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# The Role of Vascular Endothelial Growth Factor in the Pathogenesis, Diagnosis and Treatment of Malignant Pleural Effusion

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## Abstract

Malignant pleural effusions (MPEs) are a significant source of cancer-related morbidity. Over 150,000 patients in the United States suffer from breathlessness and diminished quality of life due to MPE each year. Current management strategies are of mostly palliative value and focus on symptom control; they do not address the pathobiology of the effusion, nor do they improve survival. Further elucidation of the pathophysiological mechanisms, coupled with the development of novel treatments such as intrapleural chemotherapeutics targeting this process, has the potential to greatly improve the efficacy of our current management options. Vascular endothelial growth factor-A (VEGF-A) has been implicated as a critical cytokine in the formation of malignant pleural effusions. Elevated levels of VEGF produced by tumor cells, mesothelial cells and infiltrating immune cells, result in increased vascular permeability, cancer cell transmigration, and angiogenesis. Therefore anti-angiogenic therapies such as Bevacizumab, a monoclonal antibody targeting VEGF-A, may have a potential role in the management of malignant pleural effusions. Herein we review the pathogenesis and potential treatment strategies of malignant pleural effusions, with a focus on angiogenesis and anti-angiogenic therapeutics.

### Keywords

Pleural Effusions; Angiogenesis; Vascular Endothelial Growth Factor; Lung Cancer

## Introduction

Malignant pleural effusions (MPEs) are a significant source of cancer-related morbidity, debilitating patients by impairing respiratory function and decreasing quality of life dramatically in over 150,000 patients in the US yearly [1]. MPEs are common complications in cancer patients. During the course of their disease approximately 50% of all patients with metastatic cancer develop a MPE. Virtually any cancer can cause an MPE, although greater than 75% are caused by lung -, breast -, ovarian cancer, or by malignant lymphomas [2]. MPEs are especially common complications of lung cancer, with 15% of lung cancer

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patients having an MPE at presentation and 50% developing an MPE during the course of their disease [3, 4]. Unfortunately, MPEs are associated with a bleak prognosis, heralding a rapid deterioration with a median survival of 3 months [5]. This time is typically plagued by numerous hospitalizations and multiple interventions for symptom control [6]. Furthermore, the presence of an MPE can decrease the patient's overall performance status and thereby affect their candidacy to receive potentially life-extending anticancer therapies.

Most of our current management strategies for MPE do not improve patient survival, largely fail to address the underlying cause of the effusion and consequently are predominantly palliative. Further elucidation of the pathogenic mechanisms, coupled with novel local and/or systemic treatments targeting these pathways, has the potential to improve the efficacy of our current management strategies. Herein we present a review of the pathophysiology, diagnosis and management of MPEs focusing on the role of vascular endothelial growth factor (VEGF) in the formation of MPEs and the rationale for VEGF-targeted treatment modalities.

## VEGF in the Pathogenesis of Malignant Pleural Effusions

As far back as 1939, Ide and colleagues hypothesized the production of pro-angiogenic factors by tumors [7]. In 1971, Folkman postulated that a tumors are dependent on increasing vascular supply, and suggested that antiangiogenic therapies could serve a role in cancer treatment [8]. Subsequent research led to our increasing understanding of the mechanisms of VEGF in malignancy and the development of a number of anti-angiogenic therapeutic strategies.

Among the various mediators found in malignant effusions, VEGF has drawn interest for its central role in pleural fluid accumulation [9] and for its potential as a therapeutic target [10, 11]. VEGF is a family of endothelial growth factors which includes VEGF-A –B –C –D –E and placental growth factor [12]. This family of peptides has been the focus of extensive research with applications in effusions, cancer, hypoxic injury and normal growth and development. VEGF possesses critical functions in angiogenesis [13], exerting a number of effects on the vascular endothelium including survival, proliferation, differentiation, sprouting and tube formation [14-16]. VEGF not only possesses potent vasodilatory effects [17], but also the ability to increase vascular [18] and mesothelial permeability [19]. Increased permeability as a result of VEGF stimulation is mediated by several mechanisms (see Bates, 2010[20]for an excellent review of this subject) including induction of endothelial fenestrations [21-23], loss of junctional integrity [24] and the formation of transcellular gaps [25].

