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LEADING ARTICLE

Critical Appraisal of Ramucirumab (IMC-1121B) for Cancer Treatment: From Benchside to Clinical Use

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Abstract Although antiangiogenic treatments have produced milestone advances in the treatment of several diseases, and have significantly extended the median survival of cancer patients, these agents share some weaknesses, including a limited impact on the overall cure rate, a fleeting effect because of redundant pathways or early appearance of resistance mechanisms, and the lack of predictive factors for treatment selection. Recent data suggest that antibodies targeting the vascular endothelial growth factor axis exert their activity through the inhibition of vascular endothelial growth factor receptor-2 phosphorylation, which has a pivotal role in the neoangiogenic process. Ramucirumab, a fully humanized monoclonal antibody specifically directed against the extracellular domain of the receptor, administered intravenously every 2 or 3 weeks, is emerging as a novel antiangiogenic opportunity. Starting with preclinical data and early clinical results, this concise review focuses on the development of the novel compound across multiple cancers (including gastrointestinal malignancies, breast cancer, lung carcinoma, and genitourinary tumors), and presents available data from randomized phase II and phase III trials. REGARD was the first phase III study to report on the efficacy of single-agent ramucirumab in patients with advanced cancer. Many other ongoing phase III trials are testing the efficacy of this interesting antiangiogenic compound as a single agent or in combination with chemotherapy in different cancer types.

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1 Introduction

In the last decade, antiangiogenic drugs have emerged as optimal agents to upfront treat different solid malignancies, and many class compounds have gained US Food and Drug Administration (FDA) and European Medicines Agency (EMA) approval, either alone or in combination with traditional chemotherapy [1]. While antiangiogenic inhibitors have a limited impact on the overall cure rate and lack predictive factors for treatment selection, they may significantly prolong the median survival of cancer patients, with a satisfying safety profile. Several class compounds are currently approved or in development for treatment of solid tumors. Their specific mechanisms of action differ according to their chemical structure and molecular target although they share the common biological principle of acting by reducing tumor neovascularization and promoting the formation of more stable and normalized vasculature [2]. This also results in the increased ability of anti-tumor drugs to reach cancer cells and render antiangiogenic therapy effective in combination with several chemotherapeutic drugs.

Recent data suggest that antibodies targeting the vascular endothelial growth factor (VEGF) axis exert their activity through a direct or indirect inhibition of VEGF receptor (VEGFR)-2 phosphorylation, which is suggested to have a pivotal role in the neoangiogenic process. In this scenario, ramucirumab, a fully humanized monoclonal antibody, is emerging as a novel, intravenously administered antiangiogenic drug specifically directed against the extracellular domain of VEGFR-2. Starting with preclinical data, this short review focuses on the clinical development of ramucirumab (IMC-1121B) across multiple cancers, and presents available data from phase III randomized trials.

2 VEGF Ligands and Receptors in Tumor Angiogenesis and Lymphangiogenesis

The inner complexity of angiogenesis has been studied within the scope of physiological processes such as embryonic development, organogenesis, normal tissue turnover, and wound healing. Accordingly, a parallel involvement has been revealed in the pathogenesis of neoplastic disorders. Due to the high metabolic demand for oxygen and nutrients, tumor growth is invariably accompanied by angiogenesis, a key hallmark of cancer that ultimately endorses the formation of a vastly disorganized, highly permeable neovascular network that allows more cancer cells to metastasize. This multi-faceted process depends on the activation of many pathways, is sustained by the activity of many different molecules, requires the interaction of different cell types (among others, vascular endothelial cells and cancer cells) with the stroma and the surrounding microenvironment, and is regulated by a number of cytokines and plasma factors.

Chief among the driving forces of angiogenesis is the interaction between VEGFs and their respective receptors, which are expressed both in normal and in neoplastic vasculature [3]. The VEGF family includes (i) different secreted growth factors, referred to as VEGF-A (also known as VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PlGF)-1 and -2, and (ii) a complex receptor system composed of VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1, KDR), selectively expressed on vascular endothelial cells, and VEGFR-3, expressed on both the lymphatic and the vascular endothelium (Fig. 1). Alternative splicing of the *VEGF-A* gene produces different isoforms of the VEGF protein, ranging in length from 121 to 206 amino acid residues, with the VEGF-A 165 isoform being the most important and most frequently involved in cancer-related neoangiogenesis. While VEGF promotes and regulates angiogenesis, vascular permeability, and cell migration by binding to both VEGFR-1 and VEGFR-2, PlGFs and VEGF-B peptides are selective ligands for VEGFR-1. Conversely, native VEGF-C and VEGF-D do not bind to VEGFR-1, but they may interact with both VEGFR-3 and VEGFR-2 [4, 5].

Neuropilin (NRP)-1 and NRP-2 are co-receptors that may increase the binding affinity of VEGF ligands to their respective receptors [6], with NRP-1 primarily expressed in arterial endothelial cells, and NRP-2 mainly found in venous and lymphatic endothelium. In accordance with its role in the angiogenic pathway, a biomarker study in gastric cancer patients exposed to bevacizumab found an association between low NRP-1 expression and favorable clinical outcome [7].

