chromosome 7 (the chromosome on which *MET* is located) was indeed observed ~30% of NSCLC [27] and gastric [29] tumors with an increased *MET* copy number. Furthermore, such tumors might not be MET driven, given that breast tumors with an increased copy number for the human epidermal growth factor receptor 2 (HER2) gene as a result of polysomy 17 behave as HER2-negative tumors [42]. Southern blot analysis and PCR-based assays identify a gain in gene copy number regardless of the underlying cause and are thus unable to discriminate gene amplification from polysomy (Figure 1A). This methodological limitation is sometimes overlooked in determination of the prevalence of *MET* amplification in cancer.

Table 1. Prevalence of *MET* amplification and increased *MET* gene copy number (GCN) in NSCLC.

Study	Number of Patients	Technique	Classification	Positivity (%)
Camidge *et al.* (2010) [43]	66	FISH	*MET/CEP7* ratio > 2.0	0
Onozato *et al.* (2009) [33]	148	PCR based	GCN > 2	1.4
Kubo *et al.* (2009) [34]	100	PCR based	GCN > 5	2.0
Bean *et al.* (2007) [30]	16	PCR based	GCN > 5	3.0
Go *et al.* (2010) [27]	180	FISH	*MET/CEP7* ratio > 2.0	3.9
Okamoto *et al.* (2014) [44]	229	FISH	*MET/CEP7* ratio > 2.2	3.9
Cappuzzo *et al.* (2009) [45]	447	FISH	*MET/CEP7* ratio > 2.0	4.1
Onitsuka *et al.* (2010) [32]	183	PCR based	GCN > 1.31	4.4
Okuda *et al.* (2008) [31]	213	PCR based	GCN > 3	5.6
Beau-Faller *et al.* (2008) [35]	106	PCR based	GCN > mean + 2SD of 30 normal lung DNA samples	20.8

FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction; GCN, gene copy number; CEP7, centromeric portion of chromosome 7.

Table 2. Prevalence of *MET* amplification and increased *MET* gene copy number (GCN) in gastric cancer.

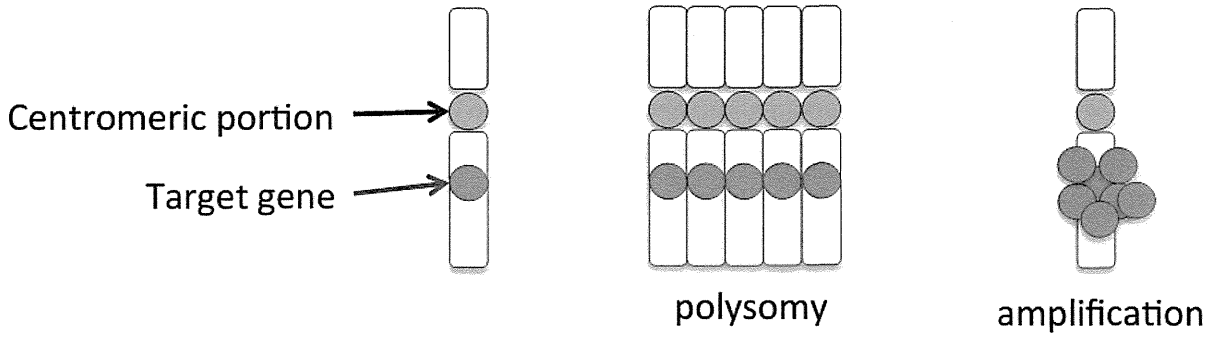
Study	Number of Patients	Technique	Classification	Positivity (%)
Janjigian *et al.* (2011) [29]	38	FISH	*MET/CEP7* ratio > 2.0	0
Kawakami *et al.* (2013) [46]	266	FISH	*MET/CEP7* ratio > 2.2	1.5
Lennerz *et al.* (2011) [28]	267 (junctional and gastric)	FISH	*MET/CEP7* ratio > 2.2	2.2
Hara *et al.* (1998) [20]	154	FISH	NA	3.9
Liu *et al.* (2014) [47]	196	FISH	*MET/CEP7* ratio > 2.0	6.1
Graziano *et al.* (2011) [40]	216	PCR based	GCN ≥ 5	9.7
Tsugawa *et al.* (1998) [21]	70	Slot blot analysis	Ratio > 2 (relative to normal mucosa)	10.0
Nakajima *et al.* (1999) [19]	128	Southern blot analysis	Ratio > 2 (relative to normal mucosa)	10.2
Lee *et al.* (2011) [39]	472	PCR based	GCN ≥ 4	21.2
Shi *et al.* (2012) [48]	128	PCR based	GCN ≥ 4	30.5

FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction; GCN, gene copy number; CEP7, centromeric portion of chromosome 7; NA, not available.

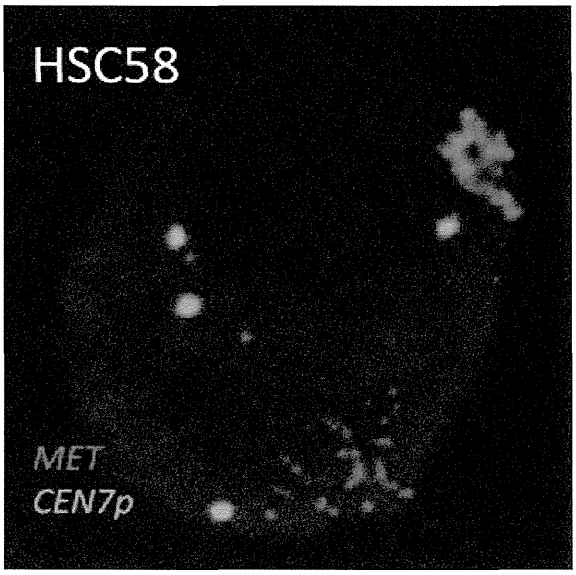
On the other hand, FISH analysis is a semiquantitative method that can be performed with two probes for determination of the number of signals both for a target gene and for the centromeric portion of the corresponding chromosome. Given that the number of centromeric signals directly indicates the copy number of the chromosome, FISH analysis reveals the copy number increase for the target gene from the ratio of the copy number of the gene to that of the chromosome (Figure 1). Comparative genomic hybridization (CGH) is another molecular cytogenetic approach to the identification of gene amplification. CGH analyzes copy number variation for whole chromosomes or subchromosomal regions relative to ploidy level in the DNA of a test sample in comparison with a reference sample [49]. Although CGH has proved to be an efficient and reproducible technique, it remains relatively expensive to perform and requires a well-equipped laboratory and a high level of operator expertise.

