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REVIEW ARTICLE

Ramucirumab Clinical Development: an Emerging Role in Gastrointestinal Tumors

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Abstract Ramucirumab (IMC-1121B, LY3009806) is a fully human G1 monoclonal antibody that specifically targets vascular endotelial growth factor receptor 2 (VEGFR-2) with a substantially greater binding affinity than that of its natural ligands. Early clinical trials in patients with advanced solid tumors demonstrated that biologically relevant blood target concentrations are achievable with tolerable doses, and also showed some preliminary evidence of clinical activity. Several pivotal phase III trials have now been concluded and have led regulatory agencies to grant marketing authorization to ramucirumab for use as second line therapy in patients with advanced gastric or gastroesophageal junction (GEJ) adenocarcinoma (as single agent or in combination with paclitaxel), in patients with advanced colorectal carcinoma (CRC) (in combination with infusional fluorouracil and irinotecan (FOLFIRI regimen)) and in patients with advanced non-small cell lung cancer (NSCLC) (in combination with docetaxel). In contrast, ramucirumab failed to significantly improve survival versus placebo as second line therapy in patients with advanced hepatocellular carcinoma (HCC). The aim of this review is to summarize the clinical development and emerging role of ramucirumab in gastrointestinal (GI) tumors, including relevant aspects of its mechanism of action, pharmacology, safety profile, and antitumor activity in gastric, HCC, and CRC carcinomas.

Key Points

Mechanism of action: Ramucirumab is a fully human monoclonal antibody that specifically targets vascular endothelial growth factor receptor 2 (VEGFR-2) with a substantially greater binding affinity than that of its natural ligands

Efficacy: Ramucirumab monotherapy or in combination with chemotherapy improved survival in advanced gastric or gastroesophageal junction adenocarcinoma and metastatic colorectal cancer, but failed to improve survival in advanced hepatocellular carcinoma

Toxicity: Ramucirumab's main side effect is hypertension, and is also associated with an increased incidence of fatigue, neutropenia, proteinuria and thrombotic/bleeding complications.

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1 Introduction

Angiogenesis is the physiological process through which new blood vessels form from pre-existing ones, a highly regulated process that is essential for organ growth and repair. Abnormal vessel growth and function are hallmarks of cancer and of different ischemic and inflammatory diseases, and contribute to disease progression [1]. Angiogenesis is tightly regulated

by a complex equilibrium among different pro- and antiangiogenic factors secreted both by tumor cells and by cells of the tumor microenvironment (pericytes, endothelial, mesenchymal or immune cells). Vascular endothelial growth factor A (VEGF-A) plays a central role in the regulation of tumor angiogenesis, as it stimulates both proliferation and migration of endotelial cells, enhances microvascular permeability, and is essential for revascularization during tumor formation [2]. It is commonly over-expressed in human tumors, and this is often associated with increased vascular density and more aggressive clinical behavior. VEGF-A and its main receptor, VEGFR2/KDR, are established targets for cancer therapy [3].

The first FDA-approved antiangiogenic agent was bevacizumab, a recombinant humanized antibody against VEGF, initially approved for the treatment of advanced CRC [4]. Subsequently, bevacizumab has demonstrated efficacy in a large number of tumor types including, among others, renal cell carcinoma, breast and ovarian cancer, non-squamous NSCLC, and glioblastoma [5–7]. Since the emergence of bevacizumab, a wide variety of drugs targeting the VEGF pathway have been developed, either directed to the ligands (i.e., aflibercept) or to their receptors (i.e., small molecule tyrosine kinase inhibitors (TKIs) such as sunitinib, sorafenib, pazopanib, axitinib, regorafenib, nintedanib, cabozantinib or vandetanib). Aflibercept is a recombinant fusion protein consisting of VEGFR-1 and VEGFR-2 extracellular domains fused with the Fc portion of human immunoglobulin G1 (IgG1), that acts as a soluble decoy receptor with high affinity to VEGF-A, VEGF-B, and placenta growth factor (PlGF), preventing these ligands from interacting with their receptors on endothelial cells. It has been aproved in combination with 5-fluorouracil and irinotecan for the treatment of pre-treated metastatic CRC patients [8]. Oral TKIs are multikinase inhibitors that act by competitive inhibition on the tyrosine kinase domain of VEGFRs and other tyrosine kinase receptors (TKRs) involved in angiogenesis regulation [9]. They differ from one another in their binding affinity to different TKRs, toxicity profile and therapeutic indications. Off-target promiscuity together with incomplete receptor blockade are the major drawbacks of this class of agents. More recently, more specific agents that target VEGFRs ligand-binding domains, such as the monoclonal antibody ramucirumab, are being developed. Based on promising results from preclinical and early clinical trials, ramucirumab is being assessed in a wide variety of tumor settings either alone or in combination with chemotherapy.

