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Review

Malignant Ascites in Ovarian Cancer and the Role of Targeted Therapeutics

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Abstract. *Ovarian cancer (OC) is the eighth most lethal gynecological malignancy and the main cause of gynecological cancer death in industrialized countries. Malignant ascites is often found in OC, with about 10% of patients suffering from recurrent OC. Tumor cells in OC-associated malignant ascites promote disease recurrence and patient mortality is mainly associated with widespread metastasis to serosal surfaces and accompanying peritoneal effusions. Targeted therapies have recently been developed as novel therapeutic options for malignant ascites. The tri-functional anti-epithelial cell adhesion molecule and anti-cluster of differentiation 3 monoclonal antibody catumaxumab has been assessed in the therapy of malignant ascites, and proven to significantly reduce the ascitic flow rate when applied into the peritoneal cavity. The anti-angiogenic targeted agent bevacizumab has also shown good effects in the symptomatic treatment of malignant ascites, significantly prolonging the time until the next paracentesis. Vascular endothelial growth factor (VEGF) Trap, or aflibercept, is a fusion protein that inhibits VEGF-receptor binding. Aflibercept has proven to be effective in reduction of ascites, diminishing clinical symptoms of ascites and prolonging the time to next paracentesis. All three agents we review in the present article are effective in symptomatic control of ascites, leading to a rapid reduction of effusion and prolonging the time interval between paracenteses. However, no improvement in overall survival was observed in any of the clinical trials reported. We, thus, conclude that further investigations on larger patient series are needed to clarify whether the reduction of ascites by these targeted agents leads to a prolongation in tumor-related survival or not.*

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Epithelial ovarian cancer (OC) is the eighth most lethal gynecological malignancy worldwide and the main cause of gynecological cancer death in industrialized countries (1). It is characterized by tumor spread to the peritoneum and the development of malignant ascites, and by a lack of specific symptoms in early disease (2). Worldwide, approximately 200,000 cases are diagnosed each year, yet 120,000 deaths are also caused by OC because of its detection at an advanced stage (3). Almost 70% of all patients present with advanced stage III and IV disease, although the late detection of OC does not stem from a lack of symptoms - however, the symptoms are rather non-specific. Most patients suffer from abdominal, gastrointestinal, urinary or pelvic pain, which rarely draws the attention of the examining clinician. This is the cause of the generally late detection of OC (4-6).

Incidences in Western countries have remained quite constant over the past three decades (7). Yet, a rise of OC incidence has been observed in Asian countries such as China and Japan (7, 8). This increase is most likely due to population expansion and the increasing proportion of the elderly population in these countries and so the number of gynecological cancers in Asia will presumably increase continuously in the coming decades (9). The mean age of patients with OC is 63 years, which varies only slightly between ethnic groups (10). Whilst until the age of 50 years OC is a rare disease, its incidence increases tremendously with the end of fertility (11); the incidence of OC continues to rise further after the age of 63 years.

Regarding subtypes of OC, serous adenocarcinoma accounts for 90-95% of all cases of OC and is a fast and aggressively growing malignancy (12). Currently, aggressive cytoreductive surgery is the standard therapy, followed by carboplatin and paclitaxel-based adjuvant chemotherapy, which is basically the same therapy procedure as for primary peritoneal cancer or fallopian tube cancer (13-15). Still, disease in most patients will eventually develop resistance to

chemotherapy, which has urged research on novel therapeutic modalities, since the overall survival for patients who undergo surgery and with adjuvant chemotherapy alone is only approximately 30% (16-18).

Early metatasis in OC occurs *via* direct expansion of the tumor to sites adjacent to the primary tumor (19). Epithelial-to-mesenchymal transition is involved in the formation of metastases, resulting in tumor cell migration to distant sites, followed by mesenchymal-to-epithelial transition for accumulation in the tissue where the metastasis is located (20, 21).

