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*Review*

# Targeted Treatment of Ovarian Cancer - The Multiple - Kinase - Inhibitor Sorafenib as a Potential Option

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**Abstract**. *Ovarian cancer (OC) is ranked as the eighth most common gynecological malignancy and is the leading cause of gynecological cancer-related deaths in women worldwide. The response to platinum- and taxane-based chemotherapy is very often poor, and targeted-therapeutics are currently being tested in patients with OC. Sorafenib is a non-selective multiple kinase inhibitor with proven antiproliferative effects in thyroid, renal and hepatocellular carcinoma. Sorafenib acts on vascular endothelial growth factor (VEGF) and on platelet-derived growth factor (PDGF) related pathways. It also influences the rat sarcoma proto-oncogene/rat fibrosarcoma protein kinase/mitogen activated protein kinase (RAS/RAF/MAPK) pathway and blocks tumor growth factor beta-1 (TGF-β-1)-mediated epithelial-mesenchymal transition (EMT). Sorafenib also acts at the epigenetic level altering the histone acetylation pattern. There have been phase I, II and III studies investigation sorafenib in OC. We review several trials in which sorafenib has been administered as single-agent or combined with other chemotherapeutics. Unfortunately, the effect of sorafenib was usually modest and complete response was rarely observed. Adverse effects occurred frequently, including rash, diarrhea, edema and weight gain. Sorafenib evidently blocks EMT in vitro. However, in the conducted trials, sorafenib was administered to patients with highly advanced tumors. We posit that blocking EMT may be more effective in early-stage disease. We also presume that sorafenib would work particularly well in the treatment of clear cell OC, since this type of OC has different molecular characteristics from usual OC and is less sensitive to standard chemotherapy. Furthermore, the combination of sorafenib with other multiple-kinase inhibiting agents, e.g. ABT-869, a targeted-agent mainly acting in the VEGF and PDGF pathways, should be investigated in further detail. It is probable that synergistic effects can be achieved.*



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Ovarian cancer (OC) is an umbrella term for malignancies that originate from the ovary, comprising various histopathological subtypes. They differ in their biological behavior and thus also in their response to current treatment modalities. Response to treatment is often poor, even though the clinical outcome normally depends more on the tumor stage than on the histological type (1). The current classification of ovarian neoplasms includes three tumor types, according to their histological differentiation, namely epithelial, sex-cord/stromal, and germ cell neoplasms. Epithelial OC is the most common sub-type, accounting for about 85% of all ovarian neoplasms (2). It is ranked as the eighth most common female malignancy and is the leading cause of gynecological cancer deaths in women worldwide. OC is especially prevalent in industrialized nations (3). Currently, radical surgery is the established treatment strategy for the management of ovarian tumors. Patients first undergo a staging procedure according to the current classification and correspondingly, cytoreductive debulking of the tumor is performed. This is usually followed by platinum- and taxane-based adjuvant chemotherapy (4, 5). The tumor mass remaining after surgery is the most important prognostic factor (6). In FIGO stage I, curative unilateral salpingo-oophorectomy (USO) can be performed in order to retain fertility (7, 8). Most patients present with FIGO stages III or IV. In these cases, patients have to undergo abdominal hysterectomy and bilateral salpingo-oophorectomy (BSO) along with omentectomy. Additionally, lymphadenectomy, as well as sampling of the peritoneal fluid, is mandatory (8). This procedure is followed by chemotherapy. In well-differentiated stage IA

or IB disease, adjuvant chemotherapy is not recommended since >90% of the patients survive progression-free longer than 10 years after tumor resection (9). Platinum-based regimens are preferred for stage IA or IB high-grade tumors (10). Today's standard first-line chemotherapy of advanced OC is the combination of platinum (usually carboplatin) and a taxane (usually paclitaxel), given intravenously every 21 days for six cycles (11-13). However, the recurrence rate of advanced OC is high despite treatment (14). Current research is, therefore, focusing on new treatment options to enhance chemotherapy outcome in OC, especially by targeting of specific molecules. In the recent past, the introduction of the VEGF antibody bevacizumab into chemotherapeutic treatment regimens has resulted in statistically significant positive effects on progression-free survival (PFS) in patients enrolled in phase II and phase III clinical studies, compared to control groups receiving standard chemotherapy only (15, 16). Studies by the International Collaboration on Ovarian Neoplasms (ICON-7) and the Gynecologic Oncology Group (GOG-218) demonstrated prolonged PFS compared to the classic platinum- and taxane-based chemotherapy for patients with OC (17). The GOG reported in a double blind randomized phase III trial that adding bevacizumab to conventional chemotherapy increased the median PFS compared to the control group by about four months, with the hazard ratio for death or progression being 0.717 [95%/CI=0.625 to 0.824; $p<0.001$] compared to the controls, who received chemotherapy only (17, 18). Aghajanian *et al*. showed in a multi-center phase III study including 484 patients, that the PFS of patients treated with bevacizumab was improved compared to the controls, with 12.4 and 8.4 months respectively; the hazard ratio of progression was 0.484 [95%/CI=0.388 to 0.605; $p<0.0001$] (19).