On April 21, 2014, the Food and Drug Administration (FDA) granted marketing authorization to ramucirumab for use as a single agent for the treatment of patients with advanced or metastatic gastric or gastroesophageal junction (GEJ) adenocarcinoma whose disease has progressed during or after prior treatment with fluoropyrimidine- or platinum-containing chemotherapy. Thereafter, ramucirumab has also been approved as second line therapy for use in combination with paclitaxel in advanced gastric or GEJ cancer (FDA: November 2014; European Medicines Agency (EMA): December 2014), with docetaxel in advanced NSCLC (FDA: December 2014; EMA: December 2015) and with FOLFIRI in advanced colorectal cancer (FDA: April 2015; EMA: December 2015). In contrast, second-line treatment with ramucirumab failed to show a survival improvement over placebo in patients with advanced hepatocellular carcinoma. The aim of this review is to summarize the clinical development of ramucirumab with a particular focus on its emerging role in GI cancer, including relevant aspects of its mechanism of action, pharmacology, toxicity profile, and antitumor activity in gastric, hepatocellular, and colorectal carcinomas (Table 1).

2 Ramucirumab Overview

2.1 Synthesis, Structure, and Mechanism of Action

Ramucirumab development began with the identification of a high affinity antibody against VEGFR-2 (clone 1121) from a bacteriophage library of Fab fragments from nonimmunized human donors [10]. The initially developed chimeric antibody IMC-1121 showed a favorable toxicity profile in early clinical trials and dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) results consistent with an antiangiogenic effect, but human antichimeric antibodies developed in half of treated patients which precluded further clinical development [11]. IMC-1121 was subsequently fully humanized to become IMC-1121B (ramucirumab) [12]. Interspecies receptor differences, however, made ramucirumab inactive in preclinical mouse models [13]. To circumvent this issue, a rat antimouse antibody directed at flk-1, the murine homolog of human VEGFR-2, was developed by hybridome technology (DC101).

Ramucirumab (IMC-1121B, LY3009806) is a fully human G1 monoclonal antibody that specifically targets VEGFR-2 with a half-maximal inhibitory concentration (IC_{50}) of 0.8–1 nM and a binding affinity of 5×10^{-11} M, eightfold greater than that of its natural ligand VEGF-A [14]. Ramucirumab effectively binds both soluble and cell-surface based VEGFR-2, thereby preventing VEGF ligands from binding and activating the receptor. VEGFR-2/KDR is expressed in adults mainly on vascular endotelial cells, megakaryocytes and hematopoietic stem cells, and is considered the main angiogenic driver.

2.2 Preclinical Development

Development of VEGFR-2 targeting agents was initiated in the late 1990s. High affinity monoclonal antibodies against

Table 1 Phase III randomized trials of ramucirumab in GI cancer

Study	Tumor	Prior TX	N	Treatment	ORR	DCR	PFS	HR	P	OS	HR	P
REACH trial	Advanced hepatocellular carcinoma	Sorafenib	283	BSC + RAMU	7.1 %*	56 %*	2.8 m*	0.625	$p<0.0001$	9.2 m	0.866	$P=0.139$
			282 (1:1)	BSC + placebo	0.7 %	46 %	2.1 m			7.6 m		
Regard trial	Advanced GEJ or gastric adenocarcinoma	FP or platinum-based CT	238	BSC + RAMU	3.4 %	49 %*	2.1 m*	0.483	$p<0.0001$	5.2 m*	0.776	$p=0.047$
			117 (2:1)	BSC + placebo	2.6 %	23 %	1.3 m			3.8 m		
Rainbow trial	Advanced GEJ or gastric adenocarcinoma	FP and platinum-based CT	330	TAX + RAMU	28 %*	80 %*	4.4 m*	0.635	$p<0.0001$	9.6 m*	0.807	$p=0.017$
			335 (1:1)	TAX + placebo	16 %	64 %	2.9 m			7.4 m		
Raise trial	Advanced CCR	Oxaliplatin, FP and Bevacizumab	536	FOLFIRI + RAMU	13.4 %	74 %	5.7 m*	0.793	$P<0.001$	13.3 m*	0.844	$p=0.022$
			536 (1:1)	FOLFIRI + placebo	12.5 %	69 %	4.5 m			11.7 m		

