Combination of Anti-angiogenics and Other Targeted Therapies

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Contents

Abstract

Angiogenesis is a hallmark of tumor development and metastasis and is now a validated target for cancer treatment. However, the overall benefits of anti-angiogenic drugs from the perspective of impacting survival have left much to desire, endorsing a need for developing more effective therapeutic regimens, e.g., combining anti-angiogenic drugs with established chemotherapeutic drugs. In this review, we discuss progress in the synergistic design of

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anti-angiogenic agents in combination with targeted therapies. Targeted cancer therapies include monoclonal antibodies and small-molecule inhibitors that have significantly changed the treatment of cancer over the past years. We focus on anti-angiogenic agents combined with targeted therapies inhibiting the epidermal growth factor receptor (EGFR) pathway and the PI3K (phosphoinositide 3-kinase)/AKT (protein kinase B)/mTOR (mammalian target of rapamycin) pathway and inhibiting immune checkpoint receptors, such as CTLA-4 (cytotoxic T lymphocyte-associated antigen 4) and PD1/PDL1 (programmed cell death protein 1/PD1 ligand). Of note, not always, encouraging preclinical data particularly of VEGF and EGFR inhibitor combinations did translate into the clinics. In addition, we highlight the rapidly developing field of VEGF-based humanized tri-specific nanobodies and novel VEGFR2-targeted antibody-based fusion proteins, potentially providing a new inspiration for antitumor treatment.

Keywords
Angiogenesis · VEGF · Angiogenesis inhibitor · Monoclonal antibodies · Bevacizumab · Cetuximab · Panitumumab · Targeted therapy · Preclinical studies · Clinical trials · Cancer · Tyrosine kinase inhibitors

Introduction

Angiogenesis, the process leading to the formation of new blood vessels, plays a central role in the survival of cancer cells, in local tumor growth, and in the development of distant metastases (Folkman 1971). Therefore, anti-angiogenic treatment in tumors is a highly promising therapeutic approach. The increasing understanding of the biological mechanisms of tumor-induced angiogenesis has stimulated the development of agents able to interfere with the molecules involved in this process (Folkman 1995). Two main approaches have been proposed for blocking vascular endothelial growth factor (VEGF)-induced endothelial cell proliferation and subsequent tumor angiogenesis:

- Monoclonal antibodies directed against specific proangiogenic growth factors and/or their receptors.
- Small molecule tyrosine kinase inhibitors (TKIs) of multiple proangiogenic growth factor receptors. Of note, anti-angiogenic TKIs often inhibit multiple tyrosine kinases because of the structural similarities between VEGFR and other receptor tyrosine kinases, thus often providing tumor growth inhibition by several independent mechanisms.

Beside these, a plethora of agents are proposed to indirectly inhibit angiogenesis through mechanisms not completely understood. These include bortezomib and thalidomide.

However, a given tumor is unlikely to be dependent on only one receptor or signaling pathway for its growth and survival. This is due to the significant level of compensatory cross talk among receptors within a signaling network as well as heterologous receptor systems. Therefore, the survival benefits of anti-angiogenic drugs have, thus far, been rather modest and, subsequently, combining drugs inhibiting different signaling pathways is currently an important strategy to achieve synergy or overcome resistance.

Synergy Between Anti-angiogenic Therapies and EGFR Inhibition

The synergy between the VEGF and epidermal growth factor receptor (EGFR) pathways lies in their close relationship and sharing common downstream signaling pathways as well as their extensive cross talk (Herbst et al. 2005). Activation of EGFR signaling in tumor cells stimulates the production of angiogenic factors such as VEGF, causing endothelial cells to proliferate and migrate, suggesting that the oncogenic properties of the EGFR-driven pathway may, at least in part, be mediated by the stimulation of tumor angiogenesis (Tabernero 2007; Larsen et al.

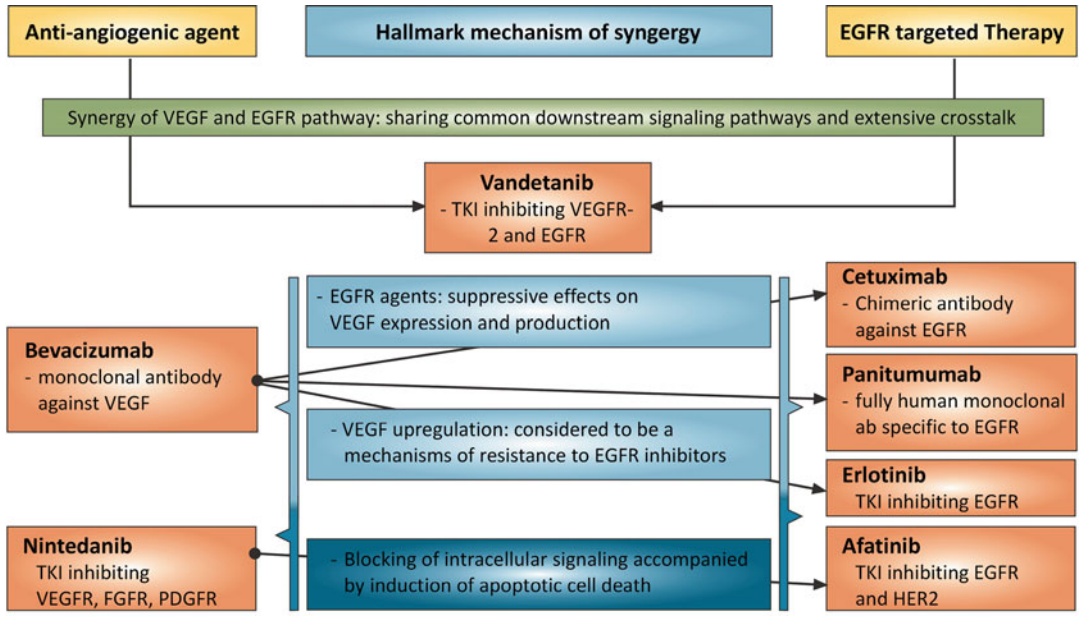

Fig. 1 Schematic representation of the main mechanisms of action postulated to mediate synergistic effects of anti-angiogenics and EGFR-targeted therapy