## Sorafenib Acts on Various Pathways Related to Carcinogenesis and Tumor Progression

Sorafenib is a non-selective multi-kinase-inhibitor, which has proven anti-proliferative effects in thyroid cancer, renal cell carcinoma (RCC) and in hepatocellular carcinoma (HCC) (20, 21). It is an antibody designed to inhibit signaling in the VEGF and PDGF receptor pathways. It exerts its effects by binding to tyrosine kinases and the Raf kinase, resulting in cell-cycle inhibition and is thereby attenuating tumor growth (22). In a randomized clinical study, Escudier *et al*. showed, that sorafenib can extend the PFS in patients by approximately 2.7 months as compared to the control group (23). Therefore, sorafenib was approved for the treatment of RCC (in 2005) and HCC (in 2007) by the US Food and Drug Administration (FDA) (23, 24). It has been shown that sorafenib effectively prolongs survival in patients with advanced HCC (20). There have been two large randomized, double-blind, placebo-controlled, multi-center phase III trials that clearly provided evidence for the efficacy of sorafenib in prolonging median overall survival and also in delaying the median time to progression in patients with HCC (24-26). Sorafenib has also been investigated in combination with bevacizumab in metastatic breast cancer (27). This combination was found to cause severe toxic effects, with 50% of the patients reporting grade 3 toxicity. The side-effects comprised of hypertension, gastrointestinal toxicity, neuropathy, rash, pain and wound complications. Complete or partial response was not observed in any of the patients. Therefore, the authors did not recommend further investigation of the sorafenib/bevacizumab combination for metastatic breast cancer (27). Schwartzberg and colleagues investigated the addition of sorafenib to either gemcitabine or capecitabine in patients with advanced, Her2-negative breast cancer who had progressive disease despite treatment with bevacizumab (28). A clinically modest, but statistically significant benefit in PFS was observed (28).

The RESILIENCE phase III trial is currently investigating the addition of sorafenib to first- or second-line capecitabine therapy for advanced stage, Her2-negative breast cancer (29). Sorafenib may also be of relevance with respect to the treatment of peritoneal cancer (30). In mouse models for epithelial growth factor receptor (egfr-) for Her2-overexpressing and for RAS/RAF mutant breast cancer, sorafenib acted synergistically with the pan-cyclin-dependent kinase-inhibitor flavopiridol. Mice treated with both drugs showed reduced primary tumor growth rates and reduced metastatic tumor loads (31). Sorafenib exerts its antitumoral activity on the one hand *via* direct effects on cancer cells, and on the other hand, *via* indirect effects on endothelial cells (32,33). Sorafenib acts at the VEGF receptor (VEGFR) the PDGF receptor (PDGFR), at the fms-related tyrosine kinase-3 (FLT3) and at v-KIT Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (c-KIT) (30).

Sorafenib is administered orally as a bisaryl urea (30). The anti-tumoral activity of sorafenib is most probably due to the inhibition of tumor growth and angiogenesis by blocking several receptor tyrosin kinases (23, 24, 34). Zhang *et al*. found, that sorafenib also inhibits the coordinated epigenetic switching in the RAS/RAF/MAPK pathway and in the ERBB signaling pathway (35). To examine whether sorafenib could impair TGF-β-induced EMT in epithelial cells, human alveolar (A549) epithelial cells, a lung adenocarcinoma cell line, have been used. A549 epithelial cells are an ideal *in vitro* model for assessing EMT and carcinogenesis. According to this investigation by Zhang and colleagues, sorafenib probably has much broader effects than currently supposed. As a matter of fact, sorafenib has been demonstrated to also act at the epigenetic level and to alter gene expression (35). Table I gives a brief drug-summary about sorafenib (Table I).