BSC best supportive care, *CCR* colorectal cancer, *CT* chemotherapy, *DCR* disease control rate, *FOLFIRI* irinotecan, folinic acid and infusional 5-fluorouracil regimen, *FP* fluoropyrimidines, *GEJ* gastroesophageal, *HR* hazard ratio, *m* months, *OS* overall survival, *ORR* objective response rate, *PFS* progression-free survival, *RAMU* ramucirumab, *TAX* paclitaxel

* statistical significance ($P<0.05$)

both the murine (flk-1) and human (KDR) variants of VEGFR-2 showed potent in vitro inhibition of VEGF receptor binding, intracelular phosphorylation and signaling, and endothelial cell proliferation and migration. In vivo, DC101 induced apoptosis, decreased vessel density, and reduced tumor growth in a wide range of murine and human tumor xenograft models, including models resistant to other antiangiogenic agents [10]. The target trough concentration affording antitumor activity in preclinical studies was 20 μg/mL. Toxicological studies with repeated weekly doses of ramucirumab were performed in cynomolgus monkeys. In a 5-week treatment duration study, doses up to 40 mg/kg were well tolerated with no adverse effects. In a longer exposure study (39 weeks), treatment with doses of 16 and 50 mg/kg caused nephrotoxicity in monkeys after 26 weeks of therapy.

2.3 Clinical Pharmacology and Early Clinical Trials

Two phase I studies with ramucirumab have been completed to date. The first one included 37 patients with advanced solid tumors, and evaluated escalating doses of ramucirumab ranging from 2 to 16 mg/kg administered as a 1-h intravenous infusion at a rate of ≤25 mg/min [15]. Cycles consisted of 4 weekly drug infusions followed by a 2-week, treatment-free pharmacokinetic (PK) sampling period (this drug-free period was subsequentely eliminated in an amendment after preliminary PK assessment). Two patients developed dose-limiting hypertension and deep venous thrombosis at the 16 mg/kg dose level, so the next lower dose level (13 mg/kg) was considered the maximum tolerated dose (MTD). Other commonly reported serious adverse events in this trial were nausea, vomiting, headache, fatigue, and proteinuria. Objective responses were documented in four of 27 patients (15 %) with measurable disease, including patients with melanoma, leiomyosarcoma, gastric and ovarian carcinoma, and 11 of 37 (30 %) had either a partial response (PR) or stable disease (SD) lasting at least 6 months across a range of doses. The PK profile was characterized by dose-dependent elimination and nonlinear exposure consistent with saturable receptor-mediated clearance. Half-life at steady-state ranged from 200 to 300 h for patients treated with doses of 8–16 mg/kg/week. Mean trough concentrations achieved throughout treatment at all dose levels exceeded biologically relevant target concentrations documented in preclinical models (20 μg/mL). Of note, no anti-ramucirumab antibodies were detected in any patient. Pharmacodynamic (PD) studies showed increases in serum VEGF-A concentrarion (1.5 to 3.5-fold over baseline values) following ramucirumab administration, particularly evident at doses ≥8 mg/kg, and such elevations were sustained for protracted periods of time throughout ramucirumab therapy. The magnitude and duration of VEGF-A elevation, therefore, could potentially serve as a useful PD marker denoting adequate VEGFR-2 blockade. In contrast, early decrements in

soluble VEGFR-1 and VEGFR-2 concentrations were generally observed after ramucirumab treatment, which soon recovered to near-pretreatment levels. Drug-induced antiangiogenic effects were also assessed by DCE-MRI in patients with appropriate liver lesions. Significant decreases in tumor perfusion and vascularity were indeed documented in nine of 13 evaluable patients (69 %) providing further preliminary support that the ramucirumab doses tested in this study were biologically relevant.

Alternative dosing regimens were explored in a second phase I trial that included 25 patients with advanced solid tumors. Ramucirumab was administered in escalating doses every 2 (6 to 10 mg/kg) or 3 weeks (15 to 20 mg/kg) to sequential cohorts of patients [16]. No DLTs were observed and the MTD was not reached. Safety and PK/PD profiles were similar to those reported in the prior trial. Three patients developed anti-ramucirumab antibodies but none of these were neutralizing. No objective responses were observed, but stable disease was documented in 60 % of patients with a median response duration of 13 months. Recommended doses for phase II studies were 8 mg/kg q2w and 10 mg/kg w3q based on results from this and the weekly dosing study. Doses within this range yielded minimum plasma concentrations well above the 20 μg/mL associated with growth inhibition in preclinical xenograft models and were well tolerated and associated with preliminary evidence of clinical activity.