2011a). Accordingly, EGFR inhibitors have a suppressive effect on VEGF expression (Prewett et al. 1998). In addition, several studies have shown the role of VEGF-A upregulation in the acquired resistance to EGFR treatment in initially EGFR inhibitor-sensitive cancer cells (Viloria-Petit et al. 2001; Ciardiello et al. 2004). Therefore, targeting both these pathways could provide a better anti-cancer therapeutic strategy, especially for overcoming the acquired resistance of cancer cells to EGFR blockade (Tortora et al. 2008). An increased level of VEGF was paralleled with an increase in both angiogenic potential in vitro and tumor angiogenesis in vivo. In addition, elevated expression of VEGF in variants of the human epidermoid carcinoma cell line A431 obtained by gene transfection rendered the cells significantly resistant to anti-EGFR antibodies in vivo. The mechanism responsible for the elevated VEGF levels detected in the anti-EGFR-resistant tumor xenografts is not fully understood. The authors hypothesize that the activation of several oncogenes such as *ras, src,* and *erbB2/neu* or the inactivation/mutation of certain tumor suppressor genes such as *p53, VHL*, or *PTEN*, respectively, may account for this finding (Kerbel et al. 1998; Yen et al. 2000; Zhong et al. 2000). Thus, elevated VEGF levels may be the result of the selection of cells possessing one or more such genetic changes during the EGFR antibody-mediated therapy. Alternatively, aberrations in signaling pathways downstream of EGFR activation that are known to effect VEGF expression could conceivably be involved. Such changes, for example, could include phosphatidylinositol 3′-kinase (PI3 kinase), and/or SRC kinase overactivation, and/or ras mutation (Kerbel et al. 1998; Maity et al. 2000; Rak et al. 2000; Sato et al. 2000; Zhong et al. 2000). However, since VEGF upregulation in tumor cells is considered to be a mechanism of resistance to EGFR inhibitors, dual inhibition of both EGFR and VEGF may exert a synergistic effect (Fig. 1).

At least in preclinical studies, combinations of VEGF and EGFR inhibitors have shown synergy in antitumor activities in lung cancer and colorectal cancer (Ciardiello et al. 2000; Martinelli et al. 2010).

However, promising preclinical data of VEGF and EGFR inhibitor combinations did not translate into the clinical practice.

One potential explanation for the lack of activity might be that dual-pathway targeting with the EGFR inhibitor panitumumab and the VEGF inhibitor bevacizumab may have caused enhanced toxicity, leading to dose reductions or dose delays (Hecht et al. 2009), although this was not observed in other studies (Tol et al. 2009). Also, pharmacokinetic interactions might have occurred between the antibodies, as was suggested by a decrease in the incidence of bevacizumab-induced hypertension in the group receiving both VEGF and EGFR inhibitor treatment (Tol et al. 2009). Furthermore, bevacizumab alters tumor vascularity of subcutaneous human xenografts in mice, thereby limiting the delivery of cetuximab to the tumor leading to reduced therapeutic efficacy (Heskamp et al. 2013). In addition, interactions may have occurred between the downstream signaling pathways, e.g., EGFR-mediated changes in downstream targets may be necessary for the antitumor activity of bevacizumab or chemotherapy (Hecht et al. 2009). In mice, it was shown that cetuximab could also hamper the delivery of bevacizumab to the tumor, potentially resulting in reduced therapeutic efficacy (Heskamp et al. 2014).

Another mechanistic reason for the clinical failure might be that strategies to block VEGF or EGFR signaling by inhibition of extracellular ligands or receptors, as is the case for the monoclonal antibodies, may only prevent part of the oncogenic signaling accompanied with limited activity on intracellular signaling events. In contrast, the combination of EGFR- and VEGF(R)-targeted small-molecule tyrosine kinase inhibitors (TKIs) such as nintedanib (targeting VEGFR) and afatinib (targeting EGFR) block intracellular EGFR and VEGFR signaling, which is accompanied by the induction of apoptotic cell death (Poindessous et al. 2011). These findings provide a rationale for clinical trials combining TKIs.

All of the abovementioned reasons might, at least partly, explain the unfavorable results in some clinical studies.

Combining Bevacizumab (VEGF) and Cetuximab (EGFR)

The encouraging preclinical data of VEGF and EGFR inhibitor combinations did not translate into the clinics. To evaluate the combination of bevacizumab and cetuximab in patients with previously untreated, metastatic colorectal cancer, a large clinical trial was conducted among 755 patients, who were assigned in either the treatment group with chemotherapy (consisting of a combination of capecitabine and oxaliplatin) plus bevacizumab or the treatment group with chemotherapy plus bevacizumab plus cetuximab. Unexpectedly, the results indicated that the combination of bevacizumab and cetuximab resulted in shortened progression-free survival and worsened quality of life. Progression-free survival was 10.7 months among patients treated with chemotherapy plus bevacizumab and 9.4 months among patients treated with chemotherapy plus bevacizumab plus cetuximab (Tol et al. 2009). These data need to be put into perspective regarding the analysis of KRAS mutations. Among patients without KRAS mutations, survival was similar in the two treatment groups. Among patients with KRAS mutations, however, treatment with the combination of bevacizumab and cetuximab significantly worsened both progression-free and overall survival. Since cetuximab later on was only approved for patients without KRAS and NRAS mutations, and also other publications have found an inferior outcome of EGFR inhibition in RAS-mutated patients (Douillard et al. 2013), the conclusion from the clinical trial is that at least there is no benefit in the combined therapy.

There is no robust explanation given why the combination failed. The authors only state that the results of the trial might be due to a negative interaction between cetuximab and bevacizumab. Further they point out that hypertension, a common side effect of bevacizumab treatment, recently shown to correlate with clinical outcome in patients with colorectal cancer (Scartozzi et al. 2009), was less frequent in the patient group receiving capecitabine, oxaliplatin, and bevacizumab plus cetuximab, potentially

suggesting decreased efficacy of bevacizumab when administered in combination with cetuximab.

Also in other studies, the addition of cetuximab to bevacizumab plus FOLFOX in metastatic colorectal carcinoma did not result in better efficacy. Even increased toxicity was observed (Ocean et al. 2010; Saltz et al. 2012). Another clinical trial was prematurely terminated after other studies reported inferior outcomes with dual antibody treatment and although terminated early, the study supports the detrimental effect of combining VEGF and EGFR inhibition in metastatic colorectal cancer (Dotan et al. 2012).

Also in a xenograft mouse model with head and neck squamous cell carcinoma, the combination of anti-EGFR (cetuximab), VEGF antibodies (bevacizumab), and cisplatin appeared less effective than bevacizumab and cisplatin alone. In this study, the triple therapy resulted in less delay in tumor growth and worse survival compared to bevacizumab and cisplatin alone. This study, therefore, also argues against the combination of the two monoclonal antibodies (Wang et al. 2010).

