Review Article

Clinical advances in the development of novel VEGFR2 inhibitors

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Abstract: Angiogenesis inhibitors have produced significant advances in the treatment of several tumors including colorectal, lung, ovarian and renal carcinomas. These agents, however, modestly impact on the overall cure rate, and their activity is often limited because of the early outbreak of redundant pathways or resistance mechanisms. Moreover, no clear predictive factor has been identified for treatment selection in the clinic. Preclinical evidence suggest that antibodies targeting the vascular endothelial growth factor (VEGF) axis may exert their activity throughout the inhibition of VEGF receptor 2 (VEGFR2) phosphorylation, a key factor in the cancer angiogenic process. Among other molecules, ramucirumab, an intravenously administered, fully humanized monoclonal antibody (mAb) targeting the extracellular domain of the receptor, and apatinib, a potent oral inhibitor of the intracellular domain, are emerging as original antiangiogenic opportunities. This up-to-date review focuses on the development of VEGFR2 inhibitors across multiple cancers and presents results of the most recent researches, ranging from early phase I studies to randomized phase III trials, in which those drugs have been tested as a single-agent or in combination with different chemotherapy regimens.

Keywords: Angiogenesis; vascular endothelial growth factor receptor 2 (VEGFR2); antibodies anti-VEGFR2; ramucirumab; apatinib; gastric cancer; lung cancer; breast cancer

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Introduction: understanding the molecular mechanisms of vascular endothelial growth factor receptor 2 (VEGFR2)-mediated angiogenesis

Cancer angiogenesis is a fundamental process for the tumor growth as it ensures oxygen and nutrients supply to proliferating cells through the development of new blood vessels, potentially causing cancer progression and metastasis (1). The vascular endothelial growth factor (VEGF) family, which includes VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PlGF), is a group of key proteins involved in the angiogenic pathway. VEGF and its receptor are highly expressed in many tumor types, including cancer of the gastrointestinal tract (2). Therefore they may represent potential targets for anticancer therapy. Sustained VEGF expression leads to the development and maintenance of a vascular network that promotes tumor growth and metastases. Environmental factors like hypoxia, inflammatory cytokines, low pH as well as the silencing of specific tumor suppressor genes (*PTEN*, *p53*, *VHL*) or the activation of oncogenes (e.g., *RAS*, *SRC*, *EGFR*, *HER2*) result in increased VEGF production (3).

The VEGF family members bind three different tyrosine kinase (TK) receptors: VEGFR1 (Flt-1), VEGFR2 (Flk-1-KDR), and VEGFR3, which is expressed on the lymphatic and vascular endothelium (*Figure 1*).

VEGFR2, a type II transmembrane TK receptor expressed on endothelial cells and on circulating bone marrow-derived endothelial progenitor cells, is the principal mediator of the VEGF-induced angiogenic signaling. This receptor contains three different parts, including an Ig-like domain extracellular region, a hydrophobic transmembrane region containing the TK domain and the carboxyl terminal tail. VEGFR2 binds all VEGF-A isoforms, VEGF-C and VEGF-D. Contrarily, VEGFR1 is a selective ligand for VEGF-B and PlGFs peptides (*Figure 1*). Also the binding affinity of VEGF

ligands to their receptors is increased by the presence of the two non-enzymatic co-receptors neuropilin (NRP)-1 and NRP-2 (4). Since NRP receptors expression correlates with tumor aggressiveness and poor prognosis, these molecules are currently studied as potential antiangiogenic targets (5). Compared to VEGFR1, VEGFR2 has a lower affinity for VEGF but at the same time it has a stronger kinase activity.

The binding of VEGF-A to VEGFR2 induces a cascade of different signaling pathways. The dimerization of the receptor and the following autophosphorylation of the intracellular TK domains lead to the simultaneous activation of PLC-γ-Raf kinase-MEK-MAP kinase and PI3K-AKT pathways, causing cellular proliferation and endothelial-cell survival.

Additionally, a soluble circulating form of VEGFR2 may be found in the human plasma (6).

Strategies for blocking these pathways include the use of specific inhibitors (antibodies or small molecules), which may either bind VEGF or interfere with the different domains of VEGFR.