Malignant Ascites in Ovarian Cancer

Malignant ascites is frequently found in OC, but also in various other solid tumor entities (22-24). No consensus has yet been reached with respect to specific treatment of malignant ascites in patients with OC (25). According to the National Cancer Institute, malignant ascites is defined by accumulation of fluid containing cancer cells in the abdomen (26). There are also commonly high levels of lactate dehydrogenase in malignant ascites compared to non-malignant peritoneal effusions, which indicates the high proliferation rate of the tumor cells and rapid disease progression (27, 28). Of patients suffering from recurrent OC, about 10% are affected by malignant ascites (25). Malignant ascites occurs more commonly in OC than in any other tumor type; OC is known to develop intraperitoneal metastases (29, 30). There are several traditional therapy options for ascites, comprising salt restriction, diuretics, radioactive isotopes, paracentesis and shunt placement (25). However, these methods have only a limited therapeutic impact and may cause significant toxic adverse effects (25).

As the capsule of the ovarian tumor disrupts, and malignant cells are dispersed into the peritoneal cavity, the cells survive as single cells or as free-floating multicellular aggregates, called spheroids, within the ascites (19). These spheroids become adherent to the mesothelial extracellular matrix, which permits them to anchor as secondary lesions on pelvic organs (31, 32). Tumor cells in OC-associated malignant ascites trigger disease recurrence. Patient mortality is mainly associated with widespread metastasis to serosal surfaces and accompanying peritoneal or pleural effusion (33-35). The effusion accumulates as a result of lymphatic obstruction, activation of native mesothelial cells by the metastatic process, and of increased vascular permeability, mediated by vascular endothelial growth factor (VEGF) and interleukin 6 and 8 (36-40). Furthermore, the tumor cells themselves accumulate at the peritoneal surface, causing mechanical obstruction and inhibiting absorption of the intraperitoneal fluid. In malignant ascites, secretion of peritoneal fluid is enhanced *via* stimulation by VEGF (41).

A study performed by Shen-Gunther and Mannel assessing the frequency of malignant ascites in ovarian malignancies found that while malignant ascites is rarely observed with FIGO stage I OC (17%), it is found in the vast majority of patients with stage II/III tumors (89%) (2). However, that study did not distinguish epithelial from non-epithelial neoplasms. Frequently reported symptoms of malignant ascites include anorexia, abdominal bloating, dyspnea and respiratory distress, fatigue, insomnia and abdominal pain (30, 42). Normal management of malignant ascites is generally unsatisfactory. Repetitive paracentesis of the intraperitoneal fluid provides only temporary relief and is not satisfactory because of the lack of causal therapy, which requires repeated drainage, depending on the severity of ascites. The loss of protein and hypovolemia also increase the frequency of circulatory problems. Finally, the risk of bowel perforation during paracentesis is of course higher if conducted more frequently (43). Thus, advances in understanding the mechanisms triggering malignant OC-associated effusion and the development of novel therapies are mandatory to improve the outcome of OC patients with malignant ascites.

Targeted-therapies have recently been developed as promising alternative therapeutic options for malignant ascites. Due to the knowledge that angiogenesis contributes considerably to ascites formation, anti-angiogenic agents have been tested for this purpose. Bevacizumab and the novel agent VEGF Trap have been investigated, and clinical efficacy has been proven in cohorts of heavily pretreated patients (25). The tri-functional antibody catumaxomab has also shown positive results in the treatment of malignant ascites.

In Depth Review of Existing Data

Pathology and biology of malignant ascites. In a study by Latifi *et al*., it was demonstrated that in malignant ascites there are adherent tumor cells as well as non-adherent ones (44). The aim of their investigation was to separate these two types in culture. Therefore, 25 patients were recruited, amongst them 11 chemonaive and 14 chemoresistant individuals. Interestingly, the adherent tumor cells within the ascites fluid expressed rather mesenchymal features whereas the non-adherent cells were of epithelial phenotype as they expressed epithelial cell adhesion molecule (EpCAM) and cytokeratin 7 (44). Mice were then intraperitoneally injected with either the adherent or the non-adherent cells. The mice that were injected with non-adherent cells developed tumors and malignant ascites within 12-14 weeks. On the contrary, mice that were injected with the adherent cells remained tumor-free for up to 20 weeks (44).

