, Cancer Res. 62 (2002) 3956–3961.
[108] E.H. Harvey, J. Webber, D. Kessel, D. Fromm, Killing tumor cells: the effect of photodynamic therapy using mono-L-aspartyl chlorine and NS-398, Am. J. Surg. 189 (2005) 302–305.
[109] M. Makowski, T. Grzela, J. Niderla, M. L.A., P. Mroz, M. Kopee, M. Legat, K. Strusinska, K. Koziak, D. Nowis, P. Mrowka, M. Wasik, M. Jakobisiak, J. Golab, Inhibition of cyclooxygenase-2 indirectly potentiates antitumor effects of photodynamic therapy in mice, Clin. Cancer Res. 9 (2003) 5417–5422.
[110] A. Ferrario, A.M. Fisher, N. Rucker, C.J. Gomer, Celecoxib and NS-398 enhance photodynamic therapy by increasing *in vitro* apoptosis and decreasing *in vivo* inflammatory and angiogenic factors, Cancer Res. 65 (2005) 9473–9478.
[111] K.K. Yee, K.C. Soo, M. Olivo, Anti-angiogenic effects of Hypericin-photodynamic therapy in combination with Celebrex in the treatment of human nasopharyngeal carcinoma, Int. J. Mol. Med. 16 (2005) 993–1002.
[112] Y. Akita, K. Kozaki, A. Nakagawa, T. Saito, S. Ito, Y. Tamada, S. Fujiwara, N. Nishikawa, K. Uchida, K. Yoshikawa, T. Noguchi, O. Miyaishi, K. Shimozato, S. Saga, Y. Matsumoto, Cyclooxygenase-2 is a possible target of treatment approach in conjunction with photodynamic therapy for various disorders in skin and oral cavity, Br. J. Dermatol. 151 (2004) 472–480.
[113] N. Hendrickx, M. Dewaele, E. Buytaert, G. Marsboom, S. Janssens, M. Van Boven, J.R. Vandenheede, P. de Witte, P. Agostinis, Targeted inhibition of p38alpha MAPK suppresses tumor-associated endothelial cell migration in response to hypericin-based photodynamic therapy, Biochem. Biophys. Res. Commun. 337 (2005) 928–935.
[114] N. Hendrickx, C. Volanti, U. Moens, O.M. Seternes, P. de Witte, J.R. Vandenheede, J. Piette, P. Agostinis, Up-regulation of cyclooxygenase-2 and apoptosis resistance by p38 MAPK in hypericin-mediated photodynamic therapy of human cancer cells, J. Biol. Chem. 278 (2003) 52231–52239.
[115] D. Ingber, T. Fujita, S. Kishimoto, K. Sudo, T. Kanamaru, H. Brem, J. Folkman, Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth, Nature 348 (1990) 555–557.
[116] Y. Hama, T. Shimizu, S. Hosaka, A. Sugenoya, N. Usuda, Therapeutic efficacy of the angiogenesis inhibitor O-(chloroacetyl-carbamoyl) fumagillol (TNP-470; AGM-1470) for human anaplastic thyroid carcinoma in nude mice, Exp. Toxicol. Pathol. 49 (1997) 239–247.
[117] J.R. Yeh, R. Mohan, C.M. Crews, The antiangiogenic agent TNP-470 requires p53 and p21CIP/WAF for endothelial cell growth arrest, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 12782–12787.
[118] N. Solban, P.K. Selbo, A.K. Sinha, S.K. Chang, T. Hasan, Mechanistic investigation and implications of photodynamic therapy induction of vascular endothelial growth factor in prostate cancer, Cancer Res. 66 (2006) 5633–5640.
[119] B. Kosharskyy, N. Solban, S.K. Chang, I. Rizvi, Y. Chang, T. Hasan, A mechanism-based combination therapy reduces local tumor growth and metastasis in an orthotopic model of prostate cancer, Cancer Res. 66 (2006) 10953–10958.
[120] M.W. Kieran, C.D. Turner, J.B. Rubin, S.N. Chi, M.A. Zimmerman, C. Chordas, G. Klement, A. Laforme, A. Gordon, A. Thomas, D. Neuberg, T. Browder, J. Folkman, A feasibility trial of antiangiogenic (metronomic) chemotherapy in pediatric patients with recurrent or progressive cancer, J. Pediatr. Hematol. Oncol. 27 (2005) 573–581.
