Antivascular Therapies: Targets beyond the vessel wall

*Kai Temming*¹,², *Robbert J. Kok*¹,³

¹ Department of Pharmacokinetics & Drug Delivery, Groningen University Institute of Drug Exploration, The Netherlands
² KREATECH Biotechnology B.V., Amsterdam, The Netherlands
³ Present affiliation: Department of Pharmaceutics, Utrecht University, The Netherlands

Abstract

Unraveling the involvement of multiple cell types in angiogenesis yields more and more cells and pathways as druggable targets. It was recently demonstrated that inhibition of the recruitment of circulating endothelial progenitor cell (CEP) improved therapeutic outcome of treatment with vascular disrupting agents (VDA). This knowledge paves the way for novel drugs and drug combinations in cancer therapy.
Introduction

Tumor angiogenesis has evolved into a widely studied field that has yielded several interesting drugs. The combination of these agents with conventional cytostatic compounds will greatly improve clinical responses, and many more anti-angiogenic compounds are under development. In parallel to new and more potent agents, the advanced insight in angiogenesis at the cellular level dictates new paradigms in drug combinations and treatment protocols. This highlight will shortly review the current status of anti-angiogenic compounds that have advanced into the clinic or into later stages of clinical testing. Second, we will discuss a recent paper by Shaked et al [1] that illustrates the important role of circulating endothelial progenitor cells (CEPs) in tumor angiogenesis and furthermore demonstrates how blockade of CEP recruitment can improve anti-vascular therapy.

Current antiangiogenic therapies

Anti-angiogenic therapies either aim for the disruption of tumor blood vessels or counteract the further outgrowth of capillaries from existing vasculature. Depending on the claimed target or mechanism of action of the applied therapeutic, compounds that have advanced into clinical testing can be divided into three different classes:

1. **Vascular disrupting agents.** Destruction of tumor endothelial cells is probably the most straightforward antivascular treatment. The destruction of the vessel wall induces hemorrhage and coagulation, resulting in occlusion of tumor blood vessels. As a consequence, all tumor cells that were fed by the occluded blood vessel are deprived of oxygen and nutrients and eventually perish. Most vascular disrupting agents (VDA) interfere with the tubulin cytoskeleton in tumor endothelium, leading to rapid changes in endothelial shape and endothelial cell death [2]. Since the cytoskeleton of non-angiogenic endothelial cells is maintained by actin rather than by tubulin, mature blood vessels are not sensitive to anti-tubulin agents. A number of VDAs have been evaluated in clinical trials with TZT1027, a dolostatin derivative, and OXi4503, a combretastatin prodrug, being the most promising compounds [2] (Figure 1).

![Figure 1. The chemical structures of two promising vascular disrupting agents (OXi4503 and TZT1027)](image)

2. **VEGF neutralizing or blocking agents.** Vascular endothelial growth factor (VEGF) is one of the most prominent proangiogenic modulators and has been the target of many anti-angiogenic strategies. Of those, the antibody Avastin directed against soluble VEGF has been approved for the treatment of metastatic colorectal cancer. Other VEGF-capturing biologics like e.g. soluble VEGF-receptor (VEGF-trap) are under clinical investigation [3, 4]. DC101, a rodent antibody...
Chapter 8

raised against VEGFR-2 that blocks binding of VEGF to its receptor, demonstrated remarkable effects in the preclinical setting and a humanized and pegylated fragment of this antibody (CDP-791) is currently in phase II clinical trials [5]. Thus far, only biologics have entered clinical trials and small molecule antagonists of VEGF receptor binding (e.g. VGA1155, GFA-116) are still in preclinical stages [6, 7] (Figure 2).

Figure 2. Two small molecule VEGF receptor antagonist (VGA1155, GFA-116) are shown. GFA-116 is composed of a central calix[4]arene scaffold (left) to which four cyclic peptides with a GKGK sequence are attached (right).