In a study by Simpson and colleagues, it was demonstrated that ovarian tumor-associated ascite fluid inhibits T-cell receptor-induced nuclear factor-kappa B (NF-κB) and nuclear

factor of activated T-cell (NFAT) signaling in tumor-associated T-cells (45). In fact, human memory T-cells present in ovarian tumor ascites do not respond properly to stimulation *via* the T-cell receptor. Thus, NF-κB and NFAT activation decreases, as does the proliferation of these immunosuppressed T-cells. Interestingly, the anergy of T-cells in ascites is due to soluble factors within the fluid. As these T-cells are assayed in the absence of ascites, they gain their normal function, and this effect is rapidly reversed when ascitic fluid is added to the T-cells (45). This might explain why human tumors grow despite the presence of T-cells and other cells of immunological response. The immunosuppressive effect of cellular or soluble biological factors on T-cells, and the accumulation of these immunosuppressed cells in tumors has already been proven (46).

Simpson *et al*. also demonstrated that the signaling arrest of NF-κB and NFAT is located upstream of phospholipase C, as the phosphorylation pattern of normal T-cell receptor signaling was compared to that of T-cells within the ascites. Moreover, T-cells derived from normal donor peripheral blood were incubated with (cirrhotic) ascitic fluid and exhibited the same signaling arrest of the T-cell receptor (45). Thus, it is suggested that ascitic fluid has an immunosuppressive effect on T-cells, causing their anergy to various stimuli. Targeting the soluble factors that cause immunosuppression of T-cells would certainly be a future therapeutic option for the treatment of OC.

Another experimental method that could be a future therapeutic option in OC-associated ascites is targeting the diphtheria toxin gene in OC cells (47). In a study by Mizrahi and colleagues, the expression of the diphtheria toxin gene was targeted under the control of H19, imprinted maternally expressed transcript, regulatory sequences. *H19* RNA occurs abundantly in human cancer tissues, including OC, whilst *H19* expression is nearly non-existent in normal tissue (47). The therapeutic value of the diphtheria toxin vector (DTA-H19) was tested in OC cell lines and also in an animal model for OC. In the animal model it was found that intratumoral injection of DTA-H19 caused 40% inhibition of tumor growth (47). A standard treatment for OC-associated ascites does not exist because a drug for the treatment of malignant ascites needs to be highly specific for tumor cells, not attacking adjacent normal cells. Thus, this novel method could help eliminate tumor cells within the fluid without affecting normal tissue, since H19 is almost exclusively expressed in malignant cells.

Davidson and colleagues have investigated active biochemical events in malignant OC-associated ascites and pleural effusion (48). Patients with malignant effusion due to OC were enrolled in that study and divided into two groups. Group 1 had effusion at presentation, whereas group 2 was diagnosed with effusion at first recurrence. Expression and activation of selected signaling proteins within the effusion samples were studied *via* protein microarrays using antibodies (48). Malignant effusions (*i.e*. >80% malignant cells) were differentiated from benign effusions. The malignant effusion samples featured higher expression of protein kinase B, activated extracellular signal-regulated kinase, cyclic adenosine monophosphate-responsive element-binding protein and c-JUN *N*-terminal kinase. Interestingly, there was no difference in signaling profiles between pleural effusion and ascites. In group-1 patients, high expression of p38 and a high phosphorylated-to-non-phosphorylated epidermal growth factor receptor (EGFR) ratio was associated with poor survival, whereas the quantity of phospho-c-JUN N-terminal kinase was associated with poor outcome in group 2.