3 Drugs Targeting the VEGF Pathway

Not surprisingly, the huge complexity of the angiogenic process makes it potentially susceptible to drug administration from multiple points [8]. Therapeutic agents that specifically target the VEGF pathway include anti-VEGF monoclonal antibodies and small molecule inhibitors, as well as more experimental compounds (such as antisense oligonucleotides, ribozymes, or soluble VEGFR), the efficacy and potential application of which are in development (Table 1). Bevacizumab, a monoclonal antibody that binds circulating VEGF-A and prevents its interaction with VEGFR, was the first clinically available antiangiogenic drug to treat patients with metastatic colorectal cancer (CRC) [9] and by far the most successful class compound in clinical practice [9–13], albeit its value for patients with advanced breast cancer (BC) remains controversial. Aflibercept (VEGF-trap) is a recombinant fusion protein consisting of VEGFR-1 and VEGFR-2 extracellular domains fused with the Fc portion of human immunoglobulin G_1 (IgG_1), that acts as a soluble decoy receptor with high affinity to not only all VEGF-A isoforms, but also to VEGF-B, PlGF-1, and PlGF-2. Aflibercept complexes VEGF in the blood stream and in the extravascular space, preventing it from interacting with its receptors on endothelial cells. Its efficacy was demonstrated in combination with 5-fluorouracil and irinotecan in pre-treated metastatic CRC patients [14].

In addition, synthetic, orally active VEGFR tyrosine kinase inhibitors (TKIs) that prevent phosphorylation and signal transduction to the downstream pathway have emerged on the clinical scene in the last few years. For example, sunitinib acts by inhibiting the VEGFR, platelet-derived growth factor receptor (PDGFR), Flt-3, c-kit, rearranged during transfection (RET), and colony stimulating factor (CSF)-1R receptor kinase [15], while sorafenib inhibits VEGFR-2 and -3, PDGFR-β and c-kit [16]. When tested in phase III trials, sunitinib and sorafenib prolonged survival in patients with advanced renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), and gastrointestinal stromal tumors (GIST) [12, 17–19]. Brivanib, a novel dual inhibitor of the fibroblast growth factor (FGF) and VEGF, produced more responses and longer time-to-progression in HCC patients who had failed sorafenib, although the overall survival (OS) was not

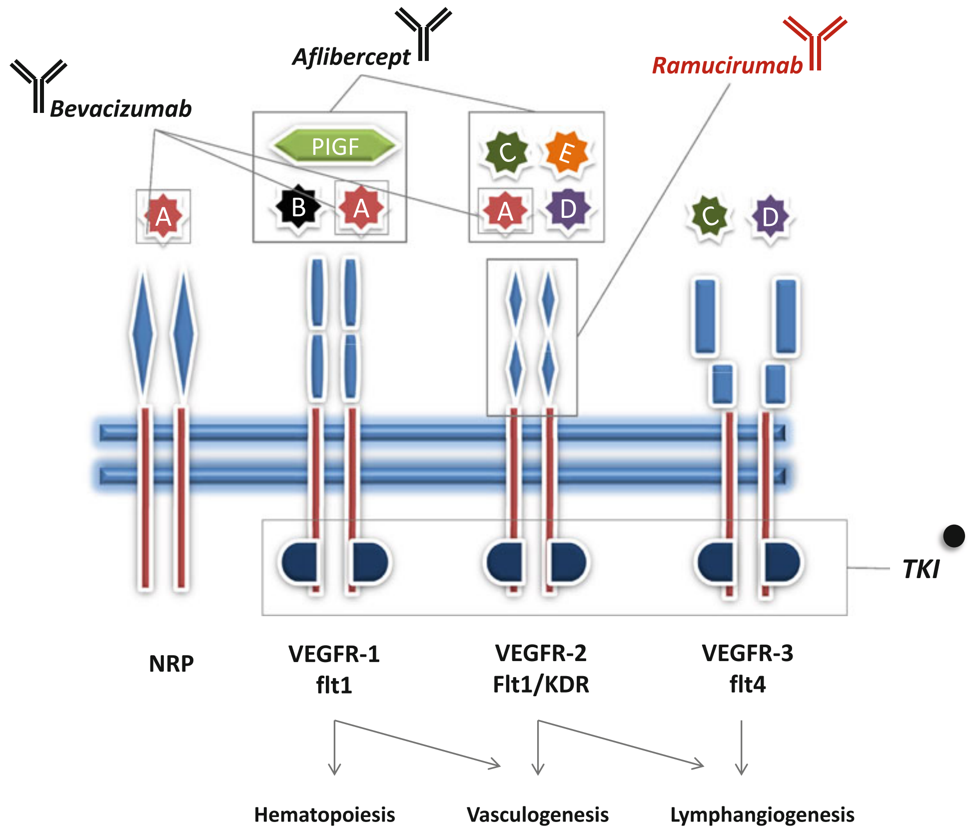

Fig. 1 Ligands and target receptors of the VEGF family. **a** VEGF-A, **b** VEGF-B, **c** VEGF-C, **d** VEGF-D, **e** VEGF-E. *NRP* neuropilin, *PlGF* placental growth factor, *TKI* tyrosine kinase inhibitor, *VEGFR* vascular endothelial growth factor receptor