Table I. *Drug summary of sorafenib.*

| Sorafenib: Drug Summary | |
|---|---|
| Name | Sorafenib |
| Chemical formula | $C_{21}H_{16}ClF_3N_4O_3$ |
| Chemical structure | |
| Administration route | Oral |
| Dosage | 400 mg b.i.d. |
| Indication | 2005: FDA approval for advanced renal cell carcinoma.<br>2006: Marketing authorization given by the European Commission for renal cell carcinoma.<br>2007: Marketing authorization given by the European Commission for hepatocellular carcinoma; FDA approval for hepatocellular carcinoma. |

## TGF-β-1-mediated EMT of Cancer Cells Is Blocked by Sorafenib

Sorafenib is the first oral chemotherapeutic agent that has the power to regulate gene expression and cellular activity *via* epigenetic reprogramming of DNA by histone modification. Thereby, sorafenib strongly inhibits tumor growth and angiogenesis (35).

Sorafenib has been demonstrated to significantly inhibit tumor growth and tumor invasion of adjacent tissue, because it prohibits EMT (35). EMT is believed to be essential for the progression of malignant diseases as it alters cell adhesion, cytoskeleton re-modeling, cell migration and MAPK-signaling (35-37). Whenever tumor cells that are derived from the epithelium lose their specific function and acquire mesenchymal features, the primary tumor progresses towards metastasis. The epithelial cells lose their characteristics in the process of EMT and become more motile and invasive (38, 39). It has been demonstrated that in OC, tumor cells undergo EMT, which is accompanied by invasive growth and metastasis. Thus, EMT plays a crucial role in ovarian carcinogenesis and blocking this process with sorafenib might be beneficial for a patient's outcome (40). TGF-β1-induced EMT is probably the most common route leading to tumor metastasis. TGF-β influences wound healing, cell proliferation and differentiation and controls apoptosis (38, 41-44). In early tumor stages, TGF-β works as a tumor suppressor as it inhibits cell growth and induces apoptosis. However, in advanced stages, TGF-β is a tumor promoter, because tumor cells cannot be growth-arrested by TGF-β, undergo EMT and become invasive. EMT is then induced by TGF-β1 (38). Sorafenib has been demonstrated to suppress TGF-β1-induced EMT in alveolar epithelial cells (35, 45).

## The Anticarcinogenic Mechanism of Histone Deacetylase Inhibitors (HDACIs) Resembles the Effect of Sorafenib

De-acetylation of histones plays an important role in various mechanisms of tumorigenesis, including cell-cycle control, apoptosis, angiogenesis and invasive growth (46, 47).

The regulation of transcription in eukaryotic cells is mainly based on acetylation and de-acetylation of histones (48). The most important regulators of the histone acetylation pattern are histone acetyltransferases (HATs) and histone deacetylases (HDACs). The acetyl group from the amino acid lysine in the histone tail is removed by HDACs. Consequently, the chromatin structure becomes more compact and gene transcription is repressed, as access to the transcription factors is then more difficult (49-51). HDACIs have been developed as therapeutic agents in cancer treatment (52). HDACIs act at the epigenetic level, inhibiting cell growth and proliferation similarly to sorafenib (47). Interestingly, HDACIs alone did not bring significant benefit in clinical use. There is even evidence for EMT induction by HDACIs, thus leading rather to tumor aggressiveness when administered as single-agents (49). Therefore, it is advisable to always combine HDACIs with drugs that specifically block EMT. As a consequence, sorafenib, as an EMT-blocking agent, is an option for a combination with HDACIs.

It has been shown by Zhang *et al*. that the expression levels of several HDACs (HDAC1, HDAC2, HDAC4, HDAC5 and HDAC8) were enhanced in cells undergoing EMT. In cells treated with sorafenib, however, there was no enhancement of these HDACs, suggesting that sorafenib probably blocks a vast variety of HDACs (35). In another trial by Tang *et al*. it was demonstrated that sorafenib synergistically acts with HDACIs in the elimination of CNS

tumor cells (53). Furthermore, Gahr *et al*. reported on a patient with metastatic HCC who was treated with both sorafenib and an HDACI, and consecutively showed a partial remission of the primary tumor and the metastasis for five months (54). A synergistic effect of HDACIs with sorafenib in clinical use is probable, however, this has to be investigated in further detail.