Many cancers have been shown to over-express VEGF, a finding associated with a poor prognosis in at least pancreatic [26, 27], gastric [28, 29] and colonic carcinomas [30, 31], as well as in lung [32], breast [33, 34] and prostate [35] cancers and melanoma [36]. The factors influencing the expression of VEGF include hypoxia; several growth factors such as epidermal growth factor, transforming growth factor, insulin-like growth factor, and others; a variety of hormones; and oncogenic mechanisms leading to the activation of proto-oncogenes and the dysfunction of tumor suppressor genes [37, 38]. Hypoxia is a well-established inducer of angiogenesis, which activates hypoxia-inducible factor-1, a transcription factor responsible for the regulation of a number of hypoxia-responsive genes [39]. Transcription of VEGF mRNA is initiated upon binding of the hypoxia-inducible factor-1/aryl hydrocarbon nuclear translocator complex to the promoter region [40]. The molecular target of rapamycin (mTOR) has been shown to play a role in the expression of VEGF through its ability to increase the expression of hypoxia-inducible factor-1 in hematologic and various solid malignancies [41-43]. Cancer cells may produce VEGF

through autocrine signaling mediated by interleukin-6, as well, indicating that there are likely a number of upstream mechanisms for initiating VEGF production in MPEs [44]. Alternative mRNA splicing of the VEGF gene produces at least six splice variants ($VEGF_{121-206}$) which have varying biologic effects, ranging from pro-angiogenic to anti-angiogenic [45, 46].

Several receptor tyrosine kinases, including VEGFR-1 -2 and -3 (also known as Flt-1, KDR/Flk-1 and Flt-4 respectively) mediate the biological response to VEGF [15]. A number of additional factors have also been shown to play a role in the cellular response to VEGF, including heparin and heparan sulfate proteoglycans, as well as co-receptors known as neuropilin-1 and -2 [46-48]. The signal transduction cascade downstream of the VEGF receptors is complex and includes at least the mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase/protein kinase-B (also referred to as PI3K/AKT) and protein kinase-C (PKC) pathways, which are central in actuating the array of VEGF effects [12]. VEGFR-2 mediates the majority of the angiogenic response to VEGF, including increased permeability [49], migration, invasion, proliferation and survival of the vascular endothelium [50, 51].

The role of VEGF in MPE formation is under investigation, with current data strongly implicating VEGF as a critical cytokine in MPE pathogenesis. Elevated levels of VEGF have been observed in pleural effusions due to both malignant and benign processes [52], but higher levels of VEGF are consistently found in pleural effusions of malignant origin [53-61, 49-51]. VEGF production by intrathoracic lung cancer cells has been shown to contribute to pleural effusion formation, tumor dissemination, and angiogenesis [62, 63]. Prager and colleagues have demonstrated that VEGF causes an increase in the permeability of an endothelial monolayer and induces transmigration of primary and cell-line derived cancer cells, effects which were both blocked by the administration of the anti-VEGF monoclonal antibody bevacizumab [43]. The addition of 20 nM rapamycin (Sirolimus) to culture medium also decreased the production of VEGF by both primary and cell line-derived cancer cells, a finding which suggests a potential benefit from co-administration of rapamycin and bevacizumab in MPE [43]. Interestingly, cancer stem cells have been isolated from malignant pleural effusions [153], a finding which prompted the hypothesis that VEGF may serve a chemotactic function for cancer stem cells [43]. It should be noted that VEGF does not function in isolation and a variety of other vasoactive mediators, such as osteopontin [64], chemokine ligand 2 [65], interleukin 5 [66], matrix metalloprotease 9 and tumor necrosis factor-alpha [67] have also been implicated in the formation of MPEs. An eloquent model of malignant pleural effusion pathogenesis which integrates tumor and host interactions has recently been presented by Stathopoulos and Kalomenidis [68].