significantly prolonged [20]. Moreover, novel insights are coming from the trials testing vandetanib [21, 22] and nintedanib (BIBF 1120) [23] in patients with non-small cell lung cancer (NSCLC). Regorafenib is another oral TKI that demonstrated efficacy in heavily pre-treated patients with advanced CRC [24] or GIST [25] by inhibiting VEGFR-2, TIE-2, FGFR-1, KIT, RET, and V600 mutant BRAF [26]. Cediranib, a highly potent adenosine triphosphate (ATP)-competitive inhibitor of recombinant VEGFR-2 tyrosine kinase [27], is being tested in the treatment of different cancers. However, clinical results were disappointing for CRC patients when the compound was administered in association with oxaliplatin-based chemotherapy [28, 29]. Apatinib (YN968D1) is an orally active small molecule that inhibits VEGFR-2 tyrosine kinase, PDGFR-β, KIT, c-src and reverses P-glycoprotein-mediated multidrug resistance [30]. Phase II/III trials in advanced gastric cancer and NSCLC are ongoing [31]. Pazopanib, an oral TKI targeting VEGFR, PDGFR, and c-kit, was tested with favorable results in phase III trials in patients with RCC [32] or soft-tissue sarcomas [33]. Axitinib is a second-generation inhibitor of VEGFR-1, VEGFR-2, VEGFR-3, PDGFR, and CSF-1. Despite the unsatisfactory results in pancreatic cancers and in NSCLC, this antiangiogenic agent is currently approved for the treatment of patients with RCCs who had previously failed systemic therapies with TKIs [34].

4 VEGFR-2: A More Specific Target in the Antiangiogenic Process?

Among all the receptors involved in tumor angiogenesis, VEGFR-2 is the most widely explored [35]. Preferentially expressed on endothelial cells and its progenitors, it is considered the main angiogenic driver and a preferential target for antiangiogenic drug development [36]. VEGFR-2 is a type II transmembrane kinase receptor and consists of 1,356 amino acids, separated into three different regions: a hydrophobic transmembrane region containing the tyrosine kinase domain, the carboxyl terminal region, and the seven extracellular Ig-like domains [37]. Although VEGFR-2 binds VEGF-A with an affinity tenfold lower than that of VEGFR-1, its phosphorylation activity is higher, suggesting a more potent role in the transduction pathway. On the plasma membrane, a fraction of the VEGFR-2 clusters with caveolin. The interaction of VEGF-A, -B, or -C with VEGFR-2 causes a rapid dissociation of the cluster and, after their binding has been completed, VEGFR-2 may either homodimerize or heterodimerize with VEGFR-1.

Table 1 Main antiangiogenic molecules of different classes, with their mechanism of actions, target cancers, and most relevant toxicities

Molecule	Class	Relevant mechanisms of action	Target cancers	Key toxicities
Bevacizumab	Monoclonal antibody	Binds circulating VEGF-A	Metastatic CRC Non-squamous NSCLC Metastatic RCC Glioblastoma	Hypertension Gastrointestinal perforation Major bleeding Proteinuria
Aflibercept	Recombinant fusion protein	Binds VEGF-A, VEGF-B, PlGF-1 and PlGF-2	Metastatic CRC	Hypertension Gastrointestinal perforation Major bleeding Proteinuria Diarrhea
Sunitinib	TKI	Inhibits VEGFR, PDGFR, c-kit, RET	GIST pNET Advanced RCC	Hepatotoxicity ECG alterations Hypertension Major bleeding Thyroid dysfunction Diarrhea Skin discoloration
Sorafenib	TKI	Inhibits VEGFR-2, VEGFR-3, PDGFR-β and c-kit	HCC Advanced RCC	Hand–foot syndrome Hypertension Major bleeding Cardiac events ECG alterations Gastrointestinal perforation
Brivanib	TKI	Inhibits FGF and VEGF	Advanced HCC	Fatigue Diarrhea Hypertension
Vandetanib	TKI	Inhibits VEGFR, EGFR and RET	NSCLC	Diarrhea Rash Hypertension Fatigue ECG alterations
Nintedanib	TKI	Inhibits VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α, PDGFR-β, FGFRs	NSCLC	Hepatotoxicity Gastrointestinal disturbances
Regorafenib	TKI	Inhibits VEGFR-2, TIE-2, FGFR-1, KIT, RET, and V600 mutant BRAF	Metastatic CRC GIST	Fatigue Hand-foot syndrome Diarrhea Hypertension Voice changes
Cediranib	TKI	Inhibits VEGFR-2	Metastatic CRC	Diarrhea Hypertension Fatigue Hematologic disorders Hand-foot syndrome
Apatinib	TKI	Inhibits VEGFR-2, PDGFR-β, KIT, c-src	NSCLC	Hand-foot syndrome Diarrhea Stomatitis

Table 1 continued

Molecule	Class	Relevant mechanisms of action	Target cancers	Key toxicities
Pazopanib	TKI	Inhibits VEGFRs, PDGFR, and c-kit	RCC Soft-tissue sarcoma	Diarrhea Hypertension Hair color changes Hepatotoxicity
Axitinib	TKI	Inhibits VEGFR-1, VEGFR-2, VEGFR-3, PDGFR	RCC	Diarrhea Hypertension Fatigue Dysphonia Hand-foot syndrome Hypothyroidism Proteinuria Rash

CRC colorectal cancer, *ECG* electrocardiograph, *EGFR* epidermal derived growth factor, *FGF* fibroblast growth factor, *GIST* gastrointestinal stromal tumors, *HCC* hepatocellular carcinoma, *NSCLC* non-small cell lung cancer, *PDGFR* platelet derived growth factor, *PlGF* placental growth factor, *pNET* primitive neuroectodermal tumors, *RCC* renal cell carcinoma, *RET* rearranged during transfection, *TKI* tyrosine-kinase inhibitor, *VEGFR* vascular endothelial growth factor receptor