## Sorafenib Unfolds Its Anti-Tumoral Activity by Interacting with EMT-Associated Genes

During EMT, sorafenib continuously restores the changes that result from histone modification (35, 55). In critical EMT-associated genes, sorafenib suppresses the coordinated epigenetic switching, *i.e*. the switch from E-cadherin to N-cadherin expression, which is a characteristic of EMT (35, 56-58). Zhang and colleagues performed a study where markers of active euchromatin (H3K4me3 and H3K9ac) were determined in the promoter region of E-cadherin and N-cadherin in A549 alveolar epithelial cells, an established *in vitro* model for EMT. The markers were assessed on the protein level *via* western-blotting and *via* chromatin immunoprecipitation followed by high throughput sequencing (ChIP-seq). As expected, both the markers were decreased in the E-cadherin promoter and increased in the N-cadherin promoter as the cells underwent EMT. Moreover, Zhang *et al*. showed an increased expression of the adhesion molecules OB-cadherin and CDH19 from the cadherin superfamily in their promoter regions. These epigenetic changes that were obviously induced by EMT were impressively blocked when the cells were treated with sorafenib (35).

Apart from E-cadherin and N-cadherin, there are many other EMT-associated genes, which we briefly describe here: One of the most important EMT-associated genes is keratin-19 (*KRT19*), which shows decreased expression in tumor tissue and is highly associated with metastasis (56). Keratins are epithelial markers whose down-regulation leads to EMT (59). Another important EMT inducer is twist basic helix-loop-helix transcription factor 1 (*TWIST1*), which promotes EMT by repressing E-cadherin expression. Loss of E-cadherin makes cells less adherent to adjacent structures and gives them the potential to migrate (60). The association of *TWIST1* expression and metastasis is a well-established phenomenon (60, 61). The transcription factor-3 (*TCF3*) gene is also known to repress E-cadherin and it is known to be involved in the cellular acquisition of mesenchymal features (62). *ERBB3*, which is a member of the EGFR family, has the potential to heterodimerize with other *ERBB* receptors. Thereby, it can switch on pathways that consecutively promote cell proliferation and differentiation (63, 64). However, some studies also indicate that *ERBB3* can promote apoptosis and therefore prohibit tumor growth and metastasis (65-67). Down-regulation of the *CDH1* gene that leads to loss of E-cadherin expression is also associated with EMT (35). Increased expression of the zinc finger E-box binding homeobox 1 and 2 genes (*ZEB1*, *ZEB2*), both repressing E-cadherin, and increased expression of the ERBB receptor feedback inhibitor-1 (*ERRFI-1*) gene, which is associated with cell growth and of sirtuin 1 (*SIRT1*), a gene that can de-acetylase histones and silence genes of cell cycle regulation, thereby blocking apoptosis and the elimination of damaged cells, also leads to EMT (35).

Additional genes that are associated with EMT are caldesmon 1 (*CALD1*), that is a promoter for cell proliferation, loss of desmoplakin (*DSP*), which is normally responsible for cell-to-cell adherence and snail homolog 2 *SNAIL2*, that encodes a protein which represses E-cadherin and thus has anti-apoptotic effects. Furthermore, secreted protein, acidic, cysteine-rich (*SPARC*), that makes cells change shape and thereby promotes tumor cell invasion and metastasis is involved in EMT (56). Members of the integrin family alter the cell-cell or cell-extracellular matrix adhesion in TGF-β-mediated EMT and also promote tumor invasion and metastasis (68).

Many genes that play a role in cell contact are known to be involved in EMT and carcinogenesis. For example, it has been demonstrated that desmoplakin (*DSP*) and plakophilin-1 (*PKP1*) are under-expressed in OSCC cells (69). Deletion of *DSP* has been shown in a mouse model of pancreatic neuroendocrine carcinogenesis (70). Occludin, claudins and junctional adhesion molecules are transmembrane proteins that form tight junctions. In the course of carcinogenesis, these tight junction proteins are altered, changing their position and the gene expression levels (71-75). For instance, claudins are up- or down-regulated depending on the tumor entity, or are located differently in breast, colorectal and pancreatic cancer (71-75). Occludin is down-regulated in various tumors and is distincty associated with EMT (76-79). Zonula occludens protein 1 (ZO-1) is also up- or down-regulated in many types of tumors (80). Furthermore, adherens junctions are important for sustaining cellular adhesion and changes in the expression or function of adherens junction components are associated with invasive growth of cancer (81-83).