Over the last decade, a number of monoclonal antibodies and small molecules that specifically target the VEGF pathway have been studied as single agents or in combination with chemotherapy. Bevacizumab, for example, is a monoclonal antibody (mAb) that binds VEGF and it has gained worldwide approval for first- or second-line treatment in several different tumor types, mainly in association with standard chemotherapies (1).

VEGFR2 is also a novel target. Biological and preclinical evidence suggests that the blockage of VEGFR2 could be a promising strategy to inhibit tumor-induced angiogenesis (7). In order to proof this hypothesis, a rat mAb against murine VEGFR2 named DC101 was developed. DC101 specifically interferes with the binding of VEGF to VEGFR2, inhibiting VEGF-induced signal, and strongly blocks tumor growth in mice (8). However, since DC101 is not able to bind the human VEGFR2, the hybridoma technology was used to produce molecules for clinical use (9). Among these antibodies, ramucirumab (IMC-1121B) was the only one tested in human subjects.

Ramucirumab is a novel human IgG1 mAb that selectively inhibits the VEGFR2 and blocks the VEGFR2-related signaling and activating pathways (*Figure 1*) (10). Preclinical models showed that ramucirumab selectively binds to the extracellular domain of human VEGFR2 with half-maximal inhibitory concentration of 0.8 to 1.0 nM (11) and it has a 8-fold higher affinity for VEGFR2 when compared with its natural ligands (12). Pharmacokinetic

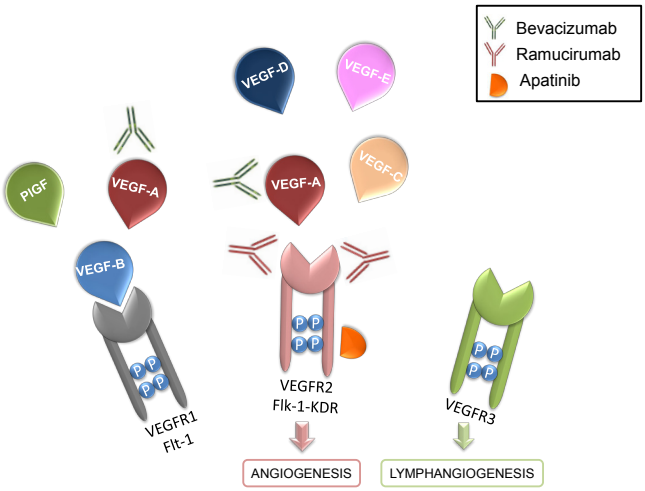

Figure 1 Natural ligands, target receptors and antiangiogenic compounds of the VEGF family. VEGF-A, vascular endothelial growth factor-A; VEGF-B, vascular endothelial growth factor-B; VEGF-C, vascular endothelial growth factor-C; VEGF-D, vascular endothelial growth factor-D; VEGF-E, vascular endothelial growth factor-E; PlGF, placental growth factor; VEGFR1, vascular endothelial growth factor receptor 1; VEGFR2, vascular endothelial growth factor receptor 2; VEGFR3, vascular endothelial growth factor receptor 3.

evaluation has demonstrated a nonlinear pharmacokinetic, with incremental doses of this agent being associated with a decrease in clearance (10). Pharmacodynamic evaluations have confirmed the increase in the VEGF ligand, along with the decrease in VEGFR2, after administration of ramucirumab (13). It has a potential therapeutic role in many different tumor types, including gastric cancer, with a favorable toxicity profile. The results of several trials demonstrated its efficacy not only in association with chemotherapy but also as a single agent (4).

Many small molecules may also inhibit VEGFR2, even if the inhibition is not specific. Among those agents we should mention sorafenib, that is approved for first-line treatment of renal cell carcinoma (RCC) and hepatocarcinoma (HCC), sunitinib, that is approved for RCC and gastrointestinal stromal tumors (GIST), and pazopanib, recently approved for RCC and soft tissue sarcoma (14). Moreover, apatinib is a small molecule that may specifically inhibit VEGFR2 (15). Several ongoing trials are testing the efficacy of VEGFR2 inhibitors. In this review we report on the main studies that investigate the use of VEGFR2 inhibitors.