This dimerization immediately induces conformational changes in the inner receptor structure, leading to autophosphorylation of the intracellular tyrosine kinase domains and the carboxyl terminal tail, and eventually triggering the downstream signaling cascade. Ultimately, the final effects of VEGFR-2 activation include a potent pro-angiogenic stimuli, causing increase in endothelial permeability as well as promotion of cell migration, proliferation, and survival. Alongside this, multiple cross-talks with other molecules, including angiopoietins and integrins, may occur and even complicate the dynamic signal landscape causing phospholipase C gamma (PLC-γ) phosphorylation as well as the activation of mitogen-activated protein (MAP) kinase and Raf-MEK-ERK pathways [4].

Therefore, putative strategies for blocking the complex VEGFR-2 pathway are multiple [38]. While small TKIs are interesting for hitting multiple targets [39], antibody-directed drugs are appreciable due to their higher specificity [40]. Targeting of the extracellular domain of VEGFR-2 is a novel approach to block tumor angiogenesis, explored so far with new antiangiogenic inhibitors such as ramucirumab [41, 42].

5 Structure and Mechanisms of Action of Ramucirumab

Ramucirumab (IMC-1121B, LY30009806, ImClone Systems Inc, NY, USA) is an intravenously administered, fully human IgG_1 monoclonal antibody, derived from phage display technology [43], the initial development of which began with the identification of an antibody with high affinity to VEGFR-2 (2111B clone) among a bacteriophage human Fab fragment library of non-immunized human donors [44]. Ramucirumab specifically targets VEGFR-2, with a half-maximal inhibitory concentration (IC_{50}) of 0.8–1.0 nM [45]. It binds with high affinity to the end of the extracellular domain, inducing steric overlap and changing of the receptor conformation, and eventually preventing the ligand from binding to VEGFR-2, thus inhibiting downstream signaling events [46].

In vitro, ramucirumab binds VEGFR-2 with an eightfold greater affinity than that of VEGF (5×10^{-11} M) and inhibits VEGF-mediated intracellular calcium mobilization, proliferation, and migration of human endothelial cells, as well as VEGF-stimulated mitogenic activity [47]. In vivo, mouse xenograft models demonstrate that DC-101, a mouse-specific antibody directed against murine VEGFR-2, prevents VEGF binding, VEGFR-2 signaling, VEGF-induced endothelial cell growth and inhibits metastasis growth after tumor surgery [48]. Pharmacodynamic studies have confirmed that the interaction between VEGF and VEGFR-2 is inhibited in the presence of ramucirumab, increasing VEGF-A plasma levels by 1.5- to 3.5-fold and decreasing VEGFR-1 and -2 levels dose-dependently [49]. Pharmacokinetic (PK) tests showed a non-linear PK, with non-proportional increase of half-life, maximum plasma concentration (C_{max}), area under the concentration–time curve (AUC), and steady-state volume of distribution, and decrease of clearance at increasing doses of ramucirumab [41]. At steady-state, the half-life of ramucirumab ranges from approximately 200 to 300 h after intravenous doses of 8–16 mg/kg [49].

6 Preclinical Development of Ramucirumab

The initial development of ramucirumab was based on studies testing the activity of DC-101, a rat anti-mouse antibody with a high affinity for the murine VEGFR-2 [50]. Due to the lack of activity against the murine VEGFR-2, ramucirumab could not be studied in mouse models, although non-obese diabetic/severe combined immunodeficiency (NOD-SCID) mice inoculated with VEGFR-2-positive leukemic cells were successfully treated with human anti-VEGFR-2 antibodies [51]. In vitro, DC-101 seemed to have both anti-angiogenic and anti-tumorigenic effects, inhibiting metastatic growth in resected tumors [46]. Preclinical studies showed that anti-Flk-1 DC-101 actively inhibited angiogenesis and tumor growth in vivo in lung, epidermoid, pancreatic, and renal cancer cells and tumor regressions were reported in glioblastomas. Although VEGFR-2 expression was expected to be present on the endothelium of normal tissues, toxicity was limited because the up-regulation of the receptor on tumor vessels made them much more susceptible to Flk-1 inhibition than normal vessels [48]. The target trough level affording antitumor activity was determined at a level of 20 μg/mL in a xenograft model, being the dose goal for testing efficacy of ramucirumab in clinical trials [52].