In contrast, as forth-line treatment, the combination of VEGF and EGFR inhibitors appears to be safe and effective (Larsen et al. 2011b). Patients with metastatic colorectal cancer who had progressed on therapy with 5-FU, oxaliplatin, and irinotecan in the first- and second-line setting and with irinotecan and cetuximab as third-line therapy independent of their KRAS mutation status received irinotecan and cetuximab combined with bevacizumab. The triple combination was well tolerated and induced a high rate of disease control in heavily pretreated patients with metastatic colorectal cancer with a progression-free survival of 8.3 months and a median overall survival of 12 months (Larsen et al. 2011b). A possible explanation for this discrepancy in response between first- or fourth-line therapy might be that monoclonal antibodies could act differently in patients that are heavily pretreated compared to patients that are chemotherapy naïve. Previous chemotherapy could induce adaptive changes in tumor cells that increase the sensitivity for EGFR- and VEGF-directed monoclonal antibodies.

Combining Bevacizumab (VEGF) and Panitumumab (EGFR)

The replacement of the EGFR inhibitor cetuximab by panitumumab provided similar results when combined with bevacizumab in patients with colorectal cancer. A study by Hecht et al. (2009) showed that the addition of panitumumab to treatment with bevacizumab and chemotherapy (oxaliplatin and irinotecan based) for first-line mCRC resulted in an inferior median overall survival (19.4 months) compared with the control group receiving bevacizumab and chemotherapy only (25.4 months). Furthermore, toxicity was increased in the group receiving the combination of antibodies; therefore, treatment was discontinued early after an interim analysis (Hecht et al. 2009). While the exact explanation for these results is unknown, the authors speculated that pharmacokinetic and pharmacodynamic interactions might be responsible for the lack of activity. Since toxicity was exacerbated by dual-pathway inhibition in combination with chemotherapy, dose delays and reductions as well as decreases in dose intensity likely might explain the similar response rates observed with worse results of time-dependent end-points. In addition, potentially, a pharmacodynamic interaction induced by EGFR inhibition could explain the lack of activity of bevacizumab and/or chemotherapy. Possible mechanisms include EGFR-mediated alterations of downstream targets required for the activity of bevacizumab and/or chemotherapy or the induction of EGFR-mediated cell-cycle arrest leading to resistance to cytotoxics.

Interestingly, in two other studies addition of panitumumab and bevacizumab to chemotherapy (FOLFIRI) as second-line treatment resulted in improvement of progression-free survival and overall survival compared to FOLFIRI alone (Xie et al. 2014b; Liu et al. 2015).

However, in a recent meta-analysis of randomized controlled trials of patients with metastatic colorectal cancer, it was concluded that addition of bevacizumab to cetuximab- or panitumumab-based therapy did not improve progression-free survival and overall survival (Lv et al. 2015).

Thus the combined therapy of bevacizumab with cetuximab or panitumumab is not recommended for the treatment of metastatic colorectal cancer.

In contrast, recently a case report showed a dramatic response to panitumumab and bevacizumab in metastatic gallbladder carcinoma (Riley and Carloss 2011). In cholangiocarcinoma, EGFR expression is significantly associated with poor prognosis (Yoshikawa et al. 2008). In addition, genomic and genetic characterization of cholangiocarcinoma identified a subgroup of patients with poor overall survival and early recurrence that was characterized by multiple aberrantly regulated oncogenic pathways, including activation of HER2 and EGFR signaling (Andersen et al. 2012). In addition, several studies have revealed overexpression of VEGF in cholangiocarcinoma (ranging from 31 to 75%), and VEGF expression has been shown to be significantly associated with intrahepatic metastasis (Yoshikawa et al. 2008). Although there is the rationale for combining EGFR and VEGF inhibitors in cholangiocarcinoma and Riley and Carloss reported a single case of a patient with metastatic gallbladder carcinoma with an important response to treatment with panitumumab and bevacizumab (Riley and Carloss 2011), further clinical studies including targeted anti-EGFR and anti-VEGF(R) therapies are warranted in this entity.

Combining Bevacizumab (VEGF) and Erlotinib (EGFR)

Recent studies have demonstrated that since the oral EGFR inhibitor erlotinib and bevacizumab act on two different pathways critical to tumor growth and dissemination, administering these drugs concomitantly may confer additional clinical benefits to cancer patients with advanced disease. The combination of bevacizumab and erlotinib has been studied in phase I and II trials in metastatic breast (Dickler et al. 2008), lung (Tanaka et al. 2011), renal (Bukowski et al. 2007), and hepatocellular cancers (Thomas et al. 2009). No pharmacokinetic interaction between the two agents was demonstrated (Thomas et al. 2009). In vitro and in murine models, EGFR agents downregulate VEGF production; the combination of bevacizumab and erlotinib is likely to be synergistic in this regard (Fig. 1).

In biliary tract cancers, VEGF and EGFR have been identified as overexpressed, and VEGF has been suggested as a potential prognostic marker and correlated with poor outcome (Park et al. 2006). Therefore, a phase II trial testing the combination of bi-weekly bevacizumab and daily erlotinib in patients with unresectable biliary cancer has been conducted. The biologic-only combination showed clinical activity with an overall response rate of 64% (31 of 49 patients) with infrequent grade 3 and 4 adverse effects. The molecular analyses performed in this study suggest that patients whose tumors show mutations in EGFR vIII or have non-wild-type KRAS may be less likely to respond to erlotinib therapy (Lubner et al. 2010). These findings are consistent with trials in lung cancer and colon cancer relative to KRAS mutants and EGFR-based biologic therapy (Karapetis et al. 2008; Zhu et al. 2008). Shortcomings of this combination (bevacizumab and erlotinib) include a lack of demonstrable improvement in overall survival compared with that of historical controls, however a problem plaguing many trials in biliary tract cancers.

In patients with advanced non-squamous non-small lung cancer harboring EGFR mutations, the combination of bevacizumab and erlotinib in the first-line setting resulted in increased PFS compared to the erlotinib monotherapy group (16 months versus 9,7 months, $p = 0.0015$) (Seto et al. 2014). Results from a retrospective study in Japan showed that the serum concentrations of EGF, hepatocyte growth factor (HGF), and VEGF in patients with NSCLC who received EGFR-TKI were significantly higher among patients with progressive disease (PD) than among those with stable disease (SD) or partial response (PR) (Kasahara et al. 2010). Furthermore, the higher concentrations of HGF and VEGF were significantly associated with shorter PFS and OS. The study suggested that the serum concentration of VEGF might be an independent prognostic factor in NSCLC.