In conclusion, it is suggested that sorafenib interferes with TGF-β1-induced EMT *via* the inhibition of targeted kinase phosphorylation and also *via* the inhibition of transcription of EMT-related genes at the epigenetic level (33, 35). This effect has been shown for some members of the E-cadherin family, but further research is needed to demonstrate the blocking of transcription by sorafenib for other EMT-associated genes.

## Histone-modifying Enzymes Are Regulated by Sorafenib

Sorafenib has an impact on the function of histone-modifying enzymes, namely HATs, and thereby potentiates histone acetylation (35, 84). As described above, Zhang *et*

*al*. have assessed markers of active euchromatin in TGF-β1 stimulated cells (35). After TGF-β1 stimulation, there was an increase of the marker H3K27me3. In TGF-β1 stimulated cells that were treated with sorafenib, this effect was reversed because of the sorafenib-mediated prohibition of epigenetic switching in these cells (35). Sorafenib prohibited the epigenetic switching by interacting with the promoters of TGF-β1, *SNAIL* and snail homolog-2 (*SLUG*) (35). In this study, significant differential histone modification regions (DHMRs) were crossmatched between the controls, TGF-β1-stimulated cells and TGF-β1-stimulated plus sorafenib-treated cells. The DHMR signals were highly divergent between control and TGF-β1-treated cells, but in TGF-β1 plus-sorafenib-treated cells there were hardly any differences compared to the controls.

Proteins of the extracellular matrix (ECM) promote the formation of carcinomas by the induction of cellular transformation and metastasis (85). Sorafenib effectively blocks the epigenetic switching in the promoter regions of several ECM genes, such as collagen type-I alpha-1 (*COL1A1*) and collagen type-V alpha-1 (*COL5A1*).

## Sorafenib in Ovarian Cancer - Review of Clinical Data

Since sorafenib inhibits the kinases c-RAF and b-RAF which participate in the MAPK pathway that is activated in OC, this drug is used in patients with OC in combination to standard (platinum and taxane-based) chemotherapy or as single-agent (86, 87). The RAS/RAF/MAPK pathway is activated *via* RAS and b-RAF, predominantly in ovarian tumors with low malignant potential, *i.e.* low-grade serous, mucinous and clear-cell OC (88-90). In high-grade lesions of the ovary, the pathway is activated rather by overexpression of c-RAF, which is a predictor of poor prognosis.

Several phase I studies have been conducted where the effect of sorafenib in treatment of solid tumors has been investigated (91-97). Six among of studies also included OC (91, 92, 94-98). In these trials, the patients were treated with sorafenib once- or twice-a-day at different dosages (about 50-800 mg per single dose). Complete response was rarely observed, and it was evident that patients only benefited from sorafenib treatment when continuously treated with a dosage around 100 mg *b.i.d.*

Phase II studies on sorafenib in OC have unfortunately demonstrated only minimal benefit (30, 99-104).

*The impact of sorafenib in combination with classic chemotherapy.* A multi-center, phase I study evaluated the efficacy of the combination of sorafenib with gemcitabine in patients with advanced solid tumors (95). In this trial, two patients with OC that had been pre-treated with taxane, platinum and anthracycline therapies experienced a partial remission (95). Welch *et al*. designed a study in which the safety and efficacy of the combination of gemcitabine with sorafenib should be tested (101). A total of 43 patients with recurrent epithelial OC were included. Two patients experienced a partial remission and 10 out of the 43 patients maintained response or stable disease for at least six months. Even though prolonged stable disease was observed, the low overall response rate (4.7%) did not reach the threshold to be considered effective (101).

In another phase I trial the safety and pharmacokinetics of the combination of sorafenib with irinotecan was evaluated (96). In 60% of the patients, sorafenib and irinotecan led to disease stabilization (96).