3 Ramucirumab in Gastric and Gastroesophageal Junction Adenocarcinoma

Results of the first pivotal study of ramucirumab in gastric cancer were published in January 2014. The REGARD study was an international, double-blind, placebo-controlled, phase III trial that included 355 patients with advanced gastric or gastroesophageal junction (GEJ) adenocarcinoma and disease progression after platinum- or fluoropyrimidine-containing chemotherapy, either within 4 months of the last dose of first-line chemotherapy for metastatic disease, or within 6 months of the last dose of adjuvant chemotherapy (NCT00917384) [17]. Patients were randomized 2:1 to receive best supportive care (BSC) plus either ramucirumab 8 mg/kg or placebo, intravenously once every 2 weeks. Randomization was stratified by weight loss (<10 % vs ≥10 % of body weight in the previous 3 months), geographic region (North America, Europe, and Australia vs South and Central America, India, South Africa, and Middle East vs Asia) and location of the primary tumor (gastric vs GEJ). Patients were treated until disease progression, unacceptable toxicity or death. No crossover between treatment groups was allowed. The primary endpoint was overall survival (OS) and secondary endpoints were progression-free survival (PFS), objective response rate (ORR), duration of response, quality of life (QoL) (assesed with the EORTC QLQ-C30 QoL questionnaire, version 3.0), safety, and immunogenicity. Treatment with ramucirumab significantly improved OS compared with placebo, with a median survival of 5.2 months in patients in the ramucirumab group and 3.8 months in those in the placebo group (HR = 0.776, $p = 0.047$). The survival benefit with ramucirumab was consistent across all subgroups, including the prespecified stratification factors, and remained unchanged after multivariable adjustment for other prognostic factors (HR = 0.774, $p = 0.042$). PFS was also significantly improved in patients treated with ramucirumab (median 2.1 vs 1.3 months, HR = 0.483, $p < 0.0001$). Moreover, although ORR was low in both treatment arms (3.4 vs 2.6 %), the disease control rate (DCR) was significantly higher in the ramucirumab group as compared to the placebo group (49 vs 23 %, $p < 0.0001$).

The incidence of severe adverse events (AEs) was very similar among study groups (57 vs 58 % developed grade 3 or higher AEs in the ramucirumab and placebo groups, respectively). Rates of hypertension were higher in the ramucirumab group compared to the placebo group (8 % vs 3 % had grade 3 hypertension; no patients developed grade 4). Grade ≥3 arterial thromboembolic events were slightly more common (1 % vs 0 %), but ramucirumab was not associated with increased rates of fatigue, anorexia, vomiting, anemia, venous thromboembolism, gastrointestinal perforation, bleeding or other relevant toxic effects. There were five (2 %) deaths in the ramucirumab group and two (2 %) in the placebo group that were considered related to the study drug. QoL was assessed at baseline in the majority of patients (>94 %), but the first follow-up 6 week assessment was only provided by 48 % of patients in the ramucirumab arm and 25 % in the placebo arm, mainly due to treatment discontinuation. Ramucirumab conferred no detrimental effect on QoL as compared to placebo, and patients receiving ramucirumab had a significantly longer time to deterioration of performance status as compared to those receiving placebo (5.1 vs 2.4 months). Anti-ramucirumab antibodies were detected in 3 % of patients receiving ramucirumab and <1 % of those receiving placebo, but no patients developed neutralising antibodies to ramucirumab.

More recently, Wilke et al. reported the results of the RAINBOW trial (NCT01170663) [18], a phase III doble-blind study comparing the combination of paclitaxel with either ramucirumab or placebo as second-line treatment of patients with advanced gastric or GEJ adenocarcinoma and disease progression after first-line platinum- and fluoropyrimidine-based chemotherapy. By the time the study was designed and initiated, results from randomized trials demonstrating a survival benefit with irinotecan or docetaxel as second-line therapy had not been communicated yet, and weekly paclitaxel was chosen as control arm due to its more favorable toxicity profile among available options in this