Since excessive angiogenesis is also associated with resistance to EGFR-TKI, several preclinical studies to overcome the resistance have suggested that a combination of an EGFR-TKI and anti-VEGF therapy could enhance antitumor activity in NSCLC cells harboring an EGFR mutation, especially in cells that express high levels of VEGF. Several mechanisms of antitumor activity of the combination therapy have been found. Tumor blood vessels are structurally and functionally abnormal because abnormal tumor vessels are hyperpermeable; the pressure gradient may be insufficient to ensure effective flow of drug from the vessel lumen to the tumor cells. Bevacizumab blocks angiogenesis by decreasing VEGF levels, and EGFR-TKI blocks synthesis of VEGF and TGF (transforming growth factor); they normalize tumor vessels transiently. The normalized vessels improve tumor oxygenations and restore delivery of drug into tumor by decreasing interstitial fluid pressure. In addition, EGFR plays a role in the regulation of cell proliferation. Partial normalization of tumor vessels by bevacizumab causes proliferation of the tumor cells, which make them more sensitive to EGFR-TKI.

In contrast to the abovementioned study with a remarkable efficacy of the erlotinib and bevacizumab combination with an increase in median PFS of 6.3 months compared to the erlotinib monotherapy group (16 months versus 9.7 months), in a small, single-arm study of 25 unselected patients who were elderly or had a performance status of 2, the bevacizumab/erlotinib combination was not encouraging with a median time to progression of 3.4 months and an overall survival rate of 5.1 months (Riggs et al. 2013). Additionally, in the TASK study, 124 patients with advanced or recurrent stage IIIB/IV NSCLC were randomized to bevacizumab plus chemotherapy versus bevacizumab plus erlotinib, and no benefit in PFS was observed for the bevacizumab/erlotinib arm at the time of interim analysis; thus the study was terminated (Ciuleanu et al. 2013). Based on these findings, the erlotinib plus bevacizumab combination is not currently recommended for first-line NSCLC. However, further results from studies currently evaluating the combination of anti-angiogenic inhibitors, such as bevacizumab and ramucirumab, in combination with targeted therapies in the EGFR mutation-positive patient population are expected within the next 5 years.

Combining Bevacizumab (VEGF) and HER2-Directed Therapy

Human epidermal growth factor receptor 2 (HER2) is a protein in the epidermal growth factor receptor (EGFR) family. Overexpression of HER2 promotes neoplastic transformation of cells making it a popular therapeutic target. Inhibition of HER2 is an established therapy for HER2-positive breast and gastric cancer. Trastuzumab is a monoclonal antibody that is FDA approved for HER2 overexpressed breast cancer and gastric or gastroesophageal (GE) junction patients, binding to the extracellular domain of the HER2/neu protein and inhibiting the proliferation of human tumor cells that overexpress HER2 (Baselga et al. 1996). While trastuzumab improves overall survival and response rate, resistance has been shown to develop in metastatic breast cancer patients (Tripathy et al. 2004). Therefore, the need to inhibit HER2 via alternate pathways exists. Lapatinib, also FDA approved for breast cancer patients, is a TKI of both EGFR and HER2R. Combining lapatinib and trastuzumab provides the opportunity to treat two members of the HER subfamily simultaneously and both the extracellular and intracellular domains.

Overexpression of HER2 has been associated with upregulation of VEGF in breast and lung cancer cell lines (Yen et al. 2000; Konecny et al. 2004). Preclinical data have shown that combining HER2 inhibition therapy and anti-VEGF therapy, bevacizumab, may bypass resistance to trastuzumab (du Manoir et al. 2006) (Fig. 2).

Clinically, two different phase II studies have shown responses in advanced HER2-positive breast cancer patients combining trastuzumab and bevacizumab (Drooger et al. 2016) and combining

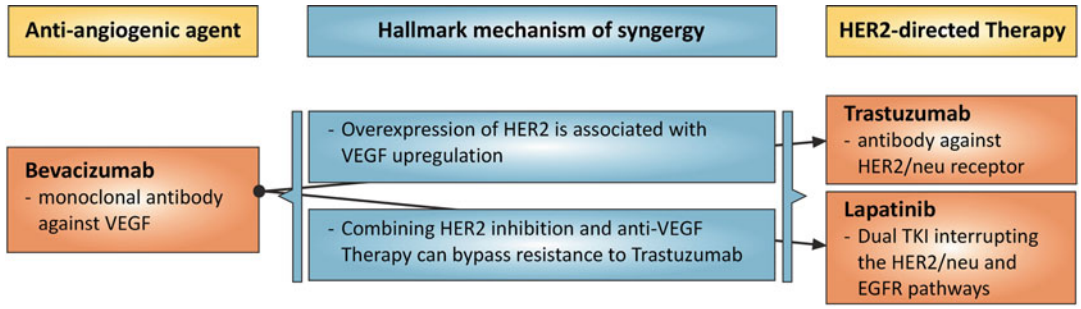

Fig. 2 Schematic representation of the main mechanisms of action postulated to mediate synergistic effects of anti-angiogenics and HER2-directed therapy

lapatinib and bevacizumab (Rugo et al. 2012). Recently, a phase I trial combined trastuzumab, lapatinib, and bevacizumab in patients with advanced cancer (Falchook et al. 2015). The combination was well tolerated with successful escalation to the FDA-approved doses of all three drugs without reaching a maximum tolerated dose (MTD). In addition, the combination has demonstrated antitumor activity in heavily pretreated patients with advanced malignancies with an overall response rate of 25% (SD > 6 months/PR/CR = 23/94 (25%). A Response (SD > 6 months/PR/CR) was achieved in 50% of heavily pretreated breast cancer patients in this study. These patients had all received prior trastuzumab and the majority prior lapatinib. Despite failing prior concurrent or sequential trastuzumab and lapatinib treatment, these patients continued to achieve SD > 6 months/PR/CR with the addition of bevacizumab to the treatment combination. Overcoming resistance to prior concurrent trastuzumab and lapatinib and achieving longer treatment duration with combining trastuzumab, lapatinib, and bevacizumab suggest that bevacizumab contributes to this HER2 treatment combination (Falchook et al. 2015). Other disease categories also achieved SD > 6 months/PR including a patient with non-small cell lung cancer harboring a HER2 mutation at exon 20, a patient with HER2-positive salivary duct cancer, and patients with HER2-negative breast and pancreatic cancer (N = 1 of each). Based on these observations, further evaluation of this combination of dual HER inhibition plus VEGF inhibition is warranted.