Figure 1. (**A**) Schematic comparison of gene amplification and polysomy. The ratio of the copy number for the target gene to that for the centromeric portion of the chromosome distinguishes an increased copy number of the target gene attributable to gene amplification from that resulting from extra copies of the chromosome (polysomy). (**B**) FISH analysis of a gastric cancer cell line (HSC58) positive for *MET* amplification. The image shows a single cancer cell, with green and red signals corresponding to *CEP7* (*CEN7p*) and the *MET* locus, respectively.

A



B



FISH is thus currently the gold standard for detection of gene amplification. According to the recent ASCO/CAP guidelines for *HER2* testing, gene amplification is defined as positive with a target gene/centromere ratio of >2.2, negative with a ratio of <1.8, and equivocal with a ratio between 1.8 and 2.2 [50]. Importantly, polysomy, which is mechanistically distinct from gene amplification, is mostly associated with a ratio in the equivocal range [51].

With the strict definition of *MET* amplification as a *MET/CEP7* (centromeric region of chromosome 7) ratio of >2.2 as determined by FISH analysis, we identified nine out of 229 patients with advanced NSCLC (3.9%) as being positive for *MET* amplification [44]. We also found that four out of 266 gastric cancer patients (1.5%) were positive for *MET* amplification as determined with a combination of PCR-based screening and FISH confirmation [46]. These results suggest that *MET* amplification identifies a small but clinically important subgroup of cancer patients who are likely to respond to MET-TKIs.

4. Clinical Response to Crizotinib in *MET* Amplification—Positive Cancer Patients

To date, at least 17 MET-TKIs with kinase selectivity profiles ranging from highly selective to multitargeted have been or are currently being subjected to clinical evaluation [52]. Although several agents including cabozantinib [53] and foretinib [54] have made good progress, they are multitargeted MET-TKIs, and so little is known of the relation between their efficacy and *MET* amplification. In NSCLC, *MET* amplification is one of the mechanisms responsible for the development of resistance to EGFR-TKIs, with dual inhibition of EGFR and MET having been shown to induce apoptosis in such resistant cells [55]. Combination treatment with an EGFR-TKI and tivantinib, a selective MET-TKI with microtubule-disrupting activity similar to that of vincristine [56], has been evaluated in clinical trials, but the efficacy of this approach remains unclear. Among the MET-TKIs examined, however, crizotinib has consistently shown efficacy in patients with cancer positive for *MET* amplification.

Preliminary reports of the clinical response of patients with *MET* amplification-positive cancer to crizotinib have come from an enriched molecular cohort of individuals with advanced cancer in a phase I trial of this drug (A8081001, ClinicalTrials.gov identifier NCT00585195). This cohort includes patients with various tumor types harboring specific genetic alterations of *MET* or *ALK*, including *MET* amplification defined as a *MET/CEP7* ratio of >2.2 (but not polysomy 7, kinase domain-activating mutations of *MET*, or other chromosomal translocations leading to altered transcriptional regulation of *MET*) as well as *ALK* chromosomal translocation or gene amplification. A patient with stage IV lung adenocarcinoma that was negative for *ALK* rearrangement but positive for high-level *MET* amplification (*MET/CEP7* ratio of >5.0) started treatment with crizotinib at a dose of 250 mg twice a day [57]. The patient achieved a maximum reduction in aggregate tumor measurement of 54.8% after 4 months of such therapy and thereafter continued the study treatment showing a partial response. A patient with *MET* amplification-positive glioblastoma was also treated with crizotinib at 250 mg twice a day [58]. After 2 months of treatment, the first scheduled cranial magnetic resonance imaging (MRI) scan revealed a 40% reduction in tumor size, and after 4 months a restaging cranial MRI examination confirmed this effect to be stable. Administration of crizotinib was continued for a total of 6 months, until the patient manifested disease progression.

Another study revealed a pronounced clinical response to crizotinib in two of four patients with gastric cancer positive for *MET* amplification (*MET/CEP7* ratio of >2.2) [28]. After 1 week of crizotinib

treatment, one patient experienced a rapid symptomatic response with an increase in appetite, reduction in pain, and improvement in performance status. A computed tomography (CT) scan at the end of treatment cycle 2 (8 weeks) revealed a partial tumor response, which was confirmed at 12 weeks. Another patient also showed rapid clinical improvement, with reduced pain and improved performance status, after 1 week of crizotinib treatment. Time to progression for these two patients on crizotinib treatment was ~112 and 105 days, respectively.

Crizotinib was approved by the U.S. Food and Drug Administration for the treatment of *ALK* rearrangement-positive NSCLC in 2011, and a recent report has addressed the clinical efficacy of this agent in a clinical practice setting [59]. A male patient with stage IV squamous cell lung cancer was found to be positive for *MET* amplification (*MET/CEP7* ratio of >2.2) and negative for *ALK* rearrangement by FISH analysis. He was treated with crizotinib monotherapy at the normal dose of 250 mg twice daily. An almost complete response of tumors in the left lung and a major response of the primary tumor to therapy were demonstrated by chest CT and positron emission tomography (PET)-CT after 8 weeks of therapy.