7 Clinical Evidence: Phase I Trials

Ramucirumab has been investigated in a number of early clinical studies. In the only fully published phase I dose-escalation trial [49], 37 heavily pre-treated cancer patients lacking further standard therapeutic options received a median number of 11 intravenous infusions to evaluate safety, maximum-tolerated dose (MTD), PKs, pharmacodynamics, and preliminary anticancer activity of the compound. Of note, seven patients had already received VEGF-targeting agents. Seven dose cohorts were analyzed with the tested weekly dose of 2–16 mg/kg. One patient experienced grade 3 hypertension after receiving the fourth dose of ramucirumab (10 mg/kg); this was considered a dose-limiting toxicity (DLT) for this dose cohort. One patient experienced grade 3 deep venous thrombosis (DVT) and one grade 3 hypertension after the fourth 16-mg/kg dose of ramucirumab. Accordingly, the MTD on a weekly schedule was determined to be 13 mg/kg. Other DLTs included grade 3 proteinuria in cycle 7 (4 mg/kg), and grade 3 vomiting in cycle 2 (2 mg/kg). The most frequently occurring potential drug-related adverse events (AEs) were hypertension (13.5 %) and abdominal pain (10.8 %). Anorexia, vomiting, increased blood alkaline phosphatase, headache, proteinuria, dyspnea, and DVT (each in 5.4 % of patients) were also reported. Mild to moderate AEs included fatigue (51.4 %), headache (51.4 %), peripheral edema (35.1 %), diarrhea (35.1 %), nausea (32.4 %), upper respiratory tract infection (32.4 %), abdominal pain (29.7 %), anorexia (29.7 %), constipation (29.7 %), epistaxis (29.7 %), proteinuria (29.7 %), arthralgia (27.0 %), cough (27.0 %), and dyspnea (27.0 %). Although preliminary, anticancer activity appeared promising, with a disease-control rate (DCR) of 73 %, including two patients (melanoma and gastric carcinoma) treated at 4 mg/kg reporting a partial response and 15 patients (41 %) with a stable disease lasting at least 6 months.

PK data demonstrated that a weekly dose of at least 8 mg/kg was required for obtaining complete VEGFR-2 inhibition.

Another phase I trial was reported in abstract form. Preliminary data from every-2-week (q2w) and every-3-week (q3w) study demonstrated that 15 of 25 included patients (60 %) had stable disease with a median duration of 12.7 months. It was derived that the 10 mg/kg q3w or the 8 mg/kg q2w treatment schedule was the optimal regimen for phase II and III study [53].

8 Clinical Evidence: Phase II and III Trials

8.1 Gastrointestinal Malignancies

8.1.1 Gastric and Gastroesophageal Junction Cancers

To improve the outcome of pre-treated advanced gastric cancer patients, ramucirumab has been compared with placebo in a large phase III randomized study. Efficacy results from the REGARD trial have been initially presented at the 2013 Gastrointestinal Cancers Symposium [54] and recently updated [55]. With a 2:1 randomization, the study compared ramucirumab (8 mg/kg given intravenously every 2 weeks) and best supportive care versus placebo and best supportive care as second-line treatment in 355 patients with metastatic gastric (75 %) or gastroesophageal junction cancers (25 %). The primary endpoint of the study was OS. Ramucirumab conferred a statistically significant benefit in OS and progression-free survival (PFS) compared with placebo. Median OS was 5.2 months for the experimental arm and 3.8 months for the control arm, with a hazard ratio (HR) of 0.77 (95 % CI 0.60–0.99; $p = 0.047$). Median PFS was 2.1 months for ramucirumab and 1.3 months for placebo, with a statistically significantly advantage resulting in an HR of 0.48 (95 % CI 0.37–0.62; $p < 0.0001$). Further, patients exposed to ramucirumab had a twofold higher DCR (49 vs. 23 %; $p < 0.0001$), although the overall response rate (RR) was similar between treatment arms (3.4 vs. 2.6 %). Importantly, the REGARD trial forms the largest springboard for discussion of the tolerability profile of the VEGFR-2 inhibitor. Overall, the safety profile of ramucirumab was good. The most frequent treatment-related grade ≥3 AEs were hypertension (7.2 vs. 2.6 % in the placebo group), anemia (6.4 vs. 7.8 %), abdominal pain (5.1 vs. 2.6 %), ascites (4.2 vs. 4.3 %), fatigue (4.2 vs. 3.5 %), decreased appetite (3.4 vs. 3.5 %), and hyponatremia (3.4 vs. 0.9 %). Subgroup analysis showed consistent treatment effect on survival benefit regardless of tumor location, previously significant weight loss, type of first-line treatment, or geographical origin. The quality-of-life assessment analysis showed that a larger proportion of patients exposed to ramucirumab reported stable or improved quality of life at 6 weeks compared with those in the placebo group (34 vs. 13 %).

The phase III randomized RAINBOW trial [56] compared intravenous ramucirumab and paclitaxel versus placebo and paclitaxel as second-line treatment in patients with metastatic gastric or junctional adenocarcinomas refractory to or progressive after first-line therapy with platinum and fluoropyrimidine. Over 650 patients from 200 study centers in 30 countries were enrolled. Final results have not yet been reported. A large phase II study comparing first-line 5-fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6) plus ramucirumab versus mFOLFOX6 plus placebo in the same patient population is ongoing. PFS is the primary endpoint, estimated enrollment was 166 patients, and the study was expected to be completed by May 2014 [57].

8.1.2 Hepatocellular Carcinoma

As reported for gastric cancer patients [7], subjects with HCC and high plasma VEGF levels may have poor prognosis or resistance to sorafenib [58, 59]. REACH [60], a large phase III multicenter randomized study, compared ramucirumab with placebo in patients with HCC who had disease progression during or following first-line therapy with sorafenib or who were intolerant to this agent. Trial enrollment has been recently completed, and results of the study are expected in the near future. Moreover, data from a single-arm multicenter phase II trial of ramucirumab as first-line monotherapy in patients with advanced HCC have been presented. Median PFS was 4.3 months, with half of the patients achieving disease control [61].