Tyrosine Kinase Inhibitors Blocking Both VEGFR and EGFR

Vandetanib is a tyrosine kinase inhibitor of both VEGFR-2 and EGFR, and preclinical studies have confirmed its antitumor effects in a range of cancer types. A randomized phase III trial demonstrated that vandetanib treatment is effective in patients with metastatic symptomatic or progressive medullary thyroid cancer (Wells et al. 2012), leading to the approval by the US Food and Drug Administration (FDA) in April 2011, followed by the European Medicines Agency (EMA) in 2012. This approval was based on a statistically significant and clinically meaningful improvement in progression-free survival. However, toxicity of vandetanib was worse than that of other kinase inhibitors, including abdominal pain and diarrhea, rashes, prolonged QT interval, hypertension, headache, and fatigue. The drug underwent clinical trials as a potential targeted treatment for non-small cell lung cancer; however, EU regulatory submissions for vandetanib were withdrawn in October 2009 after trials showed no benefit when the drug was administered along with chemotherapy.

Synergy Between Anti-angiogenics and Immune Cell Therapies

Immunotherapy has now been clinically validated as an effective treatment for many cancers. There is tremendous potential for synergistic combinations of immunotherapy agents and for

combining immunotherapy agents with conventional cancer treatments.

Emerging data indicate that abnormal tumor vasculature, resulting from the prevalence of pro- versus anti-angiogenic signals, fosters an immunosuppressive tumor microenvironment that enables the tumor to evade host immunosurveillance.

VEGF is a potent angiogenic factor that regulates angiogenesis while increasing the proliferation, migration, and metastasis of tumor cells. In addition to its proangiogenic function, mounting evidence shows that VEGF also plays a major role in the immunosuppression of innate and adaptive immune system cells (Soto-Ortiz 2016). VEGF suppresses their antitumor function due to the capability of these cells of expressing VEGF receptors once they have been activated and have migrated to the tumor site (Soto-Ortiz 2016). VEGF has immune-modulating properties, which include decreasing the influx of lymphocytes and dendritic cells (DCs) into the tumor while increasing the intratumoral frequencies of regulatory T cells (TREGs) and myeloid-derived suppressor cells (MDSCs). MDSCs have been recently identified as a further major component of the microenvironment, inversely linked with outcome, representing a heterogeneous population of myeloid progenitors and precursors of granulocytes, macrophages, and dendritic cells. MDSCs can inhibit T-cell responses limiting immune therapeutic approaches and are induced by various factors, such as VEGF, expressed or secreted in states of cancer, inflammation, or trauma. Importantly, this systemic immunosuppression induced by excess VEGF can be reversed by the blockade of VEGF/VEGFR2 signaling pathway (Gabrilovich et al. 1999). Therefore, VEGF inhibition suggests synergism of immunotherapeutic effector mechanisms.

In addition to its ability to promote an immunosuppressive local tumor microenvironment, VEGF has profound effects on immune regulatory cell function, specifically inhibiting dendritic cell maturation and antigen presentation contributing to the suppression of antitumor immune responses (Oyama et al. 1998). In patients with colorectal cancer, bevacizumab has been shown to improve the antigen-presenting capacity of circulating dendritic cells (Osada et al. 2008). Furthermore, treating mice with recombinant VEGF at concentrations similar to those observed in patients with advanced-stage cancer induced T-cell defects via inhibition of Delta ligand signaling through Notch.

Furthermore there is evidence that E-selectin expression induced by bevacizumab facilitates lymphocyte adhesion and rolling. In addition, CD31 influences adhesive and signaling functions for vascular cellular extravasation. These results are consistent with previous observations of anti-VEGF treatment increasing lymphocyte tumor infiltrates in adoptive therapy models. Further evidence for immunologic changes resulting from bevacizumab was demonstrated in the peripheral blood through increasing circulating memory T cells (Hodi et al. 2014), providing a definite role for bevacizumab in effecting broad changes in the circulating immune composition.

Thus, the concept of antagonizing VEGF accompanied by immune-modulating properties could provide an attractive approach for enhancing immune responses (Fig. 3).

Indeed, anti-angiogenic agents have the potential to modulate the tumor microenvironment and improve immunotherapy, but often they are used at high doses in the clinic to prune tumor vessels and paradoxically may compromise various therapies. Recently Huang et al. demonstrated that targeting tumor vasculature with lower vascular-normalizing doses, but not high antivascular/anti-angiogenic doses, of an anti-VEGF receptor 2 (VEGFR2) antibody results in a more homogeneous distribution of functional tumor vessels (Huang et al. 2012). In addition, lower doses are superior to the high doses in polarizing tumor-associated macrophages from an immune inhibitory M2-like phenotype toward an immune stimulatory M1-like phenotype and in facilitating CD4+ and CD8+ T-cell-dependent manner in both immune-tolerant and immunogenic murine breast cancer models. These findings indicate that vascular-normalizing lower doses of anti-VEGFR2 antibody can reprogram the immunosuppressive tumor microenvironment in a manner that augments anticancer vaccine therapy.