Given the important functions of VEGF in cancer and a number of other conditions, multiple compounds capable of antagonizing the effects of VEGF have been developed for use in the clinical setting [69]. These include several tyrosine kinase inhibitors [70] and bevacizumab, a recombinant humanized monoclonal antibody targeted to VEGF that inhibits the binding of VEGF to its receptors VEGFR-1 and -2 [71]. Aflibercept is a fusion antibody protein with affinity for VEGF-A, VEGF-B and placental growth factor that has recently been approved by the FDA for use in colorectal cancer [72]. Multiple studies have carefully followed plasma levels of VEGF after administration of bevacizumab and have shown that >97% of circulating VEGF is bound by bevacizumab within hours of administration [73, 74]. Bevacizumab has been approved for the treatment of several malignancies, including advanced colorectal and non-small cell lung carcinomas, as well as advanced renal cell carcinoma [75].

Evidence is mounting in support of the hypothesis that the VEGF found in malignant effusions is produced within the pleural space. VEGF levels in malignant effusions are consistently much higher than serum levels; and multiple studies have shown no correlation between the levels of VEGF in malignant effusions and plasma [76-78, 57, 54]. While many cancers over-express VEGF, virtually all cells have this capability, and accumulating data suggest that non-neoplastic cells may contribute to the increased levels of VEGF observed in malignant states, including platelets [79] and tumor-associated stromal and immune cells [80-83]. Mesothelial cells have been shown to express VEGF in response to TGF-beta stimulation, both in vitro and in vivo, suggesting a potential role in malignant effusion [84]. These observations support the merit of intrapleural therapeutics targeting angiogenic pathways (see below).

## Diagnosis of MPE

It is critical to accurately diagnose MPEs, as the diagnosis generally precludes the possibility of a curative resection (the seventh edition of the American Joint Committee on Cancer classifies patients with an MPE as M1a, stage IV disease) [85]. As only approximately 50% of pleural effusions in the setting of cancer are MPEs, distinguishing between malignant and para-malignant effusions significantly impacts management. Para-malignant pleural effusions can develop from such processes as lymphatic obstruction, atelectasis, pulmonary embolus or post-obstructive pneumonitis. There are several features of pleural fluid suggestive of MPE. These include lymphocytic predominance with lymphocytes representing 50-70% of nucleated cells, >10% eosinophils, the presence of erythrocytes, pH < 7.3 and glucose < 60 mg/dL [2, 86]. While the majority of MPEs are exudative effusions, it should be noted that 3-10% are transudates, according to Light's criteria [87-89]. However, definitive diagnosis of MPE requires the identification of malignant cells within the fluid or positive pleural biopsy, as the aforementioned features are relatively non-specific [2]. Roughly 50-60% of MPEs are detected by cytologic examination after a single thoracentesis [90-92, 55], and the diagnostic yield improves with up to two additional thoracenteses. Increasing the volume of a single thoracentesis does not increase the diagnostic accuracy, however, and 50 mL is likely a sufficient volume [93, 94]. As the diagnostic yield of a single thoracentesis is insufficient to rule out MPE with a single negative result, in practice three negative thoracenteses are generally required to exclude MPE.

## Diagnostic and Prognostic Implications of Biomarkers in MPE

A variety of biomarkers have been investigated as potential diagnostic and prognostic indicators of MPE including (among others) cystatin-C [95], D-dimer [96], epididymal secretory protein E1 precursor [97], lung surfactant protein-A [98], carcinoembryonic antigen [98], pigment epithelium-derived factor [99-101], pro-calcitonin and c-reactive protein [102], vascular endothelial growth factor [103, 54], and insulin-like growth factor binding protein-2 (which may also play a role in cell transmigration) [104]. While many of the biomarker levels differ between malignant and benign pleural effusions, none have yet demonstrated sufficient sensitivity and specificity to garner acceptance in the clinical setting.