Phase I/II trials: moving the first steps in the clinical development

As VEGFR2 is the predominant mediator of VEGF-induced angiogenesis, its blockade has been extensively investigated. In murine models (16), DC101 was able to reduce tumor growth, to prevent cancer dissemination and to limit the neoangiogenic sprout. Notably, DC101 produced a 30% increase in median survival in mice with peritoneal metastases (17). Moreover, when added to continuous low-dose doxorubicin, DC101 inhibits angiogenesis and tumor growth in soft tissue sarcoma mouse xenografts (18). Preclinical models evidenced the possibility to combine DC101 with standard antiblastic agents. When combined with gemcitabine and administered to nude mice implanted with human pancreatic cancers, DC101 increased the rate of tumor cell death and decreased tumor cell proliferation (19). Similarly, the combination of DC101 and paclitaxel induced significant regression of transitional cell carcinomas implanted in athymic nude mice (20).

However, since DC101 could only link and block the murine VEGFR2, the clinical development of this antibody in humans was no further possible. In order to overcome this problem, a single chain variable fragment with human VEGFR2 reactivity was isolated from a phage library and named IMC-1C11.

IMC-1C11 was tested in early phase I trial enrolling 14 pretreated metastatic colorectal cancer (CRC) patients. Even if no grade 3 or higher treatment-related toxicities were reported with a weekly cycle dose range of 0.2-4.0 mg/kg, about 50% of patients developed antibodies against IMC-1C11, limiting the opportunity for further clinical tests (21).

In the scenario, ramucirumab has emerged as a new therapeutic option in solid tumors. In a pioneer phase I trial, 37 heavily pretreated patients received weekly escalating doses of ramucirumab, from 2 to 16 mg/kg, to evaluate the maximum tolerated dose (MTD) in humans. Since patients developed dose-limiting toxicities at 16 mg/kg, MTD was set up at 13 mg/kg. A total of 60% of patients developed grade 3 or higher adverse events (AEs), mainly consisting in hypertension (13.5%), abdominal pain (10.8%), anorexia, vomiting, headache, proteinuria, dyspnea, and deep venous thrombosis. Anticancer activity was seen in 11% of treated patients, with an overall disease control rate (DCR) of 73% (13).

Recently, safety data focusing on the use of ramucirumab in other phase I trials have been presented (22,23). In particular, toxicity data registered in 6 Japanese patients with metastatic breast cancer (mBC) exposed to ramucirumab (10 mg/kg every 3 weeks) plus docetaxel (75 mg/m^2 every 3 weeks) revealed that the combination is safe in Eastern patients. Similarly, an ongoing phase I trial aims to evaluate safety of ramucirumab, administered at escalating doses from 6 to 10 mg/kg q14-21 for 6 weeks, in Chinese patients with advanced solid tumors that are resistant to standard therapy (24).

Other early clinical trials are ongoing. A phase Ib/2 trial is currently enrolling patients with advanced gastric or gastroesophageal junction (GEJ) adenocarcinoma, HCC or RCC to receive ramucirumab in combination with LY2875358 (25). Another phase I trial is investigating the safety and tolerability of the combination of ramucirumab and FOLFIRI (irinotecan with fluorouracil and folinic acid) in Japanese metastatic CRC patients.

The positive results of early clinical trials stimulated further researches, as summarized in *Table 1*. To test its activity and evaluate the safety profile, Zhu *et al*. accrued 42 previously untreated HCC patients to receive ramucirumab at the dose of 8 mg/kg every other week until disease progression or unacceptable toxicities. Median progression-free survival (PFS) was 4.0 months (95% CI, 2.6-5.7), objective response rate (RR) was 9.5% (95% CI, 2.7-22.6); and median overall survival (OS) was 12.0 months (95% CI, 6.1-19.7). Grade 3 or higher AEs included hypertension (14%), gastrointestinal bleeding (7%), idiosyncratic reactions (7%), and fatigue (5%), suggesting that ramucirumab could be an active and well tolerated first-line option in HCC patients (26).