This study shows that there is distinct dysregulation in proliferation, survival and apoptosis signaling in OC effusion samples, and that some of the signaling proteins may impact the patients' outcome. With this knowledge, the authors wanted to expedite the invention of novel targeted therapeutics against OC-associated ascites (48). In the patients with chemoresistant disease that were included in this study, selective epithelial, mesenchymal and cancer stem cell markers were investigated and compared between the adherent and the non-adherent tumor cells. The non-adherent cells demonstrated enhanced mRNA expression of E-cadherin, epithelial cell adhesion molecule, signal transducer and activator of transcription 3, and octamer-binding transcription factor 4, whereas the adherent cells showed increased mRNA expression of cluster of differentiation 44, matrix metallopeptidase 9 and also of octamer-binding transcription factor 4. Patients with chemoresistant tumors had more tumorigenic epithelial, non-adherent cells in the ascitic fluid than non-tumorigenic adherent cells (44). It was also evident that the non-adherent, epithelial cells featured increased mRNA expression of cancer stem cell-associated genes. Since cancer cells in OC-associated ascites are associated with disease recurrence, the information provided by this study might contribute to a better understanding over the cell biology of the tumor cells within the ascites.

Perhaps targeting the epithelial cells in the ascitic fluid would benefit a patient's prognosis, prolonging survival. This might also explain why the novel tri-functional antibody catumaxomab is beneficial for patients with OC, since catumaxomab selectively kills epithelial tumor cells.

Catumaxomab. The tri-functional monoclonal antibody catumaxumab has been assessed in the therapy of malignant ascites. This monoclonal antibody to epithelial cell adhesion molecule and cluster of differentiation 3 significantly reduces the ascitic flow rate when applied into the peritoneal cavity, and evidently, the frequency of paracentesis was reduced for the investigated patients (43, 49). Catumaxumab functions by selectively binding to tumor cells, since the mesothelium

that lines the peritoneal cavity does not express epithelial cell adhesion molecule. Cancer cells in OC-associated malignant ascites express epithelial cell adhesion molecule in 70%-100% of all cases (50). This leads to an effective attack of tumor cells by the immune system and reduced fluid production (43). Moreover, catumaxomab binds to accessory cells, such as dendritic cells, natural killer cells and macrophages, with its Fc fragment (22). The principle of the drug's bispecific mode of action is its hybrid character, combining two half-antibodies of mouse immunoglobulin G2a and rat immunoglobulin G2b (22). Via a tri-functional approach, catumaxomab exerts major histocompatibility complex-specific killing of epithelial tumor cells (22). Since epithelial cell adhesion molecule is expressed in most solid, but not in mesothelial-derived tumors, treatment with catumaxomab is dependent on the tumor type.

There are three events which occur when catumaxomab is used in malignant ascites: One antigen-binding site, namely mouse immunoglobulin G2a, recognizes the tumor-specific antigen; the other binding site, rat immunoglobulin G2b, binds to CD3, which is a part of the T-cell receptor complex; the Fc fragment then binds to FcγR type I- and III-positive cells, for example to macrophages, dendritic cells, or natural killer cells (51-54).

As previous trials have demonstrated, patients suffering from malignant ascites that were treated with catumaxomab experienced a significant prolongation of paracentesis-free survival (22). Catumaxomab has been approved in Europe for intraperitoneal treatment of malignant ascites, given that the tumor is of epithelial origin and features positivity for epithelial cell adhesion molecule, and given that no other treatment strategies are feasible (22).

The first trial investigating the effect of intraperitoneal catumaxomab administration was conducted with eight patients (two of them suffering from OC) with malignant ascites (55). Catumaxomab was administered intraperitoneally over 6-8 hours for at least four cycles. Seven out of the eight patients enrolled did not require any further paracentesis during the entire follow-up period or until death. The mean period of time until paracentesis was 38 weeks. In all patients, ascites completely disappeared (55).