The diagnostic yield of thoracentesis may be increased by the additional measurement of VEGF in pleural fluid, although as expected, the sensitivity and specificity vary with different cut-off concentrations [55]. A recent meta-analysis reviewed the potential role of pleural fluid VEGF levels in the diagnosis of MPEs and found modest maximum joint sensitivity and specificity of 0.72 with an area under the curve of 0.82 [105]. The authors concluded that the detection of pleural fluid VEGF may play some role in MPE diagnosis,

but with unsatisfactory diagnostic value to be used in isolation, requiring interpretation in the context of clinical findings and conventional diagnostic studies. Notably, benign exudative effusions also demonstrate elevated VEGF levels, albeit less elevated than in MPEs, and there is some evidence to support the use of VEGF in differentiating between the two [55].

Few prognostic indicators have demonstrated strong predictive value. Prognostic indicators currently include the type of cancer, cell type, tumor stage, extent of pleural tumor involvement, presence of adhesions and performance status [106-110]. Several features of pleural fluid, including low pH and low glucose are associated with a poor prognosis [111-113], as are failed attempts at pleurodesis [114, 115]. Pleural effusion fluid levels of VEGF have been shown to carry prognostic significance in malignant pleural mesothelioma, with levels greater than or equal to 2000 pg/mL associated with a poor prognosis [116].

## Standard Treatment Options in MPE

Following the diagnosis of MPE, management decisions are based on the volume and symptomatic impact of the effusion. Whereas small, asymptomatic effusions can be observed, if the patient is suffering from breathlessness, drainage of the effusion by thoracocentesis should be pursued. If there is rapid reaccumulation of a symptomatic effusion (less than 1 month), the risks and benefits of permanent pleural drainage should be considered. Treatment modalities include placement of a tunneled in-dwelling pleural catheter (TIPC, PleurX®), chemical or physical pleurodesis. Uncommon management strategies include surgical pleurectomy or the placement of a pleuroperitoneal shunt.

While talc pleurodesis has long been the preferred treatment modality, its safety and efficacy have recently been challenged [117]. A systematic review of the Cochrane database demonstrated rates of approximately 90% successful pleurodesis in patients treated with chemical pleurodesis, with talc the preferred modality [118]. However, this may be an optimistic measure, as a recently published study instituting current pleurodesis guidelines and analyzed by intention-to treat revealed that only approximately one-third of patients clearly benefit from pleurodesis [119].

Increasingly, TIPCs have drawn attention for their potential value in the management of MPEs. A recently published prospective, randomized clinical trial comparing talc pleurodesis to placement of a tunneled in-dwelling pleural catheter demonstrated improved survival, effusion control and activity without dyspnea in the catheter-treated group [120]. While use as a first-line modality continues to be debated among some clinicians, TIPCs have also been shown to be cost-effective in patients with a life expectancy of six weeks or less [121]. A retrospective review of 355 patients with 418 tunneled pleural catheters demonstrated suboptimal control of MPEs, however, with only 75% and 50% control rates at one and six months, respectively [122]. And while a recently published systematic review indicated that TIPC appear to be effective in ameliorating symptoms (95.6%) with a low rate of complications, spontaneous pleurodesis was only achieved in 45.6% and the authors note that the evidence was low-quality, being primarily based on one case series [123]. These data indicate the continued need for the development of improved treatment modalities in MPEs.

In this context, eligibility criteria for currently available clinical trials should be considered as some of these therapeutic modalities may disqualify patients from enrollment into certain trials. In patients whose effusions re-accumulate very slowly, repeated thoracocentesis represents a viable treatment option. While these treatments can provide symptomatic relief [124], they do not address the underlying cause of the MPE.