setting. The study randomized (1:1) 665 patients to receive paclitaxel (80 mg/m2 day 1, day 8 and day 15) plus placebo or ramucirumab (8 mg/kg day 1 and day 15) every 28 days. Randomization was stratified by geographic region, time to progression on first line therapy (<6 months versus ≥6 months) and disease measurability (measurable versus non-measurable). The trial met its primary endpoint. OS was significantly longer in the ramucirumab plus paclitaxel group than in the placebo plus paclitaxel group (median 9.6 vs 7.4 months, HR = 0.807, $p = 0.017$). Patients treated with the ramucirumab combination also had significantly longer PFS (4.4 vs 2.9 months, HR = 0.635, $p < 0.0001$), and a higher proportion of patients achieving an objective response (28 % vs 16 %, $p = 0.0001$) or disease control (80 % vs 64 %, $p < 0.0001$). A preplanned subgroup analysis identified a difference in treatment effect for the addition of ramucirumab to paclitaxel by geographic region, showing a greater benefit for non-Asian (HR = 0.73) than for Asian population (HR = 0.99). The reasons for this difference are not clear. However, although the proportion of patients that received at least one post-discontinuation treatment was similar among study arms (48 % vs 45 %), this proportion was notably higher in Asia (70 %) than in non-Asian regions (40 %), which may have attenuated the survival benefit in these patients. Non-Asian patients also had a higher proportion of GEJ tumors (31 % vs 21 %), a location that seemed to derive greater treatment benefit in this study. In addition, population PK analysis performed with patients included in both the REGARD and RAINBOW studies suggested greater ramucirumab exposures were associated with longer PFS and OS, improved hazard ratios, and, consistently, also increased toxicity [19]. Whether or not ramucirumab exposure differed by region has not been reported.

Severe AEs occurred more frequently in ramucirumab-treated patients than in those treated with placebo (82 % vs 63 %). The most relevant grade 3 or higher AEs occuring in more than 5 % of patients in the paclitaxel plus ramucirumab versus placebo groups were, respectively, neutropenia (41 % vs 19 %), hypertension (14 % vs 2 %), and fatigue (12 % vs 5 %). Grade 3–4 bleeding (4 % vs 2 %) and proteinuria (1 % vs 0 %) were also slightly more commonly observed in patients allocated to receive ramucirumab. The incidence of febrile neutropenia, however, was low in both treatment arms (3.1 % vs 2.4 %). Other AEs of special interest such as gastrointestinal perforation or thrombotic events were not significantly different among study groups. Importantly, this increased toxicity did not result in a greater incidence of treatment discontinuation or treatment-related deaths (4 % vs 4.6 %). Moreover, QoL was not impaired by the addition of ramucirumab. Actually, QoL was maintained for a longer period of time in ramucirumab-treated patients and more patients in the ramucirumab arm reported stable or improved scores for all QoL parameters and at all on-therapy assessments [20].

The combination of ramucirumab and chemotherapy has also been assessed in chemotherapy-naive patients with somewhat disappointing preliminary results. A phase II randomized trial conducted in 164 untreated patients with advanced esophageal, GEJ or gastric adenocarcinoma (NCT01246960) [21] randomly allocated patients to receive modified FOLFOX-6 (oxaliplatin, leucovorin, and 5-fluorouracil) with ramucirumab or placebo every 2 weeks. Ramucirumab-treated patients had a higher DCR (85 % vs 67 %, $p = 0.008$), but the addition of ramucirumab did not improve PFS (6.4 vs 6.7 months, HR = 0.98) nor OS (11.7 vs 11.5 months, HR = 1.08). Toxicity profile was consistent with that reported in other trials for these agents. Treatment discontinuation rate for reasons other than disease progression, including a notable proportion of patients discontinuing due to patient decision, was higher in the ramucirumab arm. Decreased treatment exposure in the experimental arm, particularly in the esophageal subgroup, may have negatively affected treatment effect. Consistent with these observations, exploratory analysis showed longer PFS favoring ramucirumab in the gastric/GEJ cancer subgroup (52 % of patients), censoring for treatment discontinuation for reasons other than progressive disease. The role of ramucirumab as first-line therapy in patients with metastatic gastric or GEJ adenocarcinoma is being further addressed, however, in the ongoing randomized, double-blind, placebo-controlled phase III study evaluating cisplatin plus a fluoropyrimidine with or without ramucirumab in this setting (RAINFALL, NCT02314117) [22].

In light of these results, regulatory authorities approved ramucirumab for the treatment of patients with advanced gastric or GEJ adenocarcinoma, as a single agent in patients refractory or progressive following prior treatment with fluoropyrimidine or platinum-containing chemotherapy (FDA: April 2014; EMA: December 2014), or in combination with paclitaxel following disease progression on or after prior fluoropyrimidine and platinum chemotherapy (FDA: November 2014; EMA: December 2014).