8.1.3 Colorectal Cancers

Preliminary results from a phase II trial enrolling 48 CRC patients and testing upfront ramucirumab in combination with 5-fluorouracil and oxaliplatin are available [62]. Median PFS was 11.5 months, with 1-year PFS of 48 %. RR was 67 %, and median response duration lasted 11 months (95 % CI 7–12). OS at 1 year was 85 % (95 % CI 72–93). The most frequent ramucirumab-related AEs were hypertension (46 %), diarrhea (31 %), nausea, and infusion-related reactions (19 %). Severe, treatment-related hypertension was reported in 15 % of patients. Two patients died due to cardiopulmonary arrest. With this background, the RAISE trial [63] is comparing the combination of irinotecan, leucovorin, and 5-fluorouracil (FOLFIRI) with ramucirumab versus FOLFIRI alone in patients with metastatic CRC who have failed a first-line combination therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine. Trial enrollment will be completed by February 2016.

8.2 Breast Cancers

Although the advantage of using antiangiogenic drugs in women with BCs is unclear, ramucirumab is being investigated in such patients because of its ability to reduce microvascular density, blood flow, and perfusion in animal models [46]. In an ongoing open-label, multicenter, phase II trial [64], BC patients are receiving oral capecitabine (2,000 mg/m^2 daily for 14 consecutive days) plus intravenous ramucirumab (10 mg/kg) on day 1; or capecitabine (same dose) plus IMC-18F1 (12 mg/kg) on day 1 and 8; or capecitabine alone (same dose). Estimated enrollment is of 150 patients with locally advanced or metastatic BC, previously treated with anthracycline and taxane therapy. Stratification factors are triple-negative receptor status and prior antiangiogenic therapy. The primary objective of the study is PFS. Secondary objectives include tumor response, safety, PK studies, and immunogenicity. At the time of disease progression, cross-over is permitted. The trial is expected to be completed by September 2014 [65]. Another study has been planned to evaluate the combined antitumor activity of ramucirumab (10 mg/kg every 3 weeks) and eribulin mesylate in pre-treated BC women. The trial is expected to be completed by January 2015 [66].

In metastatic BC, bevacizumab has demonstrated significantly improved PFS in combination with paclitaxel [67] or docetaxel [68], although the overall benefit appears to be modest. Following the rationale for combining chemotherapy and antiangiogenic agents, TRIO-012 [69] is a phase III study that evaluates efficacy and safety of adding ramucirumab to docetaxel for previously untreated women with human epidermal growth factor receptor (HER)-2-negative tumors. Accrual of 1,113 patients is required (assuming a 10 % drop-out rate) to have a study power of 90 %. The randomization ratio is 2:1, and patients are stratified by previous exposure to taxanes, having visceral metastasis, hormone receptor status, and geographical region. PFS is the primary endpoint; median PFS of the control group was assumed to be 6 months. Study enrollment has been completed and results are expected to be available by the end of 2015.

8.3 Lung Carcinomas

In NSCLC, therapies targeting angiogenesis are of increasing interest.

Preliminary data from two phase II trials have been presented. In the first study [70], patients with advanced NSCLC (stage IIIB unsuitable for locoregional treatments, or stage IV) received ramucirumab (10 mg/kg) combined with paclitaxel (200 mg/m^2) and carboplatin (AUC = 6) on day 1 of each 3-week cycle for up to 6 cycles, followed by maintenance with ramucirumab alone. Overall, RR was 55 % (one complete response, 21 partial responses), DCR reached 90 %, median PFS was close to 8 months and 6-month PFS was 62.5 %. Nevertheless, hematological toxicities, febrile neutropenia, fatigue, peripheral neuropathy, and pulmonary embolism were frequently reported. The second study was a randomized open-label phase II trial of ramucirumab (10 mg/kg every 3 weeks) in combination with platinum-based chemotherapy [71]. Interim median PFS was 4.3 months for control-arm patients and 6.3 months for ramucirumab-arm patients (HR 0.48, 90 % CI 0.31–0.74). DCR was 72 % for control-arm patients and 87 % for ramucirumab-arm patients. Compared with placebo, severe treatment-related AEs for patients receiving ramucirumab were more frequent and included thrombocytopenia (22 vs. 19 %), neutropenia (18 vs. 17 %), fatigue (12 vs. 17 %), anemia (10 vs. 16 %), hypertension (10 vs. 1 %), and nausea (10 vs. 7 %). The phase III REVEL trial [72] has been designed to compare the efficacy of docetaxel (75 mg/m^2 every 3 weeks) plus or minus intravenous ramucirumab (10 mg/kg every 3 weeks) as second-line treatment for over 1,200 patients with stage IV NSCLC whose disease progressed during or upfront platinum-based chemotherapy. A stratification for Eastern Cooperative Oncology Group performance status (ECOG PS), sex, previous maintenance therapy, and geographic region has been planned. The primary endpoint is OS; secondary endpoints include PFS, RR, patient-reported outcomes, quality of life, safety, and toxicity. The trial is expected to be completed in 2015.