Preliminary results of the NCT00585195 phase I study for patients with *MET* amplification-positive NSCLC were reported at the 2014 Annual Meeting of the American Society of Clinical Oncology (ASCO) [60]. Patients were categorized into three classes according to *MET* amplification status as determined by FISH analysis: low (*MET/CEP7* ratio of ≥1.8 to ≤2.2), intermediate (*MET/CEP7* ratio of >2.2 to <5.0), and high (*MET/CEP7* ratio of ≥5.0). Thirteen patients with a low (n = 1), intermediate (n = 6), or high (n = 6) *MET/CEP7* ratio received crizotinib. Of the 12 evaluable patients, four (33%) showed a partial response and were found to have an intermediate (n = 1) or high (n = 3) *MET/CEP7* ratio. These findings are thus suggestive of an association between the *MET/CEP7* ratio and the clinical benefit of crizotinib in patients with *MET* amplification-positive cancer.

The accumulating clinical evidence thus suggests that *MET* amplification as strictly defined by a *MET/CEP7* ratio of >2.2 has the potential to act as an oncogenic driver and thereby to render at least a subset of affected tumors responsive to MET-TKIs such as crizotinib. Not all *MET* amplification-positive cancer patients respond to MET-TKI treatment, however, and most such patients who do respond, even those who show an initial marked response, eventually develop resistance to MET-TKIs. Preexisting and acquired resistance to MET-TKIs is thus an important clinical problem that is shared with other targeted therapies. Several mechanisms of resistance to MET-TKIs have been identified in preclinical models, including additional mutations in the activation loop of MET [61], ligand-dependent activation of EGFR signaling [61,62], *SND1-BRAF* fusion [63], and amplification and overexpression of wild-type *KRAS* [64]. Further characterization of such mechanisms will be important to provide a basis for the development of effective therapies for patients with MET-TKI resistance.

5. Conclusions

MET amplification has been identified as a potential oncogenic driver for several neoplasms, and targeted therapy with MET-TKIs for such tumors is thus a reasonable and effective treatment. Clinical trials of such drugs are strongly warranted for patients with advanced malignancies positive for *MET* amplification as strictly defined by a *MET/CEP7* ratio of >2.2 determined by FISH.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Mok, T.S.; Wu, Y.L.; Thongprasert, S.; Yang, C.H.; Chu, D.T.; Saijo, N.; Sunpaweravong, P.; Han, B.; Margono, B.; Ichinose, Y.; *et al.* Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N. Engl. J. Med.* **2009**, *361*, 947–957.
2. Sequist, L.V.; Martins, R.G.; Spigel, D.; Grunberg, S.M.; Spira, A.; Janne, P.A.; Joshi, V.A.; McCollum, D.; Evans, T.L.; Muzikansky, A.; *et al.* First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic EGFR mutations. *J. Clin. Oncol.* **2008**, *26*, 2442–2449.
3. Porter, J. Small molecule c-Met kinase inhibitors: A review of recent patents. *Expert Opin. Ther. Pat.* **2010**, *20*, 159–177.
4. Christensen, J.G.; Schreck, R.; Burrows, J.; Kuruganti, P.; Chan, E.; Le, P.; Chen, J.; Wang, X.; Ruslim, L.; Blake, R.; *et al.* A selective small molecule inhibitor of c-Met kinase inhibits c-Met-dependent phenotypes *in vitro* and exhibits cytoreductive antitumor activity *in vivo*. *Cancer Res.* **2003**, *63*, 7345–7355.
5. Christensen, J.G.; Burrows, J.; Salgia, R. c-Met as a target for human cancer and characterization of inhibitors for therapeutic intervention. *Cancer Lett.* **2005**, *225*, 1–26.
6. Davis, I.J.; McFadden, A.W.; Zhang, Y.; Coxon, A.; Burgess, T.L.; Wagner, A.J.; Fisher, D.E. Identification of the receptor tyrosine kinase c-Met and its ligand, hepatocyte growth factor, as therapeutic targets in clear cell sarcoma. *Cancer Res.* **2010**, *70*, 639–645.
7. Di Renzo, M.F.; Olivero, M.; Martone, T.; Maffe, A.; Maggiora, P.; Stefani, A.D.; Valente, G.; Giordano, S.; Cortesina, G.; Comoglio, P.M. Somatic mutations of the Met oncogene are selected during metastatic spread of human HNSC carcinomas. *Oncogene* **2000**, *19*, 1547–1555.
8. Park, W.S.; Dong, S.M.; Kim, S.Y.; Na, E.Y.; Shin, M.S.; Pi, J.H.; Kim, B.J.; Bae, J.H.; Hong, Y.K.; Lee, K.S.; *et al.* Somatic mutations in the kinase domain of the Met/hepatocyte growth factor receptor gene in childhood hepatocellular carcinomas. *Cancer Res.* **1999**, *59*, 307–310.
9. Schmidt, L.; Duh, F.M.; Chen, F.; Kishida, T.; Glenn, G.; Choyke, P.; Scherer, S.W.; Zhuang, Z.; Lubensky, I.; Dean, M.; *et al.* Germline and somatic mutations in the tyrosine kinase domain of the Met proto-oncogene in papillary renal carcinomas. *Nat. Genet.* **1997**, *16*, 68–73.
10. Birchmeier, C.; Birchmeier, W.; Gherardi, E.; vande Woude, G.F. Met, metastasis, motility and more. *Nat. Rev. Mol. Cell Biol* **2003**, *4*, 915–925.
11. Danilkovitch-Miagkova, A.; Zbar, B. Dysregulation of Met receptor tyrosine kinase activity in invasive tumors. *J. Clin. Invest.* **2002**, *109*, 863–867.
12. Tanizaki, J.; Okamoto, I.; Okamoto, K.; Takezawa, K.; Kuwata, K.; Yamaguchi, H.; Nakagawa, K. Met tyrosine kinase inhibitor crizotinib (PF-02341066) shows differential antitumor effects in non-small cell lung cancer according to Met alterations. *J. Thorac. Oncol.* **2011**, *6*, 1624–1631.