4 Ramucirumab in Hepatocelullar Carcinoma

Sorafenib, a TKI of VEGFRs and several other receptors involved in angiogenesis control, is the worldwide standard of care for HCC, although its efficacy in this context is somewhat limited. Increased plasma VEGF levels have been associated with resistance to sorafenib in HCC and overexpression of VEGFR-2 has been correlated with rapid disease progression and a poor prognosis. VEGFR-2 anbibody-mediated inhibition has been shown to reduce tumor growth in HCC animal models. Preliminary evidence of ramucirumab anticancer activity in treatment-naïve HCC was documented in a single arm phase II study by Zhu et al. (NCT00627042) [23]. This study

included 42 patients with advanced HCC that met the following inclusion criteria: histological diagnosis of HCC, no prior systemic treatment, Cancer of the Liver Italian Programme (CLIP) score 0–3 and Child-Pugh Cirrhosis A or B. Exclusion criteria included, among other factors, gastric varices not amenable to ablative therapy or those with a bleeding episode within 3 months prior to study entry. Patients were treated with ramucirumab 8 mg/kg every two weeks until disease progression or unacceptable toxicity. Ramucirumab was associated with an ORR of 9.5 %, a DCR of 69 %, a median PFS of 4.0 months and a median OS of 12.0 months (18.0 months for patients with Child-Pugh A cirrhosis and 4.4 months for those with Child-Pugh B cirrhosis). Interestingly, PFS and particularly OS were better in patients who developed hypertension (PFS 4.2 vs 3.1 months; OS 23.1 vs 6.1 months). Treatment-related grade ≥3 AEs reported were hypertension (14 %), gastrointestinal hemorrhage (7 %, including one toxic death), infusion-related reactions (7 %), and fatigue (5 %). Grade 3–5 hemorrhagic events observed in this trial emphasize the need for a comprehensive endoscopic evaluation of patients with portal hypertension and more stringent requirements for study entry in patients with esophageal varices, as well as closer monitoring and greater restrictions on the concomitant use of antithrombotic or anticoagulant agents while on therapy in these patients. Following ramucirumab infusion, there was an increase in serum VEGF and PlGF and a transient decrease in soluble VEGFR2, consistent with VEGFR-2 blockade as observed with ramucirumab in other patient populations. Exploratory analysis showed a potential association between relative changes in sVEGFR-1 and both PFS and OS, with better outcomes in patients with documented sVEGFR-1 decreases form baseline to day 8 assesmemt.

Based on these encouraging results, the REACH randomized phase III study was designed to evaluate the safety and efficacy of ramucirumab in patients with advanced HCC following first-line therapy with sorafenib (NCT01140347). This trial included 565 patients with Child-Pugh class A HCC and Barcelona Clinic Liver Cancer (BCLC) stage B or C refractory or not amenable to locoregional therapy, who had progressed or were intolerant to prior therapy with sorafenib [24]. Patients with esophageal or gastric varices requiring therapy were excluded from study entry. Some other relevant patient characteristics were the following: 45 % of patients were from East Asia, 72 % of patients had extrahepatic disease, 88 % had BCLC stage C, 38 and 28 % had hepatitis B or C (respectively), and 44 % had baseline alpha-fetoprotein levels greater than 400 ng/mL. Patients were randomly allocated (1:1) to ramucirumab (8 mg/kg) or placebo every 2 weeks plus BSC, stratified by region and etiology of liver disease. Patients treated with ramucirumab had a significant increase in ORR (7 % vs 0.7 %, $p < 0.0001$) and PFS (HR = 0.625, $P < 0.0001$) as compared to those treated with placebo, but ramucirumab did not significantly improve OS (9.2 vs 7.6 months, HR = 0.866, $p = 0.139$). Preplanned subgroup analysis suggested that patients with elevated baseline alpha-fetoprotein (AFP) levels were more likely to benefit from ramucirumab. Indeed, OS in patients with AFP levels ≥400 ng/mL was significantly greater for patients treated with ramucirumab than for patients treated with placebo (7.8 vs 4.2 months, HR = 0.674, $p = 0.0059$). Further investigation in this subgroup of patients will be explored in future trials. In addition, QoL studies demonstrated that ramucirumab did not result in a detriment in symptoms or patient functioning [25]. A delay in symptom and performance-status deterioration coupled with survival benefit was observed in patients treated with ramucirumab in the elevated AFP population.

5 Ramucirumab in Colorectal Cancer

Ramucirumab was first evaluated in combination with the modified FOLFOX-6 regimen (mFOLFOX-6) as first-line therapy in patients with metastatic CRC (NCT00862784) [26]. Results of this phase II trial that included 48 patients suggested ramucirumab could enhance the efficacy of mFOLFOX-6 with an aceptable safety profile. A median PFS of 11.5 months, an ORR of 58 %, a DCR of 94 %, and an OS of 20.4 months were deemed encouraging in this setting. Toxicity of the combination was consistent with the known safety profile of the constituent drugs. The most frequent grade ≥3 AEs were neutropenia (42 %), hypertension (17 %), and peripheral neuropathy (13 %). One patient developed nephrotic syndrome (grade 4 proteinuria) and there were two treatment-related deaths due to cardiopulmonary arrest and myocardial infarction.