Another phase II study tested the activity of ramucirumab combined to modified FOLFOX6 (5-fluorouracil, leucovorin, and oxaliplatin) as first-line therapy in 48 advanced CRC patients. Overall RR was 58.3% (95% CI, 43.1-72.4), with a DCR approaching 95%; the median OS was 20.4 months (95% CI, 18.5-25.1). Severe AEs included neutropenia (33.3%), hypertension (16.7%), and neuropathy (12.5%) (33). Camidge *et al*. reported on the results of a phase II trial evaluating the activity of ramucirumab combined to paclitaxel and carboplatin as first-line treatment in 40 patients with stage IIIb/IV non-small cell lung cancer (NSCLC). Median PFS was 7.5 months (95% CI, 5.5-9.9), RR 55%. The most frequently reported AE were fatigue (53%), neuropathy (33%), nausea (28%), myalgias (23%) and minor bleeding (23%) (30). In another randomized phase II trial (31), 140 patients with recurrent or advanced NSCLC received a platinum-based chemotherapy with or without ramucirumab. Median PFS observed at the first pre-planned interim analysis was 6.3 months for patients

Table 1 Principal phase II trials

Trial identifier	Refs	Treatment	Schedule	Phase	Population	Endpoint	Results
NCT00627042	(26)	Ramucirumab	8 mg/kg every 14 days	II	Unresectable HCC patients with Child-Pugh score of A or B	PFS	4.0 months
NCT00515697	(27)	Ramucirumab	8 mg/kg every 14 days	II	Metastatic renal cell carcinoma patients who had previously received TKIs	Objective response rate	5.1%
NCT00721162	(28)	Ramucirumab	8 mg/kg every 14 days	II	Persistent or recurrent epithelial ovarian, fallopian tube, or primary peritoneal carcinoma in patients who had received a platinum-based regimen	Objective response rate, PFS	3.5 months
NCT00533702	(29)	Ramucirumab ± dacarbazine	10 mg/kg every 21 days ± dacarbazine 1,000 mg/m^2 every 21 days	II	Metastatic melanoma patients	PFS	2.6 *vs.* 1.7 months
NCT00735696	(30)	Ramucirumab + paclitaxel and carboplatin	10 mg/kg every 21 days + paclitaxel 200 mg/m^2 carboplatin (AUC =6)	II	Stage IIIB/IV non-small cell lung cancer patients	PFS	7.85 months
NCT01160744	(31)	Pemetrexed + carboplatin/ cisplatin/ gemcitabine + carboplatin/ cisplatin ± ramucirumab	Pemetrexed 500 mg/m^2 every 21 days/gemcitabine 1,000 mg/m^2 on day 1 and 8 every 21 days + carboplatin (AUC =6)/cisplatin 75 mg/kg every 21 days ±10 mg/kg every 21 days	II	Recurrent or advanced non-small cell lung cancer patients	PFS	4.3 *vs*. 6.3 months
NCT00683475	(32)	Ramucirumab/ IMC-A12 + mitoxantrone and prednisone	6 mg/kg on day 1, 8 and 15 every 21 days + mitoxantrone; 12 mg/m^2 every 21 days, prednisone 10 mg/day	II	AIPC patients following disease progression on docetaxel-based chemotherapy	PFS	10.8 *vs*. 13 months
NCT00862784	(33)	Ramucirumab + 5-FU/FA and oxaliplatin	8 mg/kg every 14 days + mFOLFOX6	II	Metastatic colorectal cancer patients	PFS	11.5 months
NCT01234402	(34)	Capecitabine ± ramucirumab/ IMC-18F1	Capecitabine 1,000 mg/m^2 twice a day for 14 days ± ramucirumab 10 mg/kg every 21 days/IMC-18F1 12 mg/kg on day 1 and 8 every 21 days	II	Unresectable, locally advanced or metastatic breast cancer patients previously treated with anthracycline and taxane therapy	PFS	/
NCT01427933	(35)	Eribulin ± ramucirumab	Eribulin 1.4 mg/m^2 on day 1 and 8 every 21 days ± ramucirumab 10 mg/kg every 21 days	II	Unresectable, locally-recurrent or metastatic breast cancer patients	PFS	4.4 *vs*. 4.1 months

HCC, hepatocellular carcinoma; PFS, progression free survival; TKI, tyrosine kinase inhibitor; AIPC, androgen-independent prostate cancer; 5-FU/FA, 5-fluoruracil/folinic acid.

who received ramucirumab and 4.3 months for patients enrolled in the control arm. Hypertension was more frequently reported in patients receiving ramucirumab (19% *vs.* 6%) compared to the others. A similarly designed trial is testing ramucirumab in combination with docetaxel in stage IV NSCLC patients who had reported disease progression after or during a platinum-based therapy (36).