After this study, a multicenter phase I/II clinical trial was conducted to explore the tolerability and efficacy of intraperitoneal catumaxomab in patients with OC with malignant ascites containing epithelial cell adhesion molecule-positive tumor cells (43). Twenty three women with therapy-refractory OC suffering from recurrent ascites received 4-5 intraperitoneal infusions of catumaxomab (5-200 μg over 9-13 days). Good response rates were observed, as 22 out of 23 patients did not require paracentesis between the last catumaxomab infusion and the end of the study after 37 days (43). Fever, nausea and vomiting were the most frequently reported grade 2 and 3 adverse events observed in this trial.

In a prospective, randomized Phase II/III study, the efficacy of catumaxomab-plus-paracentesis was evaluated in comparison to paracentesis alone in patients with malignant ascites (50). Catumaxomab was given *via* an intraperitoneal catheter directly after paracentesis at dosages of 10, 20, 50 and 150 μg on days 0, 3, 7 and 10. The end-point to evaluate the efficacy was paracentesis-free survival (50). Other evaluated parameters included the time to repetition of paracentesis, signs and symptoms of ascites, and overall survival. In patients treated with catumaxomab, paracentesis-free survival was significantly longer, lasting a median of 46 days, compared to controls (11 days). Furthermore, time to the next paracentesis was also significantly longer in the catumaxomab-group, with a duration of 77 days, than in the control group (13 days). Patients treated with catumaxomab also featured fewer signs and symptoms of ascites than the placebo group. The most commonly observed adverse effects were fever, pain, nausea and vomiting. One patient experienced grade 3 gastric hemorrhage.

Referring to the results from the trials listed above, catumaxomab was approved by the European Medicines Agency for the treatment of malignant ascites in patients suffering from epithelial cell adhesion molecule-positive tumors, given that standard therapy was no longer effective (56).

In a phase II/III trial conducted by Ott and colleagues, 5258 patients with malignant ascites were treated with catumaxomab (57). Evidently, catumaxomab showed distinct clinical benefit compared to paracentesis, and the adverse effects observed were manageable. Human antimouse antibodies (HAMAs) were evaluated in the blood of catumaxomab-treated patients as a parameter for humoral response, since catumaxomab is a mouserat antibody. It was shown that there were HAMA-positive and HAMA-negative patients in the catumaxomab group. HAMAs were not detected in the controls. In the HAMA-positive and HAMA-negative catumaxomab-treated patients as well as in the controls, paracentesis-free survival, time-to-next puncture and overall survival were evaluated and compared (57). A strong correlation was found between HAMA-positivity and prolonged paracentesis-free survival, prolonged time-to-next puncture and improvement in overall survival. In the HAMA-negative patients and in the controls, the evaluated parameters were significantly worse compared to the HAMA-positive group. The authors conclude that humoral response to catumaxomab, evaluated by the presence of HAMA in the patients' blood, strongly correlates with the clinical outcome (57).

Anti-angiogenic agents in malignant ascites. In healthy individuals, there is a balance in pro-angiogenic and anti-angiogenic signals (excluding wound healing and embryonic development), providing a quiescent (peri)vascular environment (25). In the tumorous

microenvironment, the pro-angiogenic signaling cascade dominates the anti-angiogenic pathway, resulting in the formation of new blood vessels (58, 59). When the tumor is more than 1-2 mm in diameter, angiogenesis becomes essential for tumor growth (60). Angiogenesis is mainly regulated by members of the VEGF family, consisting of growth factors and receptors, and ascites formation is also dependent on VEGFs. Tumor vessels are rather disorganized, contorted and tend to leak (61). As VEGF-dependent signaling is blocked, the formation of malignant ascites also decreases (62). The expression of VEGF has been detected in OC in various analyses, and furthermore, the degree of VEGF expression has been demonstrated to be associated with poor prognosis (63, 64).

As cancer cells proliferate, the secretion of VEGF is triggered and promotes neovascularization to ensure nutrient delivery to the tumor, facilitating metastasis. It has been shown that in disseminated intra-abdominal metastatic disease, cancer cells produce an increased load of peritoneal fluid and microvascular permeability is augmented (25). This results in significant ascites. According to Zebrowski and colleagues, VEGF proteins are increased in malignant peritoneal effusion in comparison to non-malignant cirrhotic ascites (65).