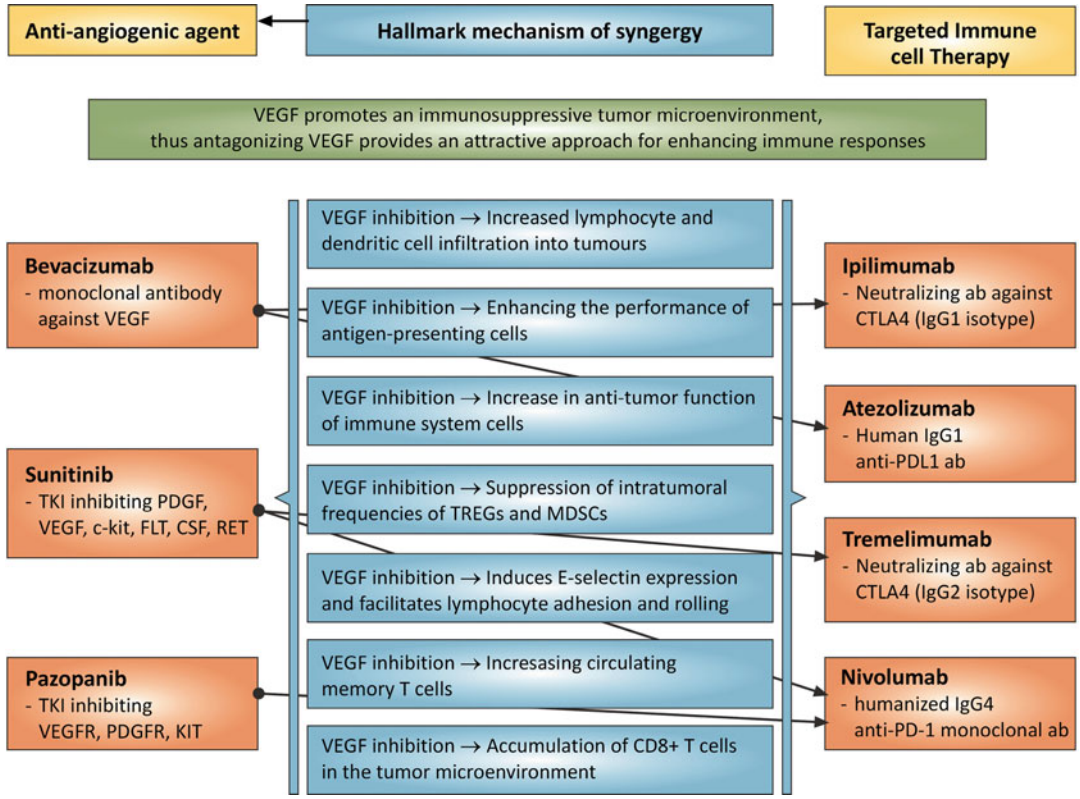

Fig. 3 Schematic representation of the main mechanisms of action postulated to mediate synergistic effects of anti-angiogenics and targeted immune cell therapy

Combining VEGF and CTLA4 Blockade

The VEGF inhibitor bevacizumab has recently been combined with ipilimumab, a monoclonal antibody that inhibits the checkpoint receptor cytotoxic T lymphocyte-associated antigen 4 (CTLA4) for advanced-stage melanoma. A total of 46 patients with metastatic melanoma were treated with this combination, and the efficacy was remarkably good, resulting in a median overall survival of more than 2 years (Hodi et al. 2014). High-grade toxicity was more common than expected for either drug alone, but it was manageable and included inflammatory events such as hypophysitis, temporal arteritis, dermatitis, hepatitis, and uveitis. Interestingly, the combination led to an accumulation of CD8+ T cells and DCs in the tumor microenvironment – suggesting synergism of immunotherapeutic effector mechanisms – and warrants further investigation of this combination.

The anti-CTLA-4 mAb tremelimumab administered with the VEGFR TKI sunitinib produced partial remissions in 9/21 evaluable patients with renal cell carcinoma but was associated with acute renal toxicity, which the authors proposed might be immune related (Rini et al. 2011).

Further investigation is needed to evaluate the mechanistic basis of bevacizumab activity and the full impact of clinical activity. Continued development of immune checkpoint and anti-angiogenic combination therapies are warranted for the treatment of melanoma and other cancers.

Combining VEGF and PDL1/PD1 Blockade

The VEGFR TKIs sunitinib and pazopanib are standard of care in the treatment of patients with metastatic renal cell carcinoma; however their antitumor effects are not durable. As it was

hypothesized that anti-VEGF strategies suppress regulatory T cells to attenuate tumor-induced immunosuppression and might sensitize tumors to immunotherapy when used in combination, nivolumab has been combined with either sunitinib or pazopanib in patients with metastatic renal cell carcinoma. Nivolumab is a fully human monoclonal antibody inhibiting the programmed death-1 immune checkpoint receptor to restore T-cell antitumor immune responses. It also demonstrated clinical activity in metastatic renal cell carcinoma (mRCC) (Motzer et al. 2015). The median progression-free survival was 48.9 versus 31.4 weeks for sunitinib plus nivolumab and pazobanib plus nivolumab, respectively. The authors concluded that combination therapy with sunitinib plus nivolumab showed encouraging antitumor activity and was associated with a manageable safety profile in patients with mRCC. They also noted that the combination therapy resulted in responses that were higher than previously reported for monotherapy of either agent. However, the combination of pazopanib plus nivolumab is not a feasible treatment option at the dose and schedule studied here, because of dose-limiting toxicities, including liver enzyme elevations and fatigue.

Atezolizumab is a human anti-PD-L1 monoclonal antibody preventing PD-L1 binding to the inhibitory receptors PD-1 and B7.1 on activated T cells and has demonstrated clinical activity in various cancers including metastatic renal cell carcinoma (McDermott et al. 2016). In April 2016, the FDA granted priority review to atezolizumab for patients with locally advanced or metastatic non-small cell lung cancer who express PD-L1 and have progressed after a platinum-containing regimen. In May 2016 it was approved by the FDA for the second-line treatment of advanced bladder cancer.

As bevacizumab has been proposed to enhance the antitumor effects of atezolizumab by blocking VEGF-related suppressive effects on immune function and lymphocyte traffic, a multicenter phase Ib study was conducted to determine the safety and activity of atezolizumab plus bevacizumab in a cohort of metastatic renal cell carcinoma patients. The combination of atezolizumab and bevacizumab showed strong antitumor activity with an overall response rate of 40% (in 4 of 10 patients). In addition, increases in tumor-infiltrating CD8+ T cells were observed on-treatment and the combination was well tolerated (Sznol et al. 2015). A phase II trial of atezolizumab +/− bevacizumab versus sunitinib in patients with previously untreated metastatic renal cell carcinoma is currently ongoing.

Interestingly, the anti-PD-L1 antibody atezolizumab was also investigated in colorectal cancer in an open-label, multicenter phase Ib study. Patients were either treated with atezolizumab plus bevacizumab in refractory metastatic colorectal cancer (Arm A) or with atezolizumab plus bevacizumab plus chemotherapy FOLFOX in oxaliplatin-naïve metastatic colorectal cancer (arm B). Both treatment combinations were well tolerated with no unexpected toxicities, and in both arms clinical activity was observed with an unconfirmed overall response rate of 8% (1/13) in arm A and 36% (9/25) in arm B (Bendell et al. 2015). Longer follow-up and randomized studies will be needed to estimate the potential benefit of adding atezolizumab to bevacizumab and chemotherapy.

Synergy Between VEGF Blockade and Temsirolimus

Temsirolimus is a mTOR (mammalian target of rapamycin) inhibitor that inhibits the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mTOR pathway, which is involved in protein synthesis, cellular proliferation, and tumor angiogenesis. mTOR inhibitors inhibit endothelial cell VEGF expression as well as VEGF-induced endothelial cell proliferation (Dormond et al. 2007) and are an important class of anti-angiogenic agents. Temsirolimus has been approved by the FDA to treat renal cell carcinoma.