13. Zou, H.Y.; Li, Q.; Lee, J.H.; Arango, M.E.; Burgess, K.; Qiu, M.; Engstrom, L.D.; Yamazaki, S.; Parker, M.; Timofeevski, S.; *et al.* Sensitivity of selected human tumor models to PF-04217903, a novel selective c-Met kinase inhibitor. *Mol. Cancer Ther.* **2012**, *11*, 1036–1047.
14. Zou, H.Y.; Li, Q.; Lee, J.H.; Arango, M.E.; McDonnell, S.R.; Yamazaki, S.; Koudriakova, T.B.; Alton, G.; Cui, J.J.; Kung, P.P.; *et al.* An orally available small-molecule inhibitor of c-Met, PF-2341066, exhibits cytoreductive antitumor efficacy through antiproliferative and antiangiogenic mechanisms. *Cancer Res.* **2007**, *67*, 4408–4417.
15. Timofeevski, S.L.; McTigue, M.A.; Ryan, K.; Cui, J.; Zou, H.Y.; Zhu, J.X.; Chau, F.; Alton, G.; Karlicek, S.; Christensen, J.G.; *et al.* Enzymatic characterization of c-Met receptor tyrosine kinase oncogenic mutants and kinetic studies with aminopyridine and triazolopyrazine inhibitors. *Biochemistry* **2009**, *48*, 5339–5349.
16. Park, W.S.; Oh, R.R.; Kim, Y.S.; Park, J.Y.; Shin, M.S.; Lee, H.K.; Lee, S.H.; Yoo, N.J.; Lee, J.Y. Absence of mutations in the kinase domain of the Met gene and frequent expression of Met and HGF/SF protein in primary gastric carcinomas. *APMIS* **2000**, *108*, 195–200.
17. Lee, J.H.; Han, S.U.; Cho, H.; Jennings, B.; Gerrard, B.; Dean, M.; Schmidt, L.; Zbar, B.; vande Woude, G.F. A novel germ line juxtamembrane Met mutation in human gastric cancer. *Oncogene* **2000**, *19*, 4947–4953.
18. Chen, J.D.; Kearns, S.; Porter, T.; Richards, F.M.; Maher, E.R.; Teh, B.T. Met mutation and familial gastric cancer. *J. Med. Genet.* **2001**, *38*, E26.
19. Nakajima, M.; Sawada, H.; Yamada, Y.; Watanabe, A.; Tatsumi, M.; Yamashita, J.; Matsuda, M.; Sakaguchi, T.; Hirao, T.; Nakano, H. The prognostic significance of amplification and overexpression of c-Met and c-Erb b-2 in human gastric carcinomas. *Cancer* **1999**, *85*, 1894–1902.
20. Hara, T.; Ooi, A.; Kobayashi, M.; Mai, M.; Yanagihara, K.; Nakanishi, I. Amplification of c-Myc, k-Sam, and c-Met in gastric cancers: Detection by fluorescence in situ hybridization. *Lab. Invest.* **1998**, *78*, 1143–1153.
21. Tsugawa, K.; Yonemura, Y.; Hirono, Y.; Fushida, S.; Kaji, M.; Miwa, K.; Miyazaki, I.; Yamamoto, H. Amplification of the c-Met, c-Erbb-2 and epidermal growth factor receptor gene in human gastric cancers: Correlation to clinical features. *Oncology* **1998**, *55*, 475–481.
22. Smolen, G.A.; Sordella, R.; Muir, B.; Mohapatra, G.; Barmettler, A.; Archibald, H.; Kim, W.J.; Okimoto, R.A.; Bell, D.W.; Sgroi, D.C.; *et al.* Amplification of Met may identify a subset of cancers with extreme sensitivity to the selective tyrosine kinase inhibitor PHA-665752. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 2316–2321.
23. Okamoto, W.; Okamoto, I.; Arao, T.; Kuwata, K.; Hatashita, E.; Yamaguchi, H.; Sakai, K.; Yanagihara, K.; Nishio, K.; Nakagawa, K. Antitumor action of the Met tyrosine kinase inhibitor crizotinib (PF-02341066) in gastric cancer positive for Met amplification. *Mol. Cancer Ther.* **2012**, *11*, 1557–1564.
24. Masuya, D.; Huang, C.; Liu, D.; Nakashima, T.; Kameyama, K.; Haba, R.; Ueno, M.; Yokomise, H. The tumour-stromal interaction between intratumoral c-Met and stromal hepatocyte growth factor associated with tumour growth and prognosis in non-small-cell lung cancer patients. *Br. J. Cancer* **2004**, *90*, 1555–1562.