8.4 Genitourinary Cancers

8.4.1 Renal Cell Carcinoma

A phase II trial has tested single-agent ramucirumab in metastatic RCC patients after disease progression or intolerance to oral TKIs; patients included in the trial had an ECOG PS of 0–1, prior nephrectomy and preserved hematopoietic function. The primary endpoint of the trial was RR, and secondary endpoints included PFS, PK studies, and safety. Enrolled patients received ramucirumab 8 mg/kg every 2 weeks. Preliminary data showed that 2 of 40 patients had a clinical response, 49 % had disease stabilization lasting more than 5 months, and four patients received ramucirumab for over 1 year without disease progression. Toxicities included headache (23 %, grade 1–2), fatigue (18 %, grade 1–3), and nausea (13 %, grade 1). Serious AEs included the development of proteinuria and hemoptysis in a patient with endobronchial metastases. Fatal cardiovascular events were reported in two patients, both with previous cardiovascular disease. Specifically, one patient had cardiac ischemia 4 months after study initiation; another had a cardiopulmonary arrest followed by death 13 months after the initiation of study. These data suggest that second- or third-line treatment with ramucirumab could be an option in antiangiogenic-resistant metastatic RCC patients [73]. Another phase II trial testing docetaxel in combination with ramucirumab or IMC-18F1 in metastatic transitional cell carcinoma patients is ongoing [74].

8.4.2 Gynecological Cancers

Ramucirumab has also been studied in a phase II open-label trial [75], in patients with persistent or recurrent epithelial ovarian, fallopian tube, or primary peritoneal carcinoma, who had already failed a platinum-based chemotherapy, and had an ECOG PS of 0–1 and preserved organ function. Patients received the VEGFR-2 inhibitor at the dose of 8 mg/kg every 2 weeks. PFS at 6 months was reached in one-third of patients (34.2 %), with a median PFS of 3.5 months and a median OS of 11.1 months. Severe AE observed in >5 % of patients included headache and fatigue. Of note, five deaths occurred while on ramucirumab or within 30 days of discontinuation, one of which was linked to intestinal perforation. Moreover, a case of bowel perforation and another report of colovaginal fistula were noted.

8.4.3 Prostate Cancer

In castration-resistant metastatic prostate cancer patients, ramucirumab has been studied with mitoxantrone and prednisone after disease progression on docetaxel therapy in a phase II randomized trial [76]. Patients received mitoxantrone 12 mg/m^2 every 3 weeks, prednisone 5 mg orally twice a day, and ramucirumab or cixutumumab (both 6 mg/kg intravenously weekly). The primary endpoint was PFS; secondary endpoints included safety, RR, and survival. Fatigue, hematological toxicity, and hypertension were frequently reported. Median PFS and OS were 4.1 months (95 % CI 3.0–5.6) and 10.8 months (95 % CI

Table 2 Key phase II/III trials testing ramucirumab in different clinical settings

Trial identifier	Refs	Treatment	Schedule	Phase	Enrolled population	Endpoint
NCT00627042	[61]	RAM	8 mg/kg q14d	II	Unresectable HCC pts with Child–Pugh score <10	PFS
NCT01140347	[60]	RAM/PL + BSC	8 mg/kg q14d	III	Unresectable HCC pts who had previously received sorafenib with Child–Pugh score <7	OS
NCT00515697	[73]	RAM	8 mg/kg q14d	II	Metastatic RCC pts who had failed TKIs	RR
NCT01282463	[74]	RAM/IMC-18F1 + DOC	10 mg/kg q21d/IMC-18F1 12 mg/kg on day 1 and 8 of each 21-day cycle + DOC 75 mg/m^2 q21d	II	Locally advanced or metastatic RCC or urothelial cancer pts following disease progression on first-line PBT	PFS
NCT00721162	[75]	RAM	8 mg/kg q14d	II	Persistent or recurrent epithelial ovarian, fallopian tube, or primary peritoneal carcinoma in pts who had received PBT	RR, PFS
NCT00533702	[77]	RAM ± dacarbazine	10 mg/kg q21d ± dacarbazine 1,000 mg/m^2 q21d	II	Metastatic melanoma pts	PFS
NCT00735696	[70]	RAM + PAC and CAR	10 mg/kg q21d + PAC 200 mg/m^2 CAR (AUC = 6)	II	Stage IIIB/IV NSCLC pts	PFS
NCT01160744	[71]	Pemetrexed + CAR/CIS/ GEM + CAR/CIS ± RAM	Pemetrexed 500 mg/m^2 q21d/GEM 1,000 mg/m^2 on day 1 and 8 q21d + CAR (AUC = 6)/CIS 75 mg/kg q21d ± 10 mg/kg q21d	II	Recurrent or advanced NSCLC pts	PFS
NCT01168973	[72]	RAM/PL + DOC	10 mg/kg q21d + DOC 75 mg/m^2 q21d	III	Stage IV NSCLC following disease progression after one prior PBT	OS
NCT00683475	[76]	RAM/IMC-A12 + mitoxantrone and prednisone	6 mg/kg on day 1,8 and 15 q21d + mitoxantrone 12 mg/m^2 q21d, prednisone 10 mg/day	II	AIPC pts following disease progression on DOC-based chemotherapy	PFS
NCT00862784	[62]	RAM + 5-FU/FA and oxaliplatin	8 mg/kg q14d + mFOLFOX6	II	Metastatic CRC pts	PFS
NCT01183780	[63]	RAM/PL + 5-FU/FA and irinotecan	8 mg/kg q14d + FOLFIRI	III	Metastatic CRC pts progressive during or following first-line combination therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine	OS
NCT01246960	[57]	RAM/PL + 5-FU/FA and oxaliplatin	8 mg/kg q14d + mFOLFOX6	II	Advanced esophageal, gastroesophageal junction, or gastric adenocarcinoma	PFS
NCT01170663	[56]	RAM/PL + PAC	8 mg/kg q14d + PAC 80 mg/m^2	III	Pts with metastatic gastric adenocarcinoma, refractory to or progressive after first-line therapy with platinum and fluoropyrimidine	OS
NCT00917384	[54, 55]	RAM/PL + BSC	8 mg/kg q14d	III	Metastatic gastric or gastroesophageal junction adenocarcinoma following disease progression on first-line platinum- or fluoropyrimidine-containing combination therapy	OS