[121] S.H. Lim, P. Nowak-Sliwinska, F.A. Kamarulzaman, H. van den Bergh, G. Wagnieres, H.B. Lee, The neovessel occlusion efficacy of 15-hydroxypurpurin-7-lactone dimethyl ester induced with photodynamic therapy, Photochem. Photobiol. 86 (2010) 397–402.
[122] S.H. Lim, C. Thivierge, P. Nowak-Sliwinska, J. Han, H. van den Bergh, G. Wagnieres, K. Burgess, H.B. Lee, *In vitro* and *in vivo* photocytotoxicity of boron dipyrromethene derivatives for photodynamic therapy, J. Med. Chem. 53 (2010) 2865–2874.
[123] P. Nowak-Sliwinska, J.R. van Beijnum, A. Casini, A.A. Nazarov, G. Wagnieres, H. van den Bergh, P.J. Dyson, A.W. Griffioen, Organometallic ruthenium(II) arene compounds with antiangiogenic activity, J. Med. Chem. 54 (2011) 3895–3902.
[124] M.F. Zuluaga, N. Lange, Combination of photodynamic therapy with anti-cancer agents, Curr. Med. Chem. 15 (2008) 1655–1673.
[125] R.P. Dings, M. Loren, H. Heun, E. McNiel, A.W. Griffioen, K.H. Mayo, R.J. Griffin, Scheduling of radiation with angiogenesis inhibitors anginex and Avastin improves therapeutic outcome via vessel normalization, Clin. Cancer Res. 13 (2007) 3395–3402.
[126] R. Murata, Y. Nishimura, M. Hiraoka, An antiangiogenic agent (TNP-470) inhibited reoxygenation during fractionated radiotherapy of murine mammary carcinoma, Int. J. Radiat. Oncol. Biol. Phys. 37 (1997) 1107–1113.
[127] J. Ma, H.Q. Mai, M.H. Hong, H.Q. Min, Z.D. Mao, N.J. Cui, T.X. Lu, H.Y. Mo, Results of a prospective randomized trial comparing neoadjuvant chemotherapy plus radiotherapy with radiotherapy alone in patients with locoregionally advanced nasopharyngeal carcinoma, J. Clin. Oncol. 19 (2001) 1350–1357.
[128] Y. Kakeji, B.A. Teicher, Preclinical studies of the combination of angiogenic inhibitors with cytotoxic agents, Invest. New Drugs 15 (1997) 39–48.
[129] B.C. Baguley, Antivascular therapy of cancer: DMXAA, Lancet Oncol. 4 (2003) 141–148.
[130] S.M. Galbraith, R.J. Maxwell, M.A. Lodge, G.M. Tozer, J. Wilson, N.J. Taylor, J.J. Stirling, L. Sena, A.R. Padhani, G.J. Rustin, Combretastatin A4 phosphate has tumor antivascular activity in rat and man as demonstrated by dynamic magnetic resonance imaging, J. Clin. Oncol. 21 (2003) 2831–2842.
[131] M.J. McKeage, B.C. Baguley, Disrupting established tumor blood vessels: an emerging therapeutic strategy for cancer, Cancer 116 (2010) 1859–1871.
[132] P. Lambin, W. Landuyt, Vascular targeting: a potential additional anti-cancer treatment, Verh. K. Acad. Geneeskd. Belg. 65 (2003) 29–46.
[133] D.W. Siemann, W. Shi, Dual targeting of tumor vasculature: combining Avastin and vascular disrupting agents (CA4P or OXi4503), Anticancer. Res. 28 (2008) 2027–2031.
[134] B.G. Siim, A.E. Lee, S. Shalal-Zwain, F.B. Pruijn, M.J. McKeage, W.R. Wilson, Marked potentiation of the antitumour activity of chemotherapeutic drugs by the antivascular agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA), Cancer Chemother. Pharmacol. 51 (2003) 43–52.
[135] P.N. Lara Jr., J.Y. Douillard, K. Nakagawa, J. von Pawel, M.J. McKeage, I. Albert, G. Losonczy, M. Reck, D.S. Heo, X. Fan, A. Fandi, G. Scagliotti, Randomized phase III placebo-controlled trial of carboplatin and paclitaxel with or without the vascular disrupting agent vadimezan (ASA404) in advanced non-small-cell lung cancer, J. Clin. Oncol. 29 (2011) 2965–2971.
[136] R. Murata, D.W. Siemann, J. Overgaard, M.R. Horsman, Improved tumor response by combining radiation and the vascular-damaging drug 5,6-dimethylxanthenone-4-acetic acid, Radiat. Res. 156 (2001) 503–509.