25. Nakamura, Y.; Niki, T.; Goto, A.; Morikawa, T.; Miyazawa, K.; Nakajima, J.; Fukayama, M. c-Met activation in lung adenocarcinoma tissues: An immunohistochemical analysis. *Cancer Sci.* **2007**, *98*, 1006–1013.
26. Zhao, X.; Weir, B.A.; LaFramboise, T.; Lin, M.; Beroukhim, R.; Garraway, L.; Beheshti, J.; Lee, J.C.; Naoki, K.; Richards, W.G.; *et al.* Homozygous deletions and chromosome amplifications in human lung carcinomas revealed by single nucleotide polymorphism array analysis. *Cancer Res.* **2005**, *65*, 5561–5570.
27. Go, H.; Jeon, Y.K.; Park, H.J.; Sung, S.W.; Seo, J.W.; Chung, D.H. High Met gene copy number leads to shorter survival in patients with non-small cell lung cancer. *J. Thorac. Oncol.* **2010**, *5*, 305–313.
28. Lennerz, J.K.; Kwak, E.L.; Ackerman, A.; Michael, M.; Fox, S.B.; Bergethon, K.; Lauwers, G.Y.; Christensen, J.G.; Wilner, K.D.; Haber, D.A.; *et al.* Met amplification identifies a small and aggressive subgroup of esophagogastric adenocarcinoma with evidence of responsiveness to crizotinib. *J. Clin. Oncol.* **2011**, *29*, 4803–4810.
29. Janjigian, Y.Y.; Tang, L.H.; Coit, D.G.; Kelsen, D.P.; Francone, T.D.; Weiser, M.R.; Jhanwar, S.C.; Shah, M.A. Met expression and amplification in patients with localized gastric cancer. *Cancer Epidemiol. Biomark. Prev.* **2011**, *20*, 1021–1027.
30. Bean, J.; Brennan, C.; Shih, J.Y.; Riely, G.; Viale, A.; Wang, L.; Chitale, D.; Motoi, N.; Szoke, J.; Broderick, S.; *et al.* Met amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 20932–20937.
31. Okuda, K.; Sasaki, H.; Yukiue, H.; Yano, M.; Fujii, Y. Met gene copy number predicts the prognosis for completely resected non-small cell lung cancer. *Cancer Sci.* **2008**, *99*, 2280–2285.
32. Onitsuka, T.; Uramoto, H.; Nose, N.; Takenoyama, M.; Hanagiri, T.; Sugio, K.; Yasumoto, K. Acquired resistance to gefitinib: The contribution of mechanisms other than the T790M, Met, and HGF status. *Lung Cancer* **2010**, *68*, 198–203.
33. Onozato, R.; Kosaka, T.; Kuwano, H.; Sekido, Y.; Yatabe, Y.; Mitsudomi, T. Activation of Met by gene amplification or by splice mutations deleting the juxtamembrane domain in primary resected lung cancers. *J. Thorac. Oncol.* **2009**, *4*, 5–11.
34. Kubo, T.; Yamamoto, H.; Lockwood, W.W.; Valencia, I.; Soh, J.; Peyton, M.; Jida, M.; Otani, H.; Fujii, T.; Ouchida, M.; *et al.* Met gene amplification or EGFR mutation activate Met in lung cancers untreated with EGFR tyrosine kinase inhibitors. *Int. J. Cancer* **2009**, *124*, 1778–1784.
35. Beau-Faller, M.; Ruppert, A.M.; Voegeli, A.C.; Neuville, A.; Meyer, N.; Guerin, E.; Legrain, M.; Mennecier, B.; Wihlm, J.M.; Massard, G.; *et al.* Met gene copy number in non-small cell lung cancer: Molecular analysis in a targeted tyrosine kinase inhibitor naive cohort. *J. Thorac. Oncol.* **2008**, *3*, 331–339.
36. Kuniyasu, H.; Yasui, W.; Kitadai, Y.; Yokozaki, H.; Ito, H.; Tahara, E. Frequent amplification of the c-Met gene in scirrhous type stomach cancer. *Biochem. Biophys. Res. Commun.* **1992**, *189*, 227–232.
37. Tsujimoto, H.; Sugihara, H.; Hagiwara, A.; Hattori, T. Amplification of growth factor receptor genes and DNA ploidy pattern in the progression of gastric cancer. *Virchows Arch.* **1997**, *431*, 383–389.