Ramucirumab is being tested in women with metastatic BC as well, and two different phase II trials are ongoing to evaluate the additional benefit of ramucirumab combined to capecitabine (34) or eribulin (35) compared to the antiblastic agent alone. Recently presented data from the latter study demonstrated that there is no statistically significant difference in OS [13.5 *vs.* 11.5 months, hazard ratio (HR) 0.8; 95% CI, 0.5-1.3; P=0.4], PFS (4.4 *vs.* 4.1 months, HR 0.8; 95% CI, 0.6-1.2; P=0.4) or overall RR (20% *vs.* 24%).

A phase II single-arm trial enrolled 39 RCC patients to receive ramucirumab at 8 mg/kg q14 days until disease progression or intolerable toxicities. Overall RR was 5.1%, with a DCR of 64.1%. Median PFS was 7.1 months (95% CI, 4.1-9.7), and the median OS was 24.8 months (95% CI, 18.9-32.6). The most frequent severe AE included hypertension (7.7%) and proteinuria (5.1%) (27).

Ramucirumab was also studied in patients with persistent or recurrent ovarian carcinoma who had failed a platinum-based first-line treatment (28). In castration-resistant metastatic prostate cancer patients, the activity of ramucirumab was evaluated in combination with mitoxantrone and prednisone after failure of a docetaxel-based therapy. A median PFS of 6.7 months (95% CI, 4.5-8.3) was reported, with median OS of 13 months (95% CI, 9.5-16.0) (32). Preliminary data on the activity of the antiangiogenic drug in patients with metastatic cutaneous melanoma have been recently presented. One hundred and six patients were randomly assigned to receive dacarbazine with or without ramucirumab. PFS was 1-month longer (2.6 *vs.* 1.7 months) in patients who received the combination, and the toxicity profile was acceptable (29).

Phase III trial results: balancing meaningful advances with disappointing missteps

So far, ramucirumab is the only antibody against VEGFR2 to have reached the last phase of clinical development (4). The most notable results have been observed in patients with pretreated advanced gastric and GEJ cancers (37). REGARD is an international phase III placebo-controlled study that enrolled 355 patients with advanced gastric (75%) or GEJ (25%) adenocarcinomas. Patients were randomized with a 2:1 ratio to ramucirumab 8 mg/kg given every 2 weeks plus best supportive care (BSC) or placebo plus BSC as second-line treatment (37). Patients with either measurable or evaluable disease were enrolled. Randomization was stratified by weight loss, anatomic location of the primary tumor and geographical region. The final results were published after 278 patients had died. The addition of ramucirumab to BSC significantly prolonged median OS from 3.8 to 5.2 months, translating into a 22% reduction in the risk of death (HR 0.77; 95% CI, 0.60-0.99; P=0.0473). The study also met its secondary endpoints. Patients enrolled in the experimental arm had a longer PFS (HR 0.48; 95% CI, 0.37-0.62; P<0.0001) and a significant increase in DCR (48.7% with ramucirumab *vs.* 23.1% with placebo; P<0.0001), although the overall RR was similarly low in both treatment arms (3.4% *vs.* 2.6%) (37). Subgroup analysis showed consistent treatment effect among almost all subgroups. Interestingly, male patients exposed to ramucirumab seemed to have a greater survival benefit compared to that reported in female patients (HR for OS 0.67; 95% CI, 0.49-0.91 *vs.* HR 1.43; 95% CI, 0.85-2.40). At the 2014 Gastrointestinal Cancers Symposium, the preliminary results of RAINBOW trial, a large study focused on pretreated patients with advanced gastric or GEJ adenocarcinoma, were reported (38). The study randomized (1:1 ratio) 665 patients who had progressed while on or within 4 months of standard first-line treatment with a platinum-based chemotherapy to receive paclitaxel 80 mg/m^2 alone or in combination with ramucirumab 8 mg/kg given every 2 weeks. OS was the primary study endpoint. Stratification factors included geographic region, disease measurability, and time to progression on first-line therapy (<6 *vs.* >6 months). In the whole trial population, 398 patients were from Europe, Australia or North America, 223 were from the East Asia, and only 44 from South America. The trial met its primary and secondary endpoints with a 19% reduction in the risk of death (P=0.0169) and a 27% reduction in the risk of disease progression (P<0.0001) with the addition of ramucirumab to paclitaxel. Median OS was 9.6 months for the combination *vs.* 7.4 months for paclitaxel alone and median PFS was 4.4 months *vs.* 2.9 months, respectively. In addition, the DCR was 80% with paclitaxel plus ramucirumab *vs.* 64% with paclitaxel alone (P<0.0001). A similar proportion of patient received any post-discontinuation treatment: 47.9% in the ramucirumab plus paclitaxel arm *vs.* 45.4% in the paclitaxel alone arm.