[137] M. Seshadri, D.A. Bellnier, The vascular disrupting agent 5,6-dimethylxanthenone-4-acetic acid improves the antitumor efficacy and shortens treatment time associated with Photochlor-sensitized photodynamic therapy *in vivo*, Photochem. Photobiol. 85 (2009) 50–56.
[138] C. He, B. Fateya, B. Chen, Combination of vascular targeting PDT with combretastatin A4 phosphate, Proc. SPIE 7380 (2009) 7380321–7380326.
[139] B. Fateye, B. Chen, combination of PI3K/Akt/mTOR inhibitors and PDT in endothelial and tumor cells, Proc. SPIE 7886 (2009) 78860B78861-78816.
[140] A. Marrero, T. Becker, U. Sunar, J. Morgan, D. Bellnier, Aminolevulinic acid-photodynamic therapy combined with topically applied vascular disrupting agent vadimezan leads to enhanced antitumor responses, Photochem. Photobiol. 87 (2011) 910–919.
[141] B. Ahmed, L.I. Van Eijk, J.C. Bouma-Ter Steege, D.W. Van Der Schaft, A.M. Van Esch, S.R. Joosten-Achjanie, P. Lambin, W. Landuyt, A.W. Griffioen, Vascular targeting effect of combretastatin A-4 phosphate dominates the inherent angiogenesis inhibitory activity, Int. J. Cancer 105 (2003) 20–25.
[142] J.R. van Beijnum, R.P. Dings, E. van der Linden, B.M. Zwaans, F.C. Ramaekers, K.H. Mayo, A.W. Griffioen, Gene expression of tumor angiogenesis dissected: specific targeting of colon cancer angiogenic vasculature, Blood 108 (2006) 2339–2348.
[143] B. St Croix, C. Rago, V. Velculescu, G. Traverso, K.E. Romans, E. Montgomery, A. Lal, G.J. Riggins, C. Lengauer, B. Vogelstein, K.W. Kinzler, Genes expressed in human tumor endothelium, Science (New York, N.Y.) 289 (2000) 1197–1202.
[144] S. Folli, P. Westermann, D. Braichotte, A. Pelegrin, G. Wagnieres, H. van den Bergh, J.P. Mach, Antibody-indocyanin conjugates for immunophotodetection of human squamous cell carcinoma in nude mice, Cancer Res. 54 (1994) 2643–2649.
[145] M.L. Yarmush, W.P. Thorpe, L. Strong, S.L. Rakestraw, M. Toner, R.G. Tompkins, Antibody Targeted Photolysis, Crit. Rev. Ther. Drug Carrier Syst. 10 (1993) 197–252.
[146] M. Santimaria, G. Moscatelli, G.L. Viale, L. Giovannoni, G. Neri, F. Viti, A. Leprini, L. Borsi, P. Castellani, L. Zardi, D. Neri, P. Riva, Immunoscintigraphic detection of the ED-B domain of fibronectin, a marker of angiogenesis, in patients with cancer, Clin. Cancer Res. 9 (2003) 571–579.
[147] M. Birchler, F. Viti, L. Zardi, B. Spiess, D. Neri, Selective targeting and photocoagulation of ocular angiogenesis mediated by a phage-derived human antibody fragment, Nat. Biotechnol. 17 (1999) 984–988.
[148] A. Palumbo, F. Hauler, P. Dziunycz, K. Schwager, A. Soltermann, F. Pretto, C. Alonso, G.F. Hofbauer, R.W. Boyle, D. Neri, A chemically modified antibody mediates complete eradication of tumours by selective disruption of tumour blood vessels, Br. J. Cancer 104 (2011) 1106–1115.
[149] M. Fabbrini, E. Trachsel, P. Soldani, S. Bindi, P. Alessi, L. Bracci, H. Kosmehl, L. Zardi, D. Neri, P. Neri, Selective occlusion of tumor blood vessels by targeted delivery of an antibody-photosensitizer conjugate, Int. J. Cancer 118 (2006) 1805–1813.

[150] A.W. Griffioen, G. Molema, Angiogenesis: potentials for pharmacologic intervention in the treatment of cancer, cardiovascular diseases, and chronic inflammation, Pharmacol. Rev. 52 (2000) 237–268.
[151] D.A. Cheresh, Structure, function and biological properties of integrin alpha v beta 3 on human melanoma cells, Cancer Metastasis Rev. 10 (1991) 3–10.