One mechanism of tumor resistance to anti-angiogenic therapy, e.g., bevacizumab is upregulation of hypoxia-inducible factor 1α (HIF-1α), which mediates adaptive responses to hypoxic conditions (Zhong et al. 1999). HIF-1α inhibition in combination with anti-angiogenic therapy is a promising strategy for targeting

tumor resistance. Temsirolimus has been shown to inhibit the activity of mTOR and has resulted in reduced levels of HIF-1α, HIF-2α, and VEGF (Zhong et al. 1999). The discovery of the HIF-1α inhibition properties of temsirolimus makes it an ideal candidate for combination with bevacizumab.

However, in treatment-naïve patients with mRCC, the combination of a VEGF pathway and a mTOR inhibitor was associated with toxicity and no apparent antitumor synergy. Some postulated that not only was the benefit of combining VEGFR TKI and mTOR inhibitors over VEGFR TKI alone affected by dose reductions required for toxicity but also that the dose reductions may negatively affect the benefit expected from first-line VEGFR TKI therapy. Also in mRCC patients previously treated with VEGFR TKI, combining bevacizumab and temsirolimus required significant dose reductions and discontinuations and even applying this combination at full doses of each drug resulted in modest activity overall and would not be recommended for routine clinical use (Mahoney et al. 2016).

In contrast, in a phase I clinical study of 41 heavily pretreated patients with gynecological malignancies, after all 37% of the patients achieved disease control (Piha-Paul et al. 2014). Of note, in this study, the combination of bevacizumab and temsirolimus showed excellent tolerance without dose-limiting toxicity even when the maximum FDA-approved dose of each drug was used in the combination. Further study of bevacizumab and temsirolimus in larger populations at least with gynecological cancers may be warranted.

Synergy of Three Targeted Agents Including VEGF Blockade

There are several compelling rationales for combining bevacizumab, temsirolimus, and cetuximab in treating advanced malignancies:

(i) Bevacizumab and cetuximab may be synergistic.
(ii) Temsirolimus inhibits mTOR and the PI3 kinase/AKT/mTOR pathway as well as CYP2A, which may be a resistance mechanism for cetuximab.
(iii) Temsirolimus attenuates upregulation of HIF-1α levels, which may be a resistance mechanism for bevacizumab.
(iv) The three agents have non-overlapping toxicities.

Liu et al. investigated safety and responses in 21 patients with advanced solid tumors treated with these combined three targeted agents (Liu et al. 2016). The authors conclude that the combination of bevacizumab, temsirolimus, and cetuximab demonstrated promising activity with an overall response rate of 33% with 11% (2/18) partial responses and 22% (4/18) stable diseases but at the expense of toxicity. Overall, 11/21 (52%) of patients treated on the trial developed grade 3 to 4 toxicities including among others hyperglycemia, hypophosphatemia, headache, fatigue, leukopenia, and anemia, respectively. This reflects synergistic toxicity that could limit further development of this combination.

Unlike these findings, the combination of cetuximab, erlotinib, and bevacizumab that was investigated in a phase I trial of 34 patients with non-small cell lung cancer (NSCLC) was well tolerated (Falchook et al. 2013). Of the NSCLC patients in this trial, the most common treatment-related grade > 2 adverse events were rash (41%), hypomagnesemia (27%), and fatigue (15%). The overall response rate in these heavily pretreated patients was 32% (11/34) and thus comparable to results of the abovementioned trial applying the triple combination of bevacizumab, temsirolimus, and cetuximab.

In another phase I trial, 32 patients with different types of solid tumors received the combination of everolimus, bevacizumab, and panitumumab (Vlahovic et al. 2012). This trial was also well tolerated and appeared to have only moderate clinical activity in refractory tumors.

In summary, the results of combined three targeted agents including VEGF inhibitors fail to come up to expectations.

Combined Blockade of VEGF and Ang2 Signaling: Humanized Tri-specific Nanobody

As already mentioned above, therapies targeting single antigens with monospecific antibodies have shown limited efficacy in patients with cancer. Advances in antibody engineering technologies have enabled strategies that simultaneously target multiple receptors to circumvent the limitations of conventional monospecific therapies and achieve enhanced therapeutic efficacy.

Besides VEGF, angiopoietin2 (Ang2) is an important player in angiogenesis. Ang2, primarily expressed by endothelial cells, is a ligand of the Tie2 receptor, and Ang2/Tie2 signaling regulates tumor vessel plasticity, allowing vessels to respond to other angiogenic factors (Fig. 4). Its in vivo inhibition results in tumor growth inhibition and vasculature changes. The inhibition of Ang2 is currently being tested in phase II/III trials of the peptibody trebananib in ovarian cancer. In a randomized phase III trial in patients with recurrent ovarian cancer, trebananib was tested in combination with paclitaxel compared with chemotherapy alone and demonstrated improvement in progression-free survival (7.2 month vs 5.4 months, HR 0.66, $p < 0.0001$) (Monk et al. 2014).

Both proangiogenic pathways (VEGF/VEGFR and Ang2/Tie-2) have been reported to synergize and to cross talk with Ang2 enhancing VEGF signaling and VEGF upregulating Ang2 expression on endothelial cells. Thus, combined inhibition of VEGF and Ang2 might well result in modulation of tumor angiogenesis and reduced tumor growth rate with improved clinical efficacy compared to VEGF pathway blockade alone.

Limited clinical experience of dual blockade is available. Recently, phase I data of the bispecific human anti-Ang2/anti-VEGF-A antibody RG7221 were reported. The maximum tolerated dose (MTD) was not reached with only one dose-limiting toxicity (DLT) reported (fatal pulmonary hemorrhage). Hypertension was the most common observed adverse event. Previous clinical experience with nanobodies in different disease showed acceptable safety profile with no specific side effect related to this technology.