In a phase II trial by Ramasubbaiah *et al*., sorafenib with weekly topotecan was investigated in patients with platinum-resistant OC and in patients with primary peritoneal cancer (99). Topotecan was administered intravenously at a dosage of 3.5 mg/m$^2$ and sorafenib was administered orally at 400 mg per day. Partial remission was observed in one patient, whereas 10 patients experienced stable disease. Nine out of the latter patients remained stable for more than three months (99).

In another phase II trial sorafenib was co-administered with carboplatin and paclitaxel in a neoadjuvant setting (100). Four patients were included using a 3-week interval schedule. Sorafenib was administered at a dosage of 400 mg *b.i.d.* and cytoreductive surgery was performed thereafter. Following surgery, one cycle of carboplatin and paclitaxel was planned followed by another three cycles of carboplatin and paclitaxel in conjunction with sorafenib. However, after surgical treatment, all patients had to be dismissed from the study because of severe toxic side-effects. They included cardiac output failure and myocardial infarction. Moreover, 2 out of 4 patients had primary progressive disease. On the basis of these observations, the study had to be stopped (100).

*Sorafenib combined with bevacizumab.* Azad *et al*. conducted a study in which the combination of sorafenib and bevacizumab was investigated in patients with advanced solid tumors, including 13 patients with OC (97). Six (46%) out of the 13 OC patients experienced partial remission (97). In a study by Lee *et al*., 19 patients with epithelial OC received sorafenib and bevacizumab at different dosages. Eight out of 19 patients (42%) experienced partial remission, on average lasting for ≥4 months, and seven out of 19 (37%) reported stable disease lasting for ≥ 4 months. Thus, an overall benefit was observed in 15 out of 18 (79%) of the patients with OC (98).

Kohn *et al*. also evaluated the combination of sorafenib and bevacizumab in a phase II trial (102). Among 25 investigated patients, 6 had partial remission, lasting for a median of 15.5 months. In 16 women, stable disease that lasted for a median of 5 months was reported.

Table II. *Common adverse effects of sorafenib, observed in a trial by Matei et al. in 71 evaluated patients (30).*

| Adverse Effect | Symptoms | Frequency (n=71) | Relative frequency (%) |
|---|---|---|---|
| Gastrointestinal | Diarrhea, nausea, vomiting, loss of appetite | 56 | 79 |
| Dermatological | Rash, redness, itching, peeling skin, hand-foot skin reaction (redness, pain, swelling or blisters on the palms of the hands or soles of the feet), wound healing promblems | 54 | 76 |
| Metabolic | Weight gain or weight loss, edema | 43 | 61 |
| Pain | Skin pain, Pain in the stomach | 32 | 45 |
| Cardiovascular | High blood pressure, failure of cardiac output, QT prolongation | 24 | 34 |
| Anemia | | 22 | 31 |
| Neuropathy | Pathological skin sensations (tingling, burning, numbness) | 18 | 25 |
| Thrombocytopenia | | 15 | 21 |
| Leukopenia | | 13 | 18 |
| Neutropenia | | 10 | 14 |

*Sorafenib as single agent*. In a phase I, dose-escalation study by Strumberg *et al*., some patients experienced stable disease for up to >1 year, and in one patient that suffered from RCC disease even stabilized for more than two years (91).

In a trial by Awada *et al*., sorafenib was administered for 21 consecutive days followed by seven days off treatment. In this study, 44 patients with refractory solid tumors were included and one of them was suffering from OC. Half of the patients experienced stable disease, but only 6% of the patients stabilized for more than 1 year (92).

In another phase I study by Moore *et al*., 41 patients with solid tumors were evaluated, including 10 patients suffering from OC. Only 22% of the patients treated with sorafenib experienced stable disease (with an average duration of 7.2 months), whereas the other 78% experienced deterioration of their disease (94).

In an open-label, multi-institutional phase II study, the impact of sorafenib in patients with recurrent OC or primary peritoneal cancer was evaluated (30). A total of 59 patients were included in the evaluation for drug efficacy and received 400 mg of sorafenib *b.i.d*. Two out of 59 patients experienced partial remission and 20 out of the 59 had stable disease, lasting for a mean of 6.14 months (30).