[152] P.C. Brooks, R.A. Clark, D.A. Cheresh, Requirement of vascular integrin alpha v beta 3 for angiogenesis, Science (New York, N.Y.) 264 (1994) 569–571.
[153] E. Garanger, D. Boturyn, P. Dumy, Tumor targeting with RGD peptide ligands-design of new molecular conjugates for imaging and therapy of cancers, Anticancer Agents Med. Chem. 7 (2007) 552–558.
[154] J.X. Chen, H.Y. Wang, C. Li, K. Han, X.Z. Zhang, R.X. Zhuo, Construction of surfactant-like tetra-tail amphiphilic peptide with RGD ligand for encapsulation of porphyrin for photodynamic therapy, Biomaterials 32 (2011) 1678–1684.
[155] M.J. Goldman, J.M. Wilson, Expression of alpha v beta 5 integrin is necessary for efficient adenovirus-mediated gene transfer in the human airway, J. Virol. 69 (1995) 5951–5958.
[156] C.M. Allen, W.M. Sharman, C. La Madeleine, J.M. Weber, R. Langlois, R. Ouellet, J.E. van Lier, Photodynamic therapy: tumor targeting with adenoviral proteins, Photochem. Photobiol. 70 (1999) 512–523.
[157] T. Asai, K. Shimizu, M. Kondo, K. Kuromi, K. Watanabe, K. Ogino, T. Taki, S. Shuto, A. Matsuda, N. Oku, Anti-neovascular therapy by liposomal DPP-CNDAC targeted to angiogenic vessels, FEBS Lett. 520 (2002) 167–170.
[158] K. Ichikawa, T. Hikita, N. Maeda, S. Yonezawa, Y. Takeuchi, T. Asai, Y. Namba, N. Oku, Antiangiogenic photodynamic therapy (PDT) by using long-circulating liposomes modified with peptide specific to angiogenic vessels, Biochim. Biophys. Acta 1669 (2005) 69–74.
[159] N. Oku, T. Ishii, Antiangiogenic photodynamic therapy with targeted liposomes, Methods Enzymol. 465 (2009) 313–330.
[160] R. Kerbel, J. Folkman, Clinical translation of angiogenesis inhibitors, Nat. Rev. Cancer 2 (2002) 727–739.
[161] K.K. Frese, D.A. Tuveson, Maximizing mouse cancer models, Nat. Rev. Cancer 7 (2007) 645–658.
[162] E. Raymond, L. Dahan, J.L. Raoul, Y.J. Bang, I. Borbath, C. Lombard-Bohas, J. Valle, P. Metrakos, D. Smith, A. Vinik, J.S. Chen, D. Horsch, P. Hammel, B. Wiedenmann, E. Van Cutsem, S. Patyna, D.R. Lu, C. Blanckmeister, R. Chao, P. Ruszniewski, Sunitinib malate for the treatment of pancreatic neuroendocrine tumors, N. Engl. J. Med. 364 (2011) 501–513.
[163] J.C. Yao, M.H. Shah, T. Ito, C.L. Bohas, E.M. Wolin, E. Van Cutsem, T.J. Hobday, T. Okusaka, J. Capdevila, E.G. de Vries, P. Tomassetti, M.E. Pavel, S. Hoosen, T. Haas, J. Lincy, D. Lebwohl, K. Oberg, Everolimus for advanced pancreatic neuroendocrine tumors, N. Engl. J. Med. 364 (2011) 514–523.
[164] M. Paez-Ribes, E. Allen, J. Hudock, T. Takeda, H. Okuyama, F. Vinals, M. Inoue, G. Bergers, D. Hanahan, O. Casanovas, Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis, Cancer Cell 15 (2009) 220–231.
[165] D. Hanahan, Heritable formation of pancreatic beta-cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes, Nature 315 (1985) 115–122.
[166] D. Tuveson, D. Hanahan, Translational medicine: cancer lessons from mice to humans, Nature 471 (2011) 316–317.
[167] J.M. Ebos, C.R. Lee, W. Cruz-Munoz, G.A. Bjarnason, J.G. Christensen, R.S. Kerbel, Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis, Cancer Cell 15 (2009) 232–239.
[168] K.J. Gotink, H.J. Broxterman, M. Labots, R.R. de Haas, H. Dekker, R.J. Honeywell, M.A. Rudek, L.V. Beerepoot, R.J. Musters, G. Jansen, A.W. Griffioen, Y.G. Assaraf, R. Pili, G.J. Peters, H.M. Verheul, Lysosomal sequestration of sunitinib: a novel mechanism of drug resistance, Clin. Cancer Res. 17 (2011) 7337–7346.