The value of systemic treatment for MPE depends upon the underlying tumor type and the expected patient survival. While for some patients systemic chemotherapy may result in increased survival—particularly if the underlying malignancy is breast-, ovarian cancer or malignant lymphoma—for others these strategies are of limited palliative value. Consequently, there is a great need for the development of improved treatments, which may have the potential to improve the currently dismal survival in patients thus afflicted.

## Rationale for anti-VEGF therapy in MPE

Intrapleural therapy, as an alternate route of administration of traditional or novel chemotherapeutics and targeted agents, represents a potential modality for the management of MPEs. Studies investigating intrapleural 5-fluorouracil, taxanes, bleomycin, cytarabine, anthracyclines, platinum agents, etoposide, adenoviral-mediated interferon-beta and OK-432 (a product of heat-killed *Streptococcus pyogenes*) have been performed in patients with MPEs with mixed results and toxicity profiles according to the agents under investigation [125-137]. A recent trial in which staphylococcal superantigen of the enterotoxin gene cluster was instilled directly into the pleural space of 14 unselected patients with non-small cell lung carcinoma demonstrated an improvement in survival compared to talc pleurodesis (median survival of 7.9 months compared to 2.0 months respectively), with minimal toxicity [138]. Studies such as these have validated the feasibility of intrapleural delivery, and have paved the way for the application of more targeted therapeutics via the intrapleural route.

Multiple pre-clinical studies have been performed investigating the potential benefit of VEGF blockade in patients with malignant effusions (Table 1; see Gerber, 2005 [10] for a focused review). Several studies have demonstrated a significant decrease in ascites formation in mice with implanted with ovarian tumor cells that were treated with bevacizumab or A4.6.1, the murine equivalent of bevacizumab [139-141]. Virally-encoded murine A4.6.1 introduced directly into the pleural space in mice demonstrated a significant decrease in metastatic lung tumor volume and improved survival with VEGF antibody undetectable outside of the pleura and lung [142]. Studies in which mice were implanted with mouse breast cancer cells and subsequently treated with AF-493-NA, a goat anti-mouse VEGF antibody, also demonstrate significant reduction in malignant ascites formation [143, 144]. Mice given intraperitoneal injections of human colon cancer cells and treated with intraperitoneal DC101 (a mouse anti-VEGFR-2 antibody) demonstrated similarly positive results with reduction in ascites as assessed by ascites grading [145]. Yano and colleagues demonstrated decreased incidence and reduced formation of MPE in a mouse model of MPE secondary to human lung adenocarcinoma cell injection [146]. New Zealand rabbits with inflammatory pleural effusions that were treated with intrapleural bevacizumab combined with talc or silver nitrate pleurodesis demonstrated a significant reduction in pleural effusion formation compared to rabbits that did not receive bevacizumab [147]. However, it should be noted that several studies in animals have suggested that treatment with anti-angiogenic agents prior to attempted pleurodesis may reduce the success of pleural symphysis [148, 149].

Early clinical studies investigating the role of antiangiogenic therapy in MPEs are promising (Table 2). A case report by Pichelmayer and colleagues describes a patient with a massive non-malignant pleural effusion which responded dramatically to treatment with a single dose of 5 mg/kg intravenous bevacizumab (one of the approved doses for patients with colorectal carcinoma) [150]. Pichelmayer and colleagues also report another set of cases in which patients with malignant effusions were treated with the same dose of bevacizumab with no significant effusion reduction, however [151]. One of these patients was found to have significantly elevated levels of plasma VEGF even after treatment with bevacizumab. This observation, combined with the elevated levels of VEGF known to be present in both plasma