The efficacy, safety and tolerance of sorafenib as single-agent used as third-line therapy was evaluated in patients with epithelial OC or primary peritoneal cancer (103). Sorafenib was administered to 11 patients at a dosage of 400 mg *b.i.d*. in a 4-week cycle. None of the patients experienced partial or complete remission, nor stable disease lasting for longer than 6 months. The study was cancelled before the planned period of time was reached (103).

Recently, a randomized, double-blind, placebo controlled phase II study was conducted to analyze the effectiveness of maintenance therapy with sorafenib in OC (104). A total of 246 patients with either epithelial OC or primary peritoneal cancer in complete remission were randomized to sorafenib 400 mg *b.i.d*. or to placebo. In this study, 39 events of progression were observed in the sorafenib group and 68 events of progression in the placebo group. Evidently, there was no significant difference between sorafenib and placebo (104).

*Phase III trials*. In a phase III trial that was carried out by Matei *et al*., sorafenib was administered at a dosage of 800 mg per day in a group of patients with recurrent or persistent OC and peritoneal cancer (30). In all patients, ERK and b-RAF were expressed in the respective tumors. A total of 24% of the investigated patients survived for at least six months without progression, but the other patients responded only partially to sorafenib therapy, or their disease remained stable or became progressive (30). Unfortunately, severe toxic side-effects were observed in this trial, most commonly metabolic side-effects, such as weight gain or increased appetite. Table II summarizes common side-effects of sorafenib. Diarrhea is one of the most frequent side-effects, occurring in up to 43% of the patients (23). Diarrhea was also observed in a trial where sorafenib was administered to patients with advanced HCC. Interestingly, in this study the occurrence of sorafenib-associated grade 2 or 3 diarrhea was significantly associated with better overall survival as compared to patients with grade 0 or 1 diarrhea, respectively (105).

None of the other side-effects correlated with better outcome (105). Hypophosphatemia may also be caused by sorafenib and occurs in up to 45% (23). It is possible that sorafenib induces exocrine pancreatic dysfunction that leads to vitamin D malabsorption and secondary hyperparathyroidism, explaining the hypophosphatemia (106). Vitamin D screening and pancreatic enzyme replacement should, therefore, be considered during sorafenib treatment (106).

Another phase III trial was set out in which the impact of sorafenib in patients with recurrent OC was evaluated. Some patients definitely responded to sorafenib therapy and many patients at least remained free of progression for several months during sorafenib intake. However, in many patients toxic effects were observed. Most commonly, dermatological toxicity and metabolic abnormalities occurred. Dermatological side-effects in this study comprised of rash, severe pain in the palms and soles, swelling in the face or tongue, or general skin pain (32, 107). The metabolic side-effects were edema, rapid weight gain or weight loss, and increased appetite (32, 107).

## Discussion and Conclusion

Sorafenib evidently blocks EMT *in vitro*. However, also the reverse mechanism, namely mesenchymal epithelial transition (MET) is crucial for tumor metastasis (108, 109). As cells have undergone EMT and migrated to the tissue where they form the metastases, they need to undergo MET, re-expressing epithelial features in order to persist and multiply in that tissue (110). It is proposed that EMT is responsible for the former steps of cancer metastasis, whereas MET induces the latter (110). Considering this sequence, in metastatic disease, it might be necessary not only to block EMT but also to reverse MET. This might explain why sorafenib did not show good effects in metastatic OC so far. We posit that a drug that predominantly inhibits EMT might exhibit a stronger tumor-damaging effect at a non-metastatic tumor stage. Sorafenib could be beneficial in first-line OC therapy, combined with conventional chemotherapeutics. Whether sorafenib brings any advantage to first-line therapy still needs to be investigated in detail.

It is furthermore advisable to investigate the impact of sorafenib in clear cell OC, which has different molecular characteristics from other types of OC (111). The tumor biology of clear cell OC differs considerably from the biology of serous adenocarcinoma and it has also been reported, that it is less sensitive to standard chemotherapy (112). Ovarian serous carcinoma is thought to originate from the fallopian tube, while clear cell carcinomas are associated with endometriosis and display mutations similar as in atypical tissues of endometriosis (113, 114). For this reason it is assumed that ovarian serous carcinomas originate from neoplastic cells within endometriotic tissue rather than from the ovarian epithelium (115). It has also been demonstrated that clear-cell OCs comprise heterogeneous subsets that feature different DNA copy number abnormalities (116). Depending on these mutations, some types of ovarian clear cell carcinomas are more chemosensitive and are associated with a better prognosis than others (117). Therefore we suggest that further clinical research should be done on targeted therapy in the subsets of clear cell OC. We posit that sorafenib would be preferentially effective in clear cell carcinoma as compared to other types of OC, effectively inhibiting tumor growth and reducing tumor size. Such a result has already been shown in an animal model of clear cell OC (118).