In both these phase III trials ramucirumab was very well tolerated, with no unexpected toxicities and a safety profile in line with those of other antiangiogenic agents. In the REGARD trial 57% of the patients exposed to ramucirumab had grade 3-4 AEs compared with 58% in the placebo group. The most frequent treatment-related severe AE was hypertension (8% with ramucirumab *vs*. 3% with placebo); no grade 4 hypertension was recorded. Ramucirumab was not associated with increased rates of proteinuria, bleeding, venous thrombosis, or gastrointestinal perforation. In both groups, 2% of deaths were considered to be treatment-related. The preplanned quality-of-life (QoL) assessment analysis showed that 34% of patients given ramucirumab reported stable or improved conditions at the 6 weeks assessment compared with 13% in the placebo group. In the RAINBOW trial, more severe AEs were reported in the ramucirumab arm compared to the standard arm (82% *vs*. 63%). More specifically, grade 3-4 neutropenia was 40.7% in the combination arm *vs*. 18.8% in the other, leucopenia was 17.4% *vs*. 6.7%, and hypertension was 14.1% *vs*. 2.4%. However, these AEs did not lead to a higher rate of treatment discontinuation. The rates of treatment-related death were similar across treatment arms (4.0% *vs*. 4.6%).

Based on these results, on April 21st, 2014 the Food and Drug Administration approved single agent ramucirumab for the treatment of patients with advanced or metastatic, gastric or GEJ adenocarcinoma with disease progression on or after prior treatment with fluoropyrimidine- or platinum-containing chemotherapy (39).

Recently, therapies targeting angiogenesis have been also investigating in NSCLC. In the REVEL trial 1,242 patients diagnosed with NSCLC who had disease progression during or soon after an upfront platinum-based chemotherapy were randomized at a 1:1 ratio to receive docetaxel 75 mg/m^2 plus either ramucirumab (10 mg/kg) or placebo every 3 weeks (40,41). The primary endpoint of the study was OS and the secondary endpoints included PFS, overall RR, DCR, patient-reported outcomes, and the assessments of safety and tolerance of ramucirumab. Stratification factors were Eastern Cooperative Group (ECOG) performance status (PS) (0 *vs.* 1), gender, prior maintenance treatment (yes *vs.* no), and geographic origin (East-Asia *vs.* rest of the world). On February 19th 2014, a press release from Eli-Lilly Oncology announced that the study met its primary as well as secondary endpoints (42). The trial results have been recently presented at the 2014 American Society of Clinical Oncology (ASCO) Annual Meeting (43). The intention-to-treat analysis included 1,253 patient allocated to ramucirumab plus paclitaxel arm (n=628) or to the control arm (n=625); 1,245 treated subjects were included in the safety analysis. As expected, patients were well balanced between treatment arms. In particular, epidermal growth factor receptor (EGFR) status showed wild-type for 33% *vs* 31.5% of treated patients, 24% of patients in both treatment arms had been previously exposed to taxanes and around 14% to bevacizumab. Although the complete RR was negligible in both treatment arms (<1%), the overall RR was statistically superior in those exposed to ramucirumab (22.9% *vs.* 13.6%, P<0.001), as was the DCR (64% *vs.* 52.6%, P<0.001). The combination of ramucirumab and docetaxel produced a significant advantage in median PFS (4.5 *vs.* 3.0 months; HR 0.76; 95% CI, 0.67-0.86; P<0.0001) and in median OS (10.5 *vs.* 9.1 months; HR 0.85; 95% CI, 0.75-0.97; P=0.02), even if the proportion of patients who received any post-discontinuation therapy was similar between treatment arms (45.4% *vs.* 48.3%). The OS and PFS were consistent in most treatment subgroups, including squamous and nonsquamous histology. Importantly, the addition of ramucirumab to docetaxel did not result in an increase of serious AEs or AEs leading to death. Accordingly, the safety profile of the combination was not different from what expected, and the increase in specific antiangiogenic-induced toxicities was limited to the doubling of grade 1-2 bleedings.