It has already been shown in mouse models that the inhibition of VEGF signaling is associated with distinct reduction of ascites formation, and a decrease in tumor burden (41, 66, 67). In animal models, it was also evident that VEGF production of cancer cells correlates directly with tumor cell-induced production of ascitic fluid (68). Referring to these findings, the use of bevacizumab for the therapy of malignant ascites was investigated in heavily pretreated individuals suffering from OC (69).

Bevacizumab. In a study by Numnum *et al*., four patients with recurrent OC and ascites were treated with the monoclonal antibody against VEGF, bevacizumab (69). All four patients responded to this therapy, experiencing symptomatic relief of ascites. After therapy with bevacizumab had been initiated, no therapeutic paracenteses were required, according to the follow-ups of up to six months (69).

Moreover, Hamilton and colleagues have reported a case where a patient with advanced, recurrent OC and severe symptomatic ascites was treated with intraperitoneal bevacizumab. After the administration of two doses of bevacizumab, the patient experienced symptomatic relief and improvement of their quality of life (70).

Two other case reports described the off-label use of bevacizumab in 10 patients suffering from therapy-refractory ascites and significant disease burden (71). Symptomatic improvement was observed in all individuals and lasted for 2-6 months approximately (68).

El Shami and colleagues conducted a case study where the safety and tolerability of bevacizumab, administered intraperitoneally, was tested in nine patients with refractory ascites due to colorectal, breast, uterine and ovarian cancer (68, 72). Astonishingly, the malignant effusion was eliminated in all of the patients after only one dosage, not featuring recurrence over an observation period of more than two months (72).

VEGF Trap. The fusion protein VEGF Trap has also been investigated in the context of refractory malignant ascites. VEGF Trap, also named aflibercept, prevents VEGF receptor binding *via* incorporation of the second binding domain on the VEGFR1 receptor and of the third domain of the VEGFR2 receptor (73). VEGF Trap is consequently fusing these protein sequences to human IgG. Thereby a chimeric protein with a high affinity for VEGF is created (73, 74). In pre-clinical xenograft models, VEGF Trap inhibited tumor growth and angiogenesis, reduced blood vessel density and inhibited metastasis (41, 75, 76).

Hu and colleagues tested VEGF Trap as therapy for ascites in a mouse model of OC (77). After the intraperitoneal administration of VEGF Trap and paclitaxel, the effusion was rapidly regressive and the tumor burden was reduced by 98% (77). In mice that were primarily treated with VEGF Trap, no measurable ascites formation was observed. Tumor vessel imaging was performed, and the vessels in the treated mice were rather sparse and short, whereas the vessels were numerous, irregular, contorted and leaky in the controls (77). Byrne and colleagues also showed that therapy with VEGF Trap as single-agent caused a significant reduction of ascites and tumor burden in experimental models of OC (41).

A phase I clinical trial was conducted to evaluate the safety and tolerability of VEGF Trap (78). In total, 47 patients were enrolled in that study. Amongst them 14 were patients suffering either from OC, peritoneal or fallopian tube cancer. VEGF Trap was administered intravenously at a dosage ranging from 0.3 mg/kg to 7.0 mg/kg, every two weeks. The recorded adverse effects comprised of fatigue, nausea and vomiting. Three out of the 14 patients showed partial responses. The study authors concluded that VEGF Trap was well-tolerated at the investigated dose levels (78).