We propose that sorafenib would act synergistically in combination with other kinase-inhibiting agents. For example, ABT-869, a new drug that competitively inhibits receptor tyrosine kinases, acting on mainly the VEGF and PDGF pathways, showed good anti-carcinogenic effects *in vitro* and in animal models (119, 120). In a phase I trial, ABT-869 showed distinct benefit in solid tumors, including lung cancer and HCC (119). Dovitinib is another multiple-kinase inhibitor and target of topoisomerase I and II that is currently being tested in phase III trials (120). Dovitinib is soon to be scheduled for the treatment of various cancer types (120). Another example is ENMD-2076, a novel small molecule kinase inhibitor that, like sorafenib, acts at various pathways (121). It has effects on angiogenesis, proliferation and on the cell cycle, and inhibits tumor growth in tumor xenograft models of breast and colon carcinoma, as well as of melanoma, leukemia and multiple myeloma (121). Provided that cumulative toxicities do not occur, we propose that sorafenib, in combination with other kinase-inhibiting drugs, might probably have higher efficacy than in some of the previous studies reported in this review.

The reviewed data still point out that sorafenib does not offer much benefit in OC treatment and there is a considerable risk that sorafenib will not be very effective in combination with other kinase inhibitors either. For OC treatment, several pathways of tumorigenesis have been found where targeted agents are available, and on-going studies are investigating these agents. Drugs with their action point in the VEGF and EGFR pathways, and also CA-125-, cell surface-associated mucin 1 (MUC1)-, folate-receptor-α- and epithelial cell adhesion molecule (EpCAM)-targeting therapeutics provide novel treatment options for OC (122). Further targeting agents are emerging which could also offer clinical benefit in OC. For example, ipilimumab, a blocker of the cytotoxic T-lymphocyte antigen 4 (CTLA4), can enhance the adaptive immune response to evolving cancer because CTLA4 normally inhibits immune effector cells (123, 124). The clinical data on CTLA4 blockers in OC shows that in some patients CA-125 levels significantly decreased due to the action of this agent and in a study where patients with FIGO stage IV were treated with ipilimumab, three out of nine patients experienced stable disease (more than 6, 4 and 2 months respectively) (125). At present, a phase II trial is ongoing, where ipilimumab as monotherapy in patients with recurrent, platinum-sensitive OC is investigated (122). The recognition of cancer by the immune effector cells can furthermore be enhanced by monoclonal antibodies that recognize cancer cells themselves

in order to activate immune effector cells. Catumaxomab is such a bi-specific antibody that recognizes EpCAM and CD3 molecules (124, 126). The data show, that intraperitoneal application of catumaxomab reduces malignant ascites with EpCAM-positive tumor cells in patients with OC (127). A positive trend in overall survival was also observed in patients with OC treated with catumaxomab (128).

To date, it is not only monoclonal antibodies that give future perspectives for a better management of OC. So-called 'peptibodies' are artificially-engineered molecules consisting of a functional peptide which is chimerized with the Fc immunoglobulin fragment carrier domain. The most promising peptibody that has been developed over the past decade is AMG 386 which interferes with angiopoietin and therefore has antiangiogenic effects, prohibiting blood vessel formation and tumor growth (122). In contrast to anti-VEGF-targeting agents, bleeding or thromboembolic effects have not been reported as adverse effects of AMG 386 in clinical trials (129). AMG 386 is currently being investigated in OC in combination with standard chemotherapy (122).

To conclude, it is evident that many targeted-agents may offer benefit in the treatment of OC. In many of the trials that have been conducted so far, the investigated patients were suffering from advanced-stage disease and the trials were not well-standardized. Further randomized, double-blind and placebo-controlled trials, enrolling large patient cohorts, are advisable to determine the benefit of emerging targeted therapeutics exactly. We posit that most of these drugs would work most effectively if administered as first-line therapy in combination with standard chemotherapy, and in patients at an early tumor stage.

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