Recently, the humanized tri-specific nanobody BI 836880 comprising two single variable domains blocking VEGF and Ang2, and an additional module for half-life extension in vivo has been generated. This VEGF/Ang2 blocking nanobody was highly potent and showed in vivo monotherapy efficacy (tumor growth inhibition) in several tumor xenograft models representing colon cancer, non-small cell lung cancer,

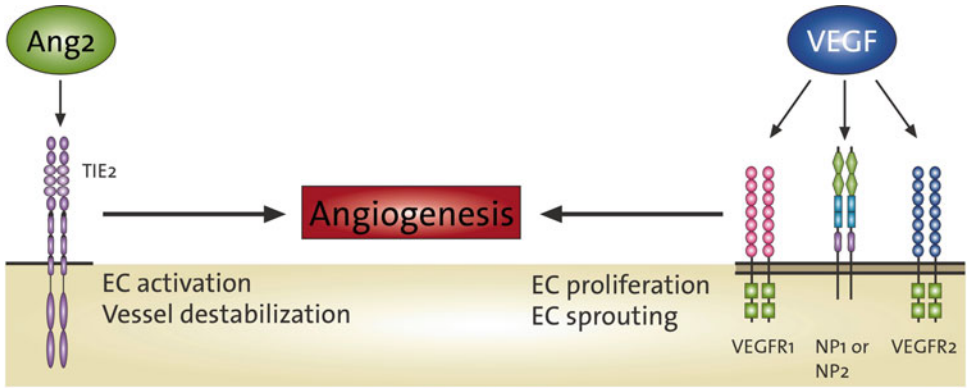

Fig. 4 Mode of action of BI 836880. BI 836880 binds the soluble ligands VEGF-A and angiopoietin Ang2 and inhibits proangiogenic signaling by their receptors, VEGFR2 and Tie2, respectively. Preclinical data demonstrate cross talk between the VEGF and Ang2 pathways, where inhibition of Ang2 increases VEGF expression, providing additional rationale for dual target inhibition

mammary cancer, ovarian cancer, pancreatic cancer, and renal cell cancer. In addition, the nanobody was found to inhibit signaling downstream of VEGF and Ang2, leading to a decrease of endothelial cell proliferation. Combined blockade of VEGF and Ang2 signaling pathways was found superior to inhibition of the individual pathways in patient-derived xenograft studies. The molecule was well tolerated in cynomolgus monkeys.

This novel VEGF/Ang2 blocking nanobody showed promising properties in vitro and in vivo, which strongly support the evaluation of this molecule in the clinic.

At present, a first-in human phase I, nonrandomized, open-label, multicenter dose escalation trial of the VEGF/Ang2 blocking nanobody BI 836880 administered by repeated intravenous infusions in patients with solid tumors is under way.

A Novel VEGFR2 Targeted Antibody-Based Fusion Protein (mAb04-MICA)

Very recently, a novel human IgG1 antibody (mAb04) specific for VEGFR2 was generated. This antibody had high affinity to VEGFR2 and exhibited anti-angiogenic activity both in vitro and in vivo (Xie et al. 2014a). To enhance the immunostimulatory activity of mAb04, this antibody was fused to MHC class I-related chain A (MICA). MICA is one of the major ligands for the NKG2D (natural killer (NK) cell receptor NK group 2, member D) which represents an activating receptor expressed on NK cells, the major effectors of antibody-dependent cellular cytotoxicity (ADCC). Thus, binding of MICA to NKG2D is thought critical for activating NK-mediated immunosurveillance.

In humans, MICA is often overexpressed in many tumor tissues from patients with epithelial tumors and some primary leukemia cells. However, since the tumors progressed despite the expression of MICA, it appeared that the MICA-NKG2D system was functionally compromised in these patients (Wu et al. 2004). Studies found that tumor cells avoid the response of NKG2D through shedding MICA from the cell surface, and this soluble MICA hinders recognition of the MICA-expressing tumor cells, thereby impairing the antitumor immune response.

Therefore, mAb04-MICA was designed and produced with the goal of reinforcing the immune surveillance activity of NK cells while retaining the anti-angiogenic and antineoplastic activity of mAb04. Indeed, mAb04-MICA localized in tumor lesions via the recognition of mAb04 to tumor cell surface VEGFR2 and attracted NK cells to the tumor lesions through the associated MICA. In human breast tumor-bearing nude mice, the antibody-based fusion protein mAb04-MICA demonstrated superior antitumor efficacy compared to combination therapy of mAb04 plus docetaxel or bevacizumab plus docetaxel, highlighting the immunostimulatory effect of MICA.

In conclusion, this novel VEGFR2 targeted antibody-based fusion protein mAb04-MICA provides a new inspiration for antitumor treatment and might have prospects for clinical application.

Conclusion

Abnormal vessel growth and function are hallmarks of cancer, and they contribute to disease progression. Therapeutic approaches to block vascular supply have reached the clinic, but limited efficacy and fast development of resistance pose unresolved challenges. A question of high priority is whether the approved anti-angiogenic regimes are optimally used in terms of dosing, duration, and combination therapy. Clinicians should acknowledge that the ability to predict which combinations are best suited for which malignant indications or clinical scenarios currently still lacks sophistication.

However, the field is developing rapidly, and the goal is to move from an era of empirical combinations to one of rational design by considering the compatibility of mechanisms that interacts synergistically, either to mediate antitumor efficacy or to reduce on-target side effects. A very promising combination approach involves delivering anti-angiogenics and targeted therapy – a newer type of cancer treatment that interferes with specific molecules involved in cancer cell growth and survival. Targeting of VEGF(R) combined with EGFR inhibition resulted in encouraging preclinical

results. However, these results did not translate into clinics, at least in patients with previously untreated, metastatic colorectal cancer, where the combined therapy of bevacizumab with cetuximab or panitumumab failed to improve progression-free survival or overall survival due to reasons that are not fully understood.

In contrast, since VEGF promotes an immunosuppressive tumor microenvironment, antagonizing VEGF provides a very attractive approach for enhancing immune responses, and thus VEGF inhibition is a very promising combination partner for targeted immunotherapy. Combining VEGF with CTLA4 blockade as well as with PDL1/PD1 blockade provided clinical activity in advanced-stage melanoma. This strategy is currently tested in clinical trials investigating nivolumab or pembrolizumab and bevacizumab in, e.g., metastatic renal cell carcinoma, high-grade glioma, or glioblastoma (clinicaltrials.gov). The combined blockade of VEGF and angiopoietin2 signaling with a humanized tri-specific nanobody and novel VEGFR2 targeted antibody-based fusion proteins are other emerging directions for the medical treatment targeting angiogenesis. In conclusion, angiogenesis-based drug combinations may provide novel, selective, safe, and reasonable future treatment options.

Cross-References

- Inhibition of Tumor Angiogenesis in GIST Therapy
- Inhibition of Tumor Angiogenesis in the Treatment of Lung Cancer
- Mechanisms of Anti-angiogenic Therapy
- Mechanisms of Tumor Angiogenesis
- The Role of the VEGF Signaling Pathway in Tumor Angiogenesis
- The Value of Anti-angiogenics in Breast Cancer Therapy
- The Value of Anti-angiogenics in Head and Neck Cancer Therapy
- The Value of Anti-angiogenics in Prostate Cancer Therapy

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