Table 2 continued

Trial identifier	Refs	Treatment	Schedule	Phase	Enrolled population	Endpoint
NCT01234402	[64, 65]	CAP ± RAM/IMC-18F1	CAP 1,000 mg/m^2 bid for 14 days ± RAM 10 mg/kg q21d/IMC-18F1 12 mg/kg on day 1 and 8 q21d	II	Unresectable, locally advanced or metastatic BC pts previously treated with anthracycline and taxanes	PFS
NCT01427933	[66]	Eribulin ± RAM	Eribulin 1.4 mg/m^2 on day 1 and 8 q21d ± RAM 10 mg/kg q21d	II	Unresectable, locally recurrent or metastatic BC pts	PFS
NCT00703326	[69]	RAM/PL + DOC	10 mg/kg q21d + DOC 75 mg/m^2 q21d	III	Previously untreated pts with HER2-negative, unresectable, locally recurrent or metastatic BC	PFS
NCT00895180	[80]	RAM/IMC-3G3	–	II	Pts with recurrent glioblastoma multiforme	PFS

AIPC androgen-independent prostate cancer, *AUC* area under the concentration–time curve, *BC* breast cancer, *bid* twice daily, *BSC* best supportive care, *CAP* capecitabine, *CAR* carboplatin, *CIS* cisplatin, *CRC* colorectal cancer, *DOC* docetaxel, *FOLFOX* 5-FU/FA and oxaliplatin, *FOLFIRI* 5-FU/FA and irinotecan, *GEM* gemcitabine, *HCC* hepatocellular carcinoma, *HER* human epidermal growth factor receptor, *NSCLC* non-small cell lung cancer, *OS* overall survival, *qxd* every x days, *PAC* paclitaxel, *PBT* platinum-based therapy, *PFS* progression-free survival, *PL* placebo, *pt(s)* patient(s), *RAM* ramucirumab, *RCC* renal cell carcinoma, *RR* response rate, *TKI* tyrosine kinase inhibitor, *5-FU/FA* 5-fluorouracil/folinic acid

6.5–13.0) for cixutumumab and 6.7 months (95 % CI 4.5–8.3) and 13.0 months (95 % CI 9.5–16.0) for ramucirumab.

8.5 Melanoma

Preliminary data regarding the use of ramucirumab combined with dacarbazine in patients with metastatic melanoma have been reported [77]. With PFS as the primary objective of the study, 102 previously untreated patients were randomized to receive ramucirumab 10 mg/kg every 3 weeks with ($n = 52$) or without ($n = 50$) dacarbazine. The median PFS was 2.8 months for the combination arm and 1.7 months for those treated with ramucirumab alone. More hematologic toxicities were reported in the combination arm: no patient treated with ramucirumab alone experienced severe neutropenia versus 19 % of those who received the combination.

8.6 Glioblastomas

Although the use of bevacizumab in the treatment of patients with recurrent glioblastoma is supported by two independent clinical studies [13, 78] and has gained FDA approval, the real efficacy of antiangiogenic drugs in this disease is still a matter of debate [79]. A trial enrolling patients with recurrent glioblastoma multiforme is ongoing and comparing ramucirumab to an anti-PDGFR antibody [80].

9 Conclusions

Owing to its specific target inhibition of VEGFR-2, ramucirumab has a favorable toxicity profile and a broad spectrum of action across different cancer types. Table 2 summarizes key phase II and phase III clinical trials. As reported in the REGARD study, ramucirumab-related toxicities were few and seemed not to impact on quality of life. In addition, its long plasma half-life after intravenous infusion [49] may sustain prolonged VEGFR-2 inhibition, potentially favoring the bound block of all VEGF ligands to VEGFR-2 throughout the disease course, even after proteolytic modifications of VEGF ligands [81]. In contrast, the greater specificity of ramucirumab for VEGFR-2 may limit its action, as it is virtually impossible for ramucirumab to simultaneously block the activation of VEGFR-1, -3, and PDGFR. From a more clinical point of view and according to REGARD efficacy outcome data, this novel antiangiogenic drug has recently emerged as a possible second-line treatment for patients with advanced gastric or gastroesophageal junction adenocarcinoma. Although the survival advantage may seem modest, a 2- to

3-month median survival gain is noteworthy in this setting, and second-line chemotherapy produced similar results [82–84] but with increased toxicity. However, the fleeting benefit highlights how the possibility of redundant pathways and the early appearance of resistance to antiangiogenic agents may limit their use in clinical practice. While the RAINBOW study will clarify if ramucirumab may add benefit on top of second-line chemotherapy for patients with advanced gastric cancer, a number of randomized trials are ongoing. Results of these studies testing ramucirumab as a single agent or in combination will hopefully help to reinforce the evidence of the drug activity in other malignancies and will show if patients exposed to ramucirumab may derive clinically meaningful results.

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