38. Seruca, R.; Suijkerbuijk, R.F.; Gartner, F.; Criado, B.; Veiga, I.; Olde-Weghuis, D.; David, L.; Castedo, S.; Sobrinho-Simoes, M. Increasing levels of Myc and Met co-amplification during tumor progression of a case of gastric cancer. *Cancer Genet. Cytogenet.* **1995**, *82*, 140–145.
39. Lee, J.; Seo, J.W.; Jun, H.J.; Ki, C.S.; Park, S.H.; Park, Y.S.; Lim, H.Y.; Choi, M.G.; Bae, J.M.; Sohn, T.S.; *et al.* Impact of Met amplification on gastric cancer: Possible roles as a novel prognostic marker and a potential therapeutic target. *Oncol. Rep.* **2011**, *25*, 1517–1524.
40. Graziano, F.; Galluccio, N.; Lorenzini, P.; Ruzzo, A.; Canestrari, E.; D'Emidio, S.; Catalano, V.; Sisti, V.; Ligorio, C.; Andreoni, F.; *et al.* Genetic activation of the Met pathway and prognosis of patients with high-risk, radically resected gastric cancer. *J. Clin. Oncol.* **2011**, *29*, 4789–4795.
41. Albertson, D.G. Gene amplification in cancer. *Trends Genet.* **2006**, *22*, 447–455.
42. Vanden Bempt, I.; van Loo, P.; Drijkoningen, M.; Neven, P.; Smeets, A.; Christiaens, M.R.; Paridaens, R.; de Wolf-Peeters, C. Polysomy 17 in breast cancer: Clinicopathologic significance and impact on Her-2 testing. *J. Clin. Oncol.* **2008**, *26*, 4869–4874.
43. Camidge, D.R.; Kono, S.A.; Flacco, A.; Tan, A.C.; Doebele, R.C.; Zhou, Q.; Crino, L.; Franklin, W.A.; Varella-Garcia, M. Optimizing the detection of lung cancer patients harboring anaplastic lymphoma kinase (ALK) gene rearrangements potentially suitable for ALK inhibitor treatment. *Clin. Cancer Res.* **2010**, *16*, 5581–5590.
44. Okamoto, I.; Sakai, K.; Morita, S.; Yoshioka, H.; Kaneda, H.; Takeda, K.; Hirashima, T.; Kogure, Y.; Kimura, T.; Takahashi, T.; *et al.* Multiplex genomic profiling of non–small cell lung cancers from the LETS phase III trial of first-line S-1/carboplatin versus paclitaxel/carboplatin: Results of a west Japan oncology group study. *Oncotarget* **2014**, *5*, 2293–2304.
45. Cappuzzo, F.; Marchetti, A.; Skokan, M.; Rossi, E.; Gajapathy, S.; Felicioni, L.; del Grammastro, M.; Sciarrotta, M.G.; Buttitta, F.; Incarbone, M.; *et al.* Increased Met gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. *J. Clin. Oncol.* **2009**, *27*, 1667–1674.
46. Kawakami, H.; Okamoto, I.; Arao, T.; Okamoto, W.; Matsumoto, K.; Taniguchi, H.; Kuwata, K.; Yamaguchi, H.; Nishio, K.; Nakagawa, K.; *et al.* Met amplification as a potential therapeutic target in gastric cancer. *Oncotarget* **2013**, *4*, 9–17.
47. Liu, Y.J.; Shen, D.; Yin, X.; Gavine, P.; Zhang, T.; Su, X.; Zhan, P.; Xu, Y.; Lv, J.; Qian, J.; *et al.* Her2, Met and FGFR2 oncogenic driver alterations define distinct molecular segments for targeted therapies in gastric carcinoma. *Br. J. Cancer* **2014**, *110*, 1169–1178.
48. Shi, J.; Yao, D.; Liu, W.; Wang, N.; Lv, H.; He, N.; Shi, B.; Hou, P.; Ji, M. Frequent gene amplification predicts poor prognosis in gastric cancer. *Int. J. Mol. Sci.* **2012**, *13*, 4714–4726.
49. Albertson, D.G.; Collins, C.; McCormick, F.; Gray, J.W. Chromosome aberrations in solid tumors. *Nat. Genet.* **2003**, *34*, 369–376.
50. Wolff, A.C.; Hammond, M.E.; Hicks, D.G.; Dowsett, M.; McShane, L.M.; Allison, K.H.; Allred, D.C.; Bartlett, J.M.; Bilous, M.; Fitzgibbons, P.; *et al.* Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *J. Clin. Oncol.* **2013**, *31*, 3997–4013.
51. Ma, Y.; Lespagnard, L.; Durbecq, V.; Paesmans, M.; Desmedt, C.; Gomez-Galdon, M.; Veys, I.; Cardoso, F.; Sotiriou, C.; di Leo, A.; *et al.* Polysomy 17 in Her-2/Neu status elaboration in breast cancer: Effect on daily practice. *Clin. Cancer Res.* **2005**, *11*, 4393–4399.

52. Zhu, K.; Kong, X.; Zhao, D.; Liang, Z.; Luo, C. c-Met kinase inhibitors: A patent review (2011–2013). *Expert Opin. Ther. Pat.* **2014**, *24*, 217–230.
53. Elisei, R.; Schlumberger, M.J.; Muller, S.P.; Schoffski, P.; Brose, M.S.; Shah, M.H.; Licitra, L.; Jarzab, B.; Medvedev, V.; Kreissl, M.C.; *et al.* Cabozantinib in progressive medullary thyroid cancer. *J. Clin. Oncol.* **2013**, *31*, 3639–3646.
54. Choueiri, T.K.; Vaishampayan, U.; Rosenberg, J.E.; Logan, T.F.; Harzstark, A.L.; Bukowski, R.M.; Rini, B.I.; Srinivas, S.; Stein, M.N.; Adams, L.M.; *et al.* Phase II and biomarker study of the dual Met/VEGFR2 inhibitor foretinib in patients with papillary renal cell carcinoma. *J. Clin. Oncol.* **2013**, *31*, 181–186.
55. Engelman, J.A.; Zejnullahu, K.; Mitsudomi, T.; Song, Y.; Hyland, C.; Park, J.O.; Lindeman, N.; Gale, C.M.; Zhao, X.; Christensen, J.; *et al.* Met amplification leads to gefitinib resistance in lung cancer by activating Erbb3 signaling. *Science* **2007**, *316*, 1039–1043.
56. Katayama, R.; Aoyama, A.; Yamori, T.; Qi, J.; Oh-hara, T.; Song, Y.; Engelman, J.A.; Fujita, N. Cytotoxic activity of tivantinib (ARQ 197) is not due solely to c-Met inhibition. *Cancer Res.* **2013**, *73*, 3087–3096.
57. Ou, S.H.; Kwak, E.L.; Siwak-Tapp, C.; Dy, J.; Bergethon, K.; Clark, J.W.; Camidge, D.R.; Solomon, B.J.; Maki, R.G.; Bang, Y.J.; *et al.* Activity of crizotinib (PF02341066), a dual mesenchymal-epithelial transition (MET) and anaplastic lymphoma kinase (ALK) inhibitor, in a non-small cell lung cancer patient with *de novo* MET amplification. *J. Thorac. Oncol.* **2011**, *6*, 942–946.
58. Chi, A.S.; Batchelor, T.T.; Kwak, E.L.; Clark, J.W.; Wang, D.L.; Wilner, K.D.; Louis, D.N.; Iafrate, A.J. Rapid radiographic and clinical improvement after treatment of a Met-amplified recurrent glioblastoma with a mesenchymal-epithelial transition inhibitor. *J. Clin. Oncol.* **2012**, *30*, e30–e33.
59. Schwab, R.; Petak, I.; Kollar, M.; Pinter, F.; Varkondi, E.; Kohanka, A.; Barti-Juhasz, H.; Schonleber, J.; Brauswetter, D.; Kopper, L.; *et al.* Major partial response to crizotinib, a dual Met/ALK inhibitor, in a squamous cell lung (SCC) carcinoma patient with *de novo* c-Met amplification in the absence of ALK rearrangement. *Lung Cancer* **2014**, *83*, 109–111.
60. Camidge, D.R.; Ou, S.-H.I.; Shapiro, G.; Otterson, G.A.; Villaruz, L.C.; Villalona-Calero, M.A.; Iafrate, A.J.; Varella-Garcia, M.; Dacic, S.; Cardarella, S.; *et al.* Efficacy and safety of crizotinib in patients with advanced *c-MET*-amplified non-small cell lung cancer (NSCLC). *J. Clin. Oncol.* **2014**, *32*, 5s.
61. Qi, J.; McTigue, M.A.; Rogers, A.; Lifshits, E.; Christensen, J.G.; Janne, P.A.; Engelman, J.A. Multiple mutations and bypass mechanisms can contribute to development of acquired resistance to Met inhibitors. *Cancer Res.* **2011**, *71*, 1081–1091.
62. McDermott, U.; Pusapati, R.V.; Christensen, J.G.; Gray, N.S.; Settleman, J. Acquired resistance of non-small cell lung cancer cells to Met kinase inhibition is mediated by a switch to epidermal growth factor receptor dependency. *Cancer Res.* **2010**, *70*, 1625–1634.
63. Lee, N.V.; Lira, M.E.; Pavlicek, A.; Ye, J.; Buckman, D.; Bagrodia, S.; Srinivasa, S.P.; Zhao, Y.; Aparicio, S.; Rejto, P.A.; *et al.* A novel SND1-BRAF fusion confers resistance to c-Met inhibitor PF-04217903 in GTl16 cells through MAPK activation. *PLoS One* **2012**, *7*, e39653.