More recently, results from the pivotal RAISE study have provided further evidence for VEGFR as a relevant therapeutic target in CRC (NCT01183780) [27]. This global, doble-blind, phase 3 study randomized 1072 patients with metastatic CRC following first-line combination therapy with bevacizumab, oxaliplatin and a fluoropyrimidine, to receive FOLFIRI with either ramucirumab (8 mg/kg) or placebo every 2 weeks. Randomization was stratified by geographic region (North America vs Europe vs all other regions), KRAS exon 2 status (mutant vs wild-type), and time to first-line disease progression (<6 months vs ≥6 months). The addition of ramucirumab to the standard second-line FOLFIRI regimen significantly improved OS as compared to placebo (median 13.3 vs 11.7 months, HR = 0.84, $p = 0.0219$), which was the primary endpoint of the study. The OS benefit was maintained after adjustment for other significant baseline prognostic factors and across all pre-specified sensitivity and subgroup analyses, including stratification factors. PFS was also significantly improved in ramucirumab- vs placebo-treated patients (median 5.7 vs 4.5 months, HR = 0.79, $p < 0.001$). Investigator-

assessed ORR did not differ, however, by treatment arm (13.4 % vs 12.5 %, $p=0.634$). The incidence of grade ≥3 treatment-emergent AEs (TEAEs) was higher in the ramucirumab-FOLFIRI arm than in the placebo-FOLFIRI arm (79 % vs 62 %, respectively), and severe TEAEs occurring in >5 % of patients included neutropenia (38.4 % vs 23.3 %), hypertension (11.2 vs 2.8 %), diarrhea (10.8 % vs 9.7 %), and fatigue (11.5 % vs 7.8 %). Other AEs of special interest, such as severe thrombocytopenia (3 % vs 0.8 %), bleeding events (2.5 % vs 1.7 %), thromboembolic events (4.2 % vs 2.1 %), and proteinuria (3 % vs 0.2 %) were only modestly increased with the addition of ramucirumab. The number of deaths due to AEs was similar on both arms (2 %). Patient-reported outcomes were assessed with the EORTC QLQ-C30 (version 3.0) and the EQ-5D. Questionnaire completion rates were high, with over 90 % of patients providing baseline and post-baseline data in both treatment arms. Baseline scores for all scales were similar between study groups. Accross multiple analyses, a transient worsening of QoL was suggested for the ramucirumab combination as compared to the placebo arm. However, when assessing sustained deterioration, no significant differences were appreciated in the majority of QoL scales [28]. Exposure-response analyses suggest, as in gastric cancer, that higher ramucirumab exposure is associated with improved outcomes (smaller hazard ratios for PFS and OS), and potentially increased neutropenia.

Finally, ramucirumab is also being assesed in combination with irinotecan and cetuximab (IC) in patients with KRAS wild type CRC progressive to one prior oxaliplatin- and bevacizumab-containing regimen. The study was designed as a randomized phase II trial with 147 patients assigned to IC (irinotecan 180 mg/m^2 iv plus cetuximab 500 mg/m^2 IV q2w) or IC plus ramucirumab (8 mg/kg iv q2w) (NCT01079780) [29]. Following inclusion of the first 35 patients accrual was held due to toxicity (excess of severe mucositis, diarrhea, neutropenia, and perforation events in the ramucirumab arm). The study was then modified (mICR: irinotecan 150 mg/m^2, cetuximab 400 mg/m^2, and ramucirumab 6 mg/kg iv q2w) and accrual resumed in May 2014. Results of this trial have not been communicated to date.

6 Conclusions and Perspectives

Ramucirumab has demonstrated that specific VEGFR-2 target inhibition is a useful therapeutic strategy in several tumor types, and may likely change the standard of care in certain disease contexts. Indeed, ramucirumab has shown to improve survival of patients with progressive gastric or colon cancer, either alone or in combination with different chemotherapy regimens. These results are relevant, particularly in gastric cancer, as there were no standard approved agents for the treatment of patients in the second-line setting. The survival benefit observed with single-agent ramucirumab versus BSC (5.3 vs 3.8 months, HR = 0.776) is similar to that reported for other cytotoxic agents (docetaxel, irinotecan) in patients with advanced previously treated GEJ or gastric adenocarcinoma, with a more favorable toxicity profile [30, 31]. Moreover, results of ramucirumab in combination with paclitaxel in advanced progressive gastric/GEJ cancer, with a median overall survival of 9.6 months for patients treated with both agents versus 7.4 months for patients treated only with paclitaxel (HR = 0.807), is certainly remarkable in this setting, particularly considering that the addition of ramucirumab did not negatively impact QoL. Ongoing studies will provide further information regarding safety and efficacty of ramucirumab in combination with other chemotherapy regimens and in other treatment settings, such as cisplatin and fluoropyrimidines as first-line therapy in patients with advanced gastric/GEJ cancer, among others. Also intriguing were results reported for HCC, particularly in patients with elevated baseline alpha-fetoprotein levels, in which ramucirumab may still play a role that certainly deserves to be further explored.