Furthermore, there were several single-agent and combined phase II clinical trials, investigating the safety and efficacy of VEGF Trap in malignant ascites. Patients with various solid tumors causing ascites were tested and amongst them, patients suffering from OC (79, 80). VEGF Trap was also tested in two recent studies where only patients with epithelial OC suffering from malignant ascites were tested: Colombo and colleagues assessed the safety and efficacy of VEGF Trap in 16 patients with advanced chemoresistant epithelial OC and symptomatic ascites in an open-label phase II trial (81). The 16 patients that were enrolled in the study

all required paracentestes 1-4 times per month. Treatment consisted of intravenous VEGF Trap at a dosage of 4 mg/kg every two weeks (81). The primary end-point of this trial was the time until paracentesis was required, and response was defined as at least two-fold increase of the period of time until paracentesis compared with the period of time required at baseline. Ten out of 16 patients experienced response according to this definition. The median time-to-paracentesis was 76 days, being 4.5-times longer than the period at baseline. The observed adverse events were hypertension, headache, anorexia and dysphonia. Two individuals experienced high-grade treatment-related adverse effects, namely severe hypertension and weight loss, and in one of the patients, intestinal perforation occurred (81).

In another trial that was conducted by Gotlieb *et al*., VEGF Trap was explored in the treatment of malignant ascites in patients with advanced OC. In this double-blind, placebo-controlled, parallel-group phase II study, patients with OC and recurrent symptomatic malignant ascites were randomly enrolled, and received either intravenous VEGF Trap at a dose of 4 mg/kg every two weeks or placebo (82). The time-to-repeat paracentesis was considered as the primary end-point of this trial. Fifty-five patients were enrolled, and 26 of them received placebo, whereas 29 received VEGF Trap. The median time until paracentesis had to be repeated and was significantly longer with VEGF Trap, compared to placebo. Interestingly, two patients receiving VEGF Trap did not need any repeat paracentesis for a period of six months. However, three out of the 29 patients treated with VEGF Trap experienced gastrointestinal perforation (82).

Discussion and Conclusion

It is evident that malignant ascites play a key role in ovarian tumorigenesis, especially in the formation of metastases to peritoneal organs, and distant organs (19, 33-35). Referring to this, novel therapeutic opportunities have been found for the treatment of malignant ascites, aiming to improve not only signs and symptoms of ascites, but also disease-free and overall survival of patients with OC.

Catumaxomab has shown good effects in the treatment of malignant ascites (22, 43, 50, 55, 57). Most notably, paracentesis-free survival and the time to the next paracentesis were prolonged significantly under catumaxomab treatment (22). However, only one investigation showed an effect on overall survival, namely that patients responding to catumaxomab therapy by expressing HAMAs have a significant prolongation in overall survival compared to patients not expressing HAMAs (57). No investigation has shown that catumaxomab prolongs overall survival compared to other treatment modalities for malignant ascites. The side-effects of catumaxomab were manageable in most reported cases. However, there is the risk of a high-grade gastric hemorrhage, as was reported by Heiss and colleagues (49).

For the use of bevacizumab in patients suffering from malignant ascites, no effect on overall survival has been demonstrated (68-72). Bevacizumab has proven effects on ascites formation, prolonging the time to paracentesis, and the patients treated with bevacizumab experienced improvement of their quality of life (70).

VEGF Trap has been shown to be effective against malignant ascites in an animal model (78). In clinical trials, aflibercept significantly prolonged the median time to paracentesis, as reported by Colombo *et al*., where the period of time was increased 4.5-fold (81). Notably, treatment with VEGF Trap can cause severe adverse effects, such as severe hypertension, weight loss and gastric or intestinal perforation (81, 82).

The novel therapeutic approaches for OC-associated malignant ascites that we have discussed in this review evidently cause symptom relief. However, treatment goals always have to be weighed against patient discomfort and, first of all, against potentially severe adverse effects. For the use of targeted therapeutics in malignant ascites, it is mandatory to carefully select patients, and to identify their risk factors so that the incidence of adverse effects can be minimized. Further comparative analyses and the assessment of the patients' quality of life are the next steps that need to be taken before these novel agents are incorporated into daily clinical practice.

Furthermore, clinical trials on larger patient series need to be conducted to clarify whether catumaxomab, bevacizumab and VEGF Trap are useful not only for the symptomatic relief but also for the prolongation of tumor-related overall survival.

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