3. VEGF receptor kinase inhibitors. Of the three identified VEGF receptors, VEGFR-2 (KDR) induces the most prominent proangiogenic stimuli upon binding of soluble VEGF-A. The VEGFR-2 kinase is a strong activator of the c-Raf-MEK-MAP-kinase pathway, PI3-kinase, and focal adhesion kinase, among others [8]. Two of the signal transduction inhibitors aiming at VEGFR signaling, sorafenib (BAY 43-9006) and sunitinib (SU11248), have been clinically approved and others like e.g. vatalanib (PTK787) are in late stage of clinical investigation [9] (Figure 3). Most signal transduction inhibitors block the activity of kinases by occupation of the ATP pocket. Owing to the relative similarity of ATP pockets on tyrosine kinases, these VEGF kinase inhibitors often display inhibitory activity on several other kinases like e.g., PDGFR-β (Sunitinib, Vatalanib), c-Kit (Sunitinib, Vatalanib) or Raf (Sorafenib). Although a common drug design strategy is to design an inhibitor with high target specificity, i.e., aiming for a specific kinase, multikinase inhibition may be advantageous since it provides a more complete blockade of activation pathways.
Figure 3. Signal transduction inhibitors that block signaling via the VEGF receptor kinase (SU11248, PTK787, BAY 43-9006)

Obviously, the above listing of compounds is only a global summary of anti-angiogenic compounds. Many other compounds have been studied for their anti-angiogenic properties, and many cytostatics exert part of their therapeutic activity via anti-angiogenic mechanisms [10].

The success of anti-angiogenic therapies illustrates that clinical anti-tumor responses can be achieved by targeting non-tumor cells. Also other non-malignant cells within the tumor microenvironment (e.g. dendritic cells, tumor associated macrophages) have been recognized as key players, as they are involved in different stages of tumor development and progression [11, 12]. Likewise, such tumor-associated cells are potential druggable targets. A recent paper illustrates that we should consider cells beyond the tumor microenvironment as potential targets for cancer therapy since also circulating progenitor cells contribute to tumor growth [1]. Although this concept is not new, the role of circulating progenitor cells in angiogenesis remained controversial since only low levels of such cells were detectable in tumors [13]. Shaked et al demonstrated that levels of CEPs were rapidly elevated upon treatment with the anti-vascular agent OXi4503, and that these cells contributed to the rapid outgrowth of remaining tumor cells at the rim of the tumor. Since tumor cells at the border of a tumor obtain nutrients and oxygen from normal tissue, those cells are not eradicated when tumor blood vessels are obstructed. The accelerated outgrowth of this so-called ‘tumor rim’ after cessation of the treatment with anti-vascular therapy opposes complete remission of the tumor burden. Understandably, progression of many solid tumors relies on the formation of new blood vessels, and it was shown that mobilization of CEPs from the bone marrow and their incorporation into the newly formed tumor blood vessels can promote this [13]. To confirm that CEPs homed to the tumor rim, lethally irradiated mice were rescued by transplantation of green fluorescent protein-positive (GFP) bone marrow cells. Such mice were used as recipients of a syngenic Lewis Lung carcinoma and treated with OXi4503. Untreated mice showed only minor incorporation of GFP+ bone marrow cells into the tumor periphery, while animals treated with the vascular damaging agent showed a substantial number of GFP+ cells colocalizing with CD31 staining for tumor blood vessels. Further experiments with Id-1+/−Id-3− mutant mice that are incapable of mobilizing CEPs confirmed that CEPs contributed significantly to the regrowth of the tumor after VDA treatment.
In order to improve VDA therapy the authors combined OXi4503 with the VEGFR blocking antibody DC101. Using the mice with GFP+ bone marrow cells, the addition of the VEGF-blocking agent prevented the mobilization of progenitor cells into the tumor periphery. Most importantly, the combination therapy improved the tumor growth inhibitory effect and slowed recurrence of the tumor, in parallel with a reduced tumor rim. The combination of standard chemotherapy with VDA comprises also interesting possibilities. The logic sequence of treatments would be to start with a chemotherapy and thereafter occlude the blood vessel with VDA therapy, thereby trapping the cytotoxic compound in the tumor. However, the chemotherapeutic needs to be administered subsequent to VDA treatment as well, in order to prevent the mobilization of CEPs and their recruitment to the tumor blood vessels.

Awareness of how tumors progressively recruit non-malignant cells is of utmost importance in order to develop innovative cancer treatments. Endothelial cells have been recognized as a non-malignant target in cancer and successful treatments have followed. Further understanding of the interaction between different non-malignant cells types will yield new targets, as for example CEPs. Some of these new targets will require novel drugs but, as shown by Shaked et al, the recruitment of CEPs can be prevented by rational combination of existing anti-angiogenic agents.

Acknowledgements

We thank Prof. Dr. Grietje Molema for critical reviewing of the manuscript.

Reference List