64. Cepero, V.; Sierra, J.R.; Corso, S.; Ghiso, E.; Casorzo, L.; Perera, T.; Comoglio, P.M.; Giordano, S. Met and Kras gene amplification mediates acquired resistance to Met tyrosine kinase inhibitors. *Cancer Res.* **2010**, *70*, 7580–7590.

Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised phase 3 trial

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See Comment page 1182

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Summary

Background VEGFR-2 has a role in gastric cancer pathogenesis and progression. We assessed whether ramucirumab, a monoclonal antibody VEGFR-2 antagonist, in combination with paclitaxel would increase overall survival in patients previously treated for advanced gastric cancer compared with placebo plus paclitaxel.

Methods This randomised, placebo-controlled, double-blind, phase 3 trial was done at 170 centres in 27 countries in North and South America, Europe, Asia, and Australia. Patients aged 18 years or older with advanced gastric or gastro-oesophageal junction adenocarcinoma and disease progression on or within 4 months after first-line chemotherapy (platinum plus fluoropyrimidine with or without an anthracycline) were randomly assigned with a centralised interactive voice or web-response system in a 1:1 ratio to receive ramucirumab 8 mg/kg or placebo intravenously on days 1 and 15, plus paclitaxel 80 mg/m^2 intravenously on days 1, 8, and 15 of a 28-day cycle. A permuted block randomisation, stratified by geographic region, time to progression on first-line therapy, and disease measurability, was used. The primary endpoint was overall survival. Efficacy analysis was by intention to treat, and safety analysis included all patients who received at least one treatment with study drug. This trial is registered with ClinicalTrials.gov, number NCT01170663, and has been completed; patients who are still receiving treatment are in the extension phase.

Findings Between Dec 23, 2010, and Sept 23, 2012, 665 patients were randomly assigned to treatment—330 to ramucirumab plus paclitaxel and 335 to placebo plus paclitaxel. Overall survival was significantly longer in the ramucirumab plus paclitaxel group than in the placebo plus paclitaxel group (median 9·6 months [95% CI 8·5–10·8] *vs* 7·4 months [95% CI 6·3–8·4], hazard ratio 0·807 [95% CI 0·678–0·962]; p=0·017). Grade 3 or higher adverse events that occurred in more than 5% of patients in the ramucirumab plus paclitaxel group versus placebo plus paclitaxel included neutropenia (133 [41%] of 327 *vs* 62 [19%] of 329), leucopenia (57 [17%] *vs* 22 [7%]), hypertension (46 [14%] *vs* eight [2%]), fatigue (39 [12%] *vs* 18 [5%]), anaemia (30 [9%] *vs* 34 [10%]), and abdominal pain (20 [6%] *vs* 11 [3%]). The incidence of grade 3 or higher febrile neutropenia was low in both groups (ten [3%] *vs* eight [2%]).

Interpretation The combination of ramucirumab with paclitaxel significantly increases overall survival compared with placebo plus paclitaxel, and could be regarded as a new standard second-line treatment for patients with advanced gastric cancer.

Funding Eli Lilly and Company.

Introduction

Gastric cancer is the fifth most common malignancy, and the third leading cause of cancer mortality worldwide.[1] Currently, platinum-based and fluoropyrimidine-based combinations are accepted worldwide as established first-line drug regimens.[2] There are not many treatment options after failure of first-line therapy. In randomised trials, selected second-line chemotherapy significantly improved overall survival compared with best supportive care,[3–5] however, median survival was less than 6 months. Therefore, new, more active second-line treatment options are needed.