Somewhat more complicated is the clinical scenario in CRC. The RAISE study showed that, following progression on first-line bevacizumab, oxaliplatin and fluoropyrimidine combination therapy, the addition of ramucirumab to FOLFIRI significantly prolonged survival compared to FOLFIRI alone (13.3 vs 11.7 months, HR = 0.844). These findings provide further evidence that continued angiogenic pathway inhibition is an important therapeutic strategy in CRC, in line with results reported in the TML or VELOUR studies with bevacizumab and aflibercept, respectively [8, 32]. However, some differences among these studies deserve to be discussed. In the VELOUR study, which compared FOLFIRI with aflibercept or placebo as second line therapy in patients with advanced CRC, only ~30 % of patients had been treated with bevacizumab in first-line. Although subgroup analysis indicated that bevacizumab pre-treated patients also appeared to derive a benefit from the addition of aflibercept, the magnitude of the effect seemed smaller in this subgroup of patients and was only statistically significant for the PFS endpoint, although the study was likely underpowered to assess OS in this subgroup. The TML study, which assessed continued use of bevacizumab plus standard second-line chemotherapy in patients with metastatic CRC progressing after standard first-line bevacizumab-based treatment, permited a wide spectrum of chemotherapy backbones at the physicians discretion (both first and second line), and excluded patients with fast-growing tumors (defined by first-line progression in <3 months), patients who were given <3 months of bevacizumab treatment and those with progressive disease being documented >3 months after bevacizumab therapy. In contrast, eligibility criteria for the RAISE study were more homogeneous

regarding prior therapy administered (all patients required to have progressed to oxaliplatin, fluoropyrimidines, and bevacizumab) and also defined a consistent dose and schedule of second-line chemotherapy (FOLFIRI) for all patients. On the other hand, they were less restrictive regarding tumor biology, as the RAISE study only required a minimum of two prior doses of bevacizumab and included patients that had progressed up to 6 months following first line therapy. Beyond these subtle differences in study design, the magnitude of the effect does not seem to substantially differ among the different angiogenesis inhibitors in this setting. Therefore, and in the absence of head-to-head comparisons or validated biomarkers, other important issues to be considered in order to make sensible decisions in the clinic include tolerability of the drugs and costs. In terms of safety, bevacizumab is likely the angiogenesis inhibitor with the most favorable toxicity profile and aflibercept the one that induces higher rates of severe hypertension, proteinuria, and diarrhea. Aflibercept and ramucirumab are also associated with greater incidence of fatigue, neutropenia, and infectious complications. Besides toxicity, comparative cost-effective analysis shall also influence drug selection, although they may widely vary among different countries depending upon drug and health-care related costs, as well as different reimbursement issues that are beyond the scope of this review.

Ramucirumab clinical development continues in these and other tumor types. Other relevant issues to be ellucidated include the most adequate dosing regimen for patients. Doses explored in phase III studies, although below the MTD of phase I clinical trials, were selected as they were found to yield minimum plasma concentrations well above those associated with growth inhibition in preclinical xenograft models, and were well tolerated and associated with preliminary evidence of clinical activity. However, retrospective exposure-response analysis in gastric and CRC have suggested that patients with higher ramucirumab exposure may derive greater clinical benefit and, in fact, the FDA has recommended the company to conduct a post-marketing clinical trial to explore the benefits and safety of a higher dosing regimen of ramucirumab. Finally, in the era of targeted therapy, the continuous failure to identify biomarkers for appropriate selection of patients most likely to benefit from the different antiangiogenic strategies, is certainly a major pending subject that deserves intense collaborative research to be pursued. Indeed, although a number of circulating and tissue biomarkers have shown predictive potential in some studies (i.e., genetic variants in VEGFA or its receptors, expression of neuropilin-1 in tumors or plasma, …), there is currently no validated biomarker that can be routinely utilized in the clinic, illustrating the great complexity, overlap, and redundancy of angiogenesis pathways in the contexto of tumor biology.

Compliance With Ethical Standards

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