and effusion fluid of patients with MPE, prompted the treatment of two other patients with higher doses of bevacizumab (15 mg/kg). Both patients treated with this elevated dose experienced successful resolution of their effusions and dramatic reduction of serum and plasma VEGF levels (serum levels were evaluated in order to assess total VEGF load, including VEGF stored in platelets). Numnum and colleagues treated four patients with malignant ascites with 15 mg/kg bevacizumab every three weeks for palliative purposes. All four patients experienced symptomatic ascites relief with no grade 3 toxicities [11]. One of these patients also had a pleural effusion, although response of the pleural effusion to bevacizumab cannot be evaluated as this patient was treated with concomitant pleurodesis [personal correspondence]. More recently, a retrospective review of bevacizumab plus chemotherapy in patients with non-small cell lung cancer and MPE was published [152]. In this study, twelve of thirteen patients achieved MPE control for greater than 8 weeks, with a median progression-free survival time without effusion reaccumulation of 312 days. Such studies have demonstrated the clinical merit of targeted antiangiogenic therapies in MPEs and prospective clinical trials are currently in development.

## Conclusions

In conclusion, accumulating evidence implicates VEGF in the formation of MPEs. The preclinical data described herein suggest that there may be a potential benefit of intrapleural anti-VEGF therapeutics in the treatment of malignant pleural effusions.

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## Table 1

Pre-Clinical Studies of VEGF Blockade in Malignant and Non-malignant effusions

| Model (n) | Antiangiogenic Treatment | Outcome |
|---|---|---|
| Rabbits NMPE (50) [147] | Bev | Reduced effusion |
| Murine ovarian MA (35) [140] | Bev | Virtually no formation |
| Murine ovarian MA (21) [139] | A4.6.1 | No formation in 20/21 |
| Murine ovarian MA (6) [141] | A4.6.1 | Reduced formation in 6/6 |
| Murine breast MA (12) [143] | IP AF-493-NA | Reduced formation |
| Murine colon MA (10) [145] | IP DC101 | Reduced formation |
| Murine lung MPE (18) [146] | PTK 787 | No formation in 9/18 Reduced in remaining 9 |

Bev: Bevacizumab; IP: intrapleural; MA: malignant ascites; MPE: malignant pleural effusion; NMPE: nonmalignant pleural effusion. A4.6.1: murine equivalent of bevacizumab; AF-493-NA = Goat anti-mouse VEGF monoclonal antibody; DC101 = anti-mouse VEGFR-2 antibody; PTK 787 = VEGFR-2 tyrosine kinase inhibitor.

## Table 2

Early Clinical Studies of VEGF Blockade in Malignant and Non-malignant Effusions

| Patients (n) | Antiangiogenic Treatment | Outcome |
|---|---|---|
| NMPE (1) [150] | Bev 5 mg/kg[A] | Reduced in 1/1 |
| NMPE (1) [154] | Bev 5 mg/kg[B] | Resolved in 1/1 |
| MPE (2) [151] | Bev 5 mg/kg[C] | Not Resolved in 2/2 |
| MPE (13) [152] | Bev 15 mg/kg[B] | Controlled in 12/13 (92.3%) |
| MA (4) [11] | Bev 15 mg/kg[B] | Controlled in 4/4 |
| MA (2) [151] | Bev 15 mg/kg[A] | Resolved or Reduced in 2/2 |
| MA (1) [155] | Bev 15 mg/kg[B] | Resolved in 1/1 |
| MA (1) [156] | IP Bev 5 mg/kg[D] | Reduced in 1/1 |
| MA (9) [157] | IP Bev 5 mg/kg[D] | Resolved in 9//9 |

Bev: Bevacizumab; IP: intraperitoneal; MA: malignant ascites; MPE: malignant pleural effusion; NMPE: nonmalignant pleural effusion. Treatment schedule as follows:

[A] once;

[B] every three weeks;

[C] 5 mg/kg initial dose was followed by one more dose of 5 mg/kg in one patient and 10 mg/kg in the other;

[D] monthly.