Over the last decade, the use of antiangiogenic agents in metastatic BC has been intensively tested (44) and the most encouraging data come from the combination of bevacizumab with taxane, either paclitaxel (45) or docetaxel (46).

Accordingly, the ROSE/TRIO-12 trial randomized at a 2:1 ratio 1,144 human epidermal growth factor receptor (HER) 2-negative mBC patients to upfront docetaxel 75 mg/m^2 plus ramucirumab 10 mg/kg or placebo every 3 weeks. The primary endpoint of the study was PFS. After a median follow-up of 16.2 months, data from an interim analysis presented at the 2013 San Antonio Breast Cancer Symposium (SABCS) showed no significant advantage in PFS or OS (47). The median PFS as assessed by the investigators was 9.5 months in the combination group and 8.2 months in the docetaxel alone group (HR 0.88; 95% CI, 0.95-1.01; P=0.077); the median OS was 27.3 and 27.2, respectively (HR 1.01; 95% CI, 0.83-1.23; P=0.915). No significant advantage in survival was detected in any subgroup analyzed, including those assessed by age, race, PS, prior taxane treatment, metastases location, hormone receptor status, and geographical region. Moreover, more grade 3-4 AEs were reported with docetaxel plus ramucirumab (61.7%) compared with docetaxel alone

(52.4%). In particular, fatigue, hypertension, bleeding, febrile neutropenia, and stomatitis were significantly higher in patients assigned to ramucirumab. So far, no antiangiogenic strategy has improved OS in metastatic BC. The negative results of ROSE mirror those of other trials that failed in demonstrating the benefit of antiangiogenic drugs in this disease (48).

In the near future, the results from the REACH (49) and the RAISE (50) trial will also be available; these two large second-line, phase III studies are evaluating the use of ramucirumab in HCC and advanced CRC patients, respectively. The design of both trials has been presented at the 2012 ASCO Annual Meeting (51).

REACH randomizes to either ramucirumab or placebo 544 HCC patients whose disease progressed during or following first-line therapy with sorafenib or who were intolerant to the latter agent (50,52). The trial enrollment has been recently completed, and results of the study are expected in 2015. However, a press notice released on Jun 11th 2014, announced that OS results favored the ramucirumab arm, but the advantage was not statistically significant.

In the RAISE trial, over 1,000 patients with advanced CRC who have failed a first-line combination including bevacizumab, oxaliplatin, and a fluoropyrimidine are randomized at a 1:1 ratio to receive irinotecan, leucovorin and 5-fluorouracil (FOLFIRI) plus ramucirumab *vs.* FOLFIRI alone (50). The trial enrollment will be completed by early 2016.