[169] A.W. Griffioen, L. Mans, A.M. de Graaf, P. Nowak-Sliwinska, S. Bosch, C. de Hoog, T. Dellemijn, F. Vyth-Dreese, A. Bex, E. Jonasch, Preoperative sunitinib treatment of RCC patients inhibits angiogenesis in the primary tumor; rapid angiogenesis onset after discontinuation of treatment, Clin. Cancer Res., in press.
[170] E. Kabingu, L. Vaughan, B. Owczarczak, K.D. Ramsey, S.O. Gollnick, CD8 + T cell-mediated control of distant tumours following local photodynamic therapy is independent of CD4 + T cells and dependent on natural killer cells, Br. J. Cancer 96 (2007) 1839–1848.
[171] P.S. Thong, K.W. Ong, N.S. Goh, K.W. Kho, V. Manivasager, R. Bhuvaneswari, M. Olivo, K.C. Soo, Photodynamic-therapy-activated immune response against distant untreated tumours in recurrent angiosarcoma, Lancet Oncol. 8 (2007) 950–952.
[172] P.S. Thong, M. Olivo, K.W. Kho, R. Bhuvaneswari, W.W. Chin, K.W. Ong, K.C. Soo, Immune response against angiosarcoma following lower fluence rate clinical photodynamic therapy, J. Environ. Pathol. Toxicol. Oncol. 27 (2008) 35–42.
[173] S.O. Gollnick, C.M. Brackett, Enhancement of anti-tumor immunity by photodynamic therapy, Immunol. Res. 46 (2010) 216–226.
[174] A.P. Castano, P. Mroz, M.R. Hamblin, Photodynamic therapy and anti-tumour immunity, Nat. Rev. Cancer 6 (2006) 535–545.
[175] E. Kabingu, A.R. Oseroff, G.E. Wilding, S.O. Gollnick, Enhanced systemic immune reactivity to a Basal cell carcinoma associated antigen following photodynamic therapy, Clin. Cancer Res. 15 (2009) 4460–4466.
[176] F. Li, Y. Cheng, J. Lu, R. Hu, Q. Wan, H. Feng, Photodynamic therapy boosts anti-glioma immunity in mice: a dependence on the activities of T cells and complement C3, J. Cell. Biochem. 112 (2011) 3035–3043.
[177] C. Framme, H.G. Sachs, B. Flucke, D. Theisen-Kunde, R. Birngruber, Evaluation of the new photosensitizer Tookad (WST09) for photodynamic vessel occlusion of the choroidal tissue in rabbits, Invest. Ophthalmol. Vis. Sci. 47 (2006) 5437–5446.
[178] C.M. Moore, C.A. Mosse, C. Allen, H. Payne, M. Emberton, S.G. Bown, Light penetration in the human prostate: a whole prostate clinical study at 763 nm, J. Biomed. Opt. 16 (2011) 015003.
[179] J.R. van Beijnum, P. Nowak-Sliwinska, E. van den Boezem, P. Hautvast, W. Buurman, A. Griffioen, Tumor angiogenesis is enforced by autocrine regulation of high-mobility group box 1, Oncogene, in press, PMID:22391561.
[180] E. Kluza, D.W. van der Schaft, P.A. Hautvast, W.J. Mulder, K.H. Mayo, A.W. Griffioen, G.J. Strijkers, K. Nicolay, Synergistic targeting of alphavbeta3 integrin and galectin-1 with heteromultivalent paramagnetic liposomes for combined MR imaging and treatment of angiogenesis, Nano Lett. 10 (2010) 52–58.
[181] V.L. Thijssen, R. Postel, R.J. Brandwijk, R.P. Dings, I. Nesmelova, S. Satijn, N. Verhofstad, Y. Nakabeppu, L.G. Baum, J. Bakkers, K.H. Mayo, F. Poirier, A.W. Griffioen, Galectin-1 is essential in tumor angiogenesis and is a target for antiangiogenesis therapy, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 15975–15980.
[182] A.W. Griffioen, D.W. van der Schaft, A.F. Barendsz-Janson, A. Cox, H.A. Struijker Boudier, H.F. Hillen, K.H. Mayo, Anginex, a designed peptide that inhibits angiogenesis, Biochem. J. 354 (2001) 233–242.