A novel small molecule primarily targeting VEGFR2

Apatinib (YN968D1) is a novel, potent VEGFR inhibitor with an intriguing biologic rationale (15) that is able to circumvent cancer cell resistance to other antineoplastic agent (53). A recently published phase II randomized trial tested apatinib in heavily pretreated gastric cancer patients. Primary objectives were to assess the activity and safety of the daily administration of apatinib and to compare the tolerability of a once-daily *vs.* a twice-daily regimen. Patients were allocated to placebo (arm A), apatinib 850 mg once daily (arm B), or apatinib 425 mg twice daily (arm C). Outcome results were statistically improved in those patients exposed to apatinib (P<0.001 both for OS and PFS). Specifically, median OS was 2.5 months for arm A (95% CI, 1.87-3.70), 4.8 months for arm B (95% CI, 4.03-5.97), and 4.3 months for arm C (95% CI, 3.83-4.77). Similarly, the median PFS was 1.4 months (95% CI, 1.20-1.83) for arm A, 3.7 months (95% CI, 2.17-6.80) for arm B, and 3.2 months (95% CI, 2.37-4.53) for arm C. Interestingly, 9 patients exposed to apatinib had a partial response. The drug was overall well tolerable, with a limited number of patients complaining of severe AEs (hand-foot syndrome and hypertension); severe hematologic toxicities were occasional (54). Results of a phase 3 randomized trial of apatinib *vs.* placebo have been recently presented. In the study, 273 heavily pretreated patients were randomized 2:1 to apatinib (at a continuous oral dose of 850 mg per day, n=181) or matching placebo (n=92). The only stratification factor was the number of metastatic sites (less than 3 *vs.* 3 or above). Key inclusion criteria were age between 18 and 70 years, histologically confirmed advanced or metastatic adenocarcinoma of the stomach, ECOG PS of 0 or 1, the presence of measurable disease according to RECIST, and adequate organ function. Also, patients were required to be able of oral ingestion. Patients with evidence of SNC metastases, prior exposition to any VEGFR inhibitor, uncontrolled hypertension, coronary artery disease or any other significant cardiovascular concurrent morbidity were excluded. OS was the primary endpoint of the study; secondary endpoints included PFS, overall RR, DCR, quality of life, and safety. Median age of included patients was approximately 60 years, and over 75% of the patients had an ECOG PS of 1 (there were 10% more patients with PS 0 in the apatinib arm); 92% had stage IV disease The cohort was clearly heavily pretreated: 65% of the patients had received 2 or 3 previous lines of therapy, 35% were exposed to more than three previous treatments. In the experimental arm, OS was prolonged by 1.8 months compared to placebo (median OS 6.5 *vs.* 4.7 months, HR 0.71, P=0.01). Accordingly, median PFS was also significantly extended (2.6 *vs.* 1.8 months, HR 0.44, P<0.001). In the experimental arm, hematological toxicity was increased, but serious AE were equally reported. A significant incidence of grade 3 or 4 HFS reaction (9%) suggests that, despite its specificity, apatinib may have off-target effects. The average survival benefit of the drug to pretreated Chinese patients (1.8 months) appears to be in the same range of that of ramucirumab in the Western population (1.6 months). This successful drug is currently being tested in patients with breast or lung cancers.

Conclusions

In the last decade, the importance of angiogenic inhibitors in cancer therapy has become increasingly clear. VEGF has been recognized as a key angiogenic mediator, and

its increased level of expression has been associated with disease aggressiveness. Many preclinical and early clinical studies have provided insights into mechanisms that underlie the complexity of neoplastic angiogenesis. Also they have posed the foundation to develop novel drugs that target VEGF or VEGFR2. Among the scope of these drugs, ramucirumab, the only human antibody that specifically blocks the VEGFR2, has produced notable results in different diseases including gastric cancers and lung carcinomas. In these poor prognosis diseases, even a small absolute survival benefit of 1.5-2 months is clinically valuable. Disappointing clinical results reported for the ROSE study confirm that breast cancer may limitedly benefit from angiogenic inhibitors. While ongoing studies will clarify the role of ramucirumab in CRC, translational research will provide more details about how to properly select optimal candidates and corroborate the evidence for an ethnical difference in benefit. Despite huge efforts have been made to identify a predictive biomarker, no validated predictor is currently available for selecting optimal candidates to antiangiogenic therapy or monitoring treatment response. Future research will possibly increase our knowledge on how to select patients who are more likely to be responsive to antiangiogenic treatment. As well, the role of VEGF in reverting immunosuppression should also be better elucidated. Moreover, novel oral VEGFR2 inhibitors will possibly add some value to this strategy.

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