VEGF and VEGFR-2-mediated signalling and angiogenesis contribute to the pathogenesis of gastric cancer. In patients with gastric cancer, circulating VEGF levels are associated with increased tumour aggressiveness and reduced survival.[6,7] In animal models of gastric adenocarcinoma, VEGFR-2 inhibition reduced tumour growth and vascularity.[8] First-line treatment with bevacizumab, a VEGF-A-directed monoclonal antibody, in combination with chemotherapy was associated with significantly improved proportions of patients achieving an objective response and progression-free survival, and non-significantly improved overall survival in patients with metastatic gastric cancer.[9,10] Ramucirumab, a human IgG1 monoclonal antibody VEGFR-2 antagonist, prevents ligand binding and receptor-mediated pathway activation in endothelial cells.[11] Paclitaxel was chosen for the

combination based on single-agent second-line trials;[12–14] the results of a retrospective analysis in gastric cancer indicated similar efficacy between frequently used second-line drugs (taxanes or irinotecan).[15] Weekly paclitaxel is better tolerated and more efficacious than 3-weekly paclitaxel in metastatic breast cancer.[16] More recently, in a Japanese randomised trial, weekly paclitaxel was associated with a good toxicity profile compared with irinotecan as second-line therapy in patients with gastric cancer.[17]

We assessed the safety and efficacy of ramucirumab plus paclitaxel in patients with advanced gastric or gastro-oesophageal junction adenocarcinoma with disease progression after first-line combination chemotherapy.

Methods

Study design and patients

RAINBOW was a double-blind, placebo-controlled phase 3 trial. Eligibility criteria included age 18 years and older; having metastatic or non-resectable, locally advanced gastric or gastro-oesophageal junction adenocarcinoma; documented objective radiological or clinical disease progression during or within 4 months of the last dose of first-line platinum and fluoropyrimidine doublet with or without anthracycline; an Eastern Cooperative Oncology Group (ECOG) performance status score of 0 or 1; and measureable or non-measurable evaluable disease (defined with Response Evaluation Criteria In Solid Tumors [RECIST], version 1.1).[18] Exclusion criteria included having squamous or undifferentiated gastric cancer; gastrointestinal perforation, fistulae, or any arterial thromboembolic event within 6 months, or any significant gastrointestinal bleeding or any significant venous thromboembolism within 3 months before randomisation; or poorly controlled hypertension. The appendix provides the full inclusion and exclusion criteria.

Each centre's institutional review board or independent ethics committee approved the study. The trial followed the principles of the Declaration of Helsinki and the Good Clinical Practice Guidelines of the International Conference on Harmonisation. All patients provided written informed consent.

Randomisation and masking

Patients were randomly assigned in a 1:1 ratio to ramucirumab plus paclitaxel or placebo plus paclitaxel using a randomisation sequence generated using the permuted blocks method within each stratum by a statistician not involved in the study activities. Randomisation was stratified by geographic region (region 1, Europe, Israel, Australia, and the USA; region 2, Argentina, Brazil, Chile, and Mexico; and region 3, Japan, South Korea, Hong Kong, Singapore, and Taiwan), time to progression after first dose of first-line therapy (<6 months *vs* ≥6 months), and disease measurability (measurable *vs* non-measurable). This sequence was programmed into a centralised interactive voice or web-response system. Study sites enrolled patients by accessing the centralised interactive voice or web-response system. The interactive voice or web-response system then assigned a unique identification number to each patient, and randomly assigned patients to one of the two treatment groups.

Patients, medical staff, study investigators, individuals who handled and analysed the data, and the funder were masked to treatment assignment. Ramucirumab and placebo for infusion were identical in appearance to preserve masking. Unmasking could be done for individual patients only on the request of a study physician in case knowledge of the identity of study drug was important for the treatment of serious adverse events.

Procedures

Patients received either ramucirumab 8 mg/kg (ImClone Systems, Branchburg, NJ, USA) or placebo intravenously on days 1 and 15, plus paclitaxel 80 mg/m² intravenously on days 1, 8, and 15 of a 28-day cycle. Patients received study treatment until disease progression, unacceptable toxicity, or withdrawal of consent. Crossover between treatment groups was not allowed. Criteria for discontinuation of patients from study treatment, and for dose modifications to manage treatment-related toxicities are presented in the appendix. All patients received supportive care if indicated.

CT scans were done every 6 weeks. Safety data were gathered continuously and local laboratory assessments (including haematology, clinical chemistry, coagulation, and urinalysis [appendix]) were done at baseline, before each treatment, at the end of therapy, and 30 days after the last dose of study drug; adverse events were graded in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE; version 4.02).[19] We planned to assess quality of life every 6 weeks until progression using the European Organisation for Research and Treatment of Cancer quality-of-life questionnaire (EORTC QLQ-C30, version 3.0)[20] and the EuroQoL five-dimension, three-level health status questionnaire (EQ-5D-3L).[21] Performance status was assessed at the start of each cycle, at the end of therapy, and at the 30-day follow-up. Blood for analysis of anti-ramucirumab antibodies (immunogenicity) was obtained at baseline, day 15 of cycle 2 and day 1 of cycle 4, and at the 30-day follow-up, and patients' sera were analysed as detailed in the appendix.

Outcomes

The primary outcome was overall survival, defined as the time from randomisation to death from any cause. Secondary outcomes were progression-free survival, defined as time from randomisation to radiographic progression or death; objective tumour response, defined as the proportion of patients who had a best response of complete response or partial response; disease control, defined as the proportion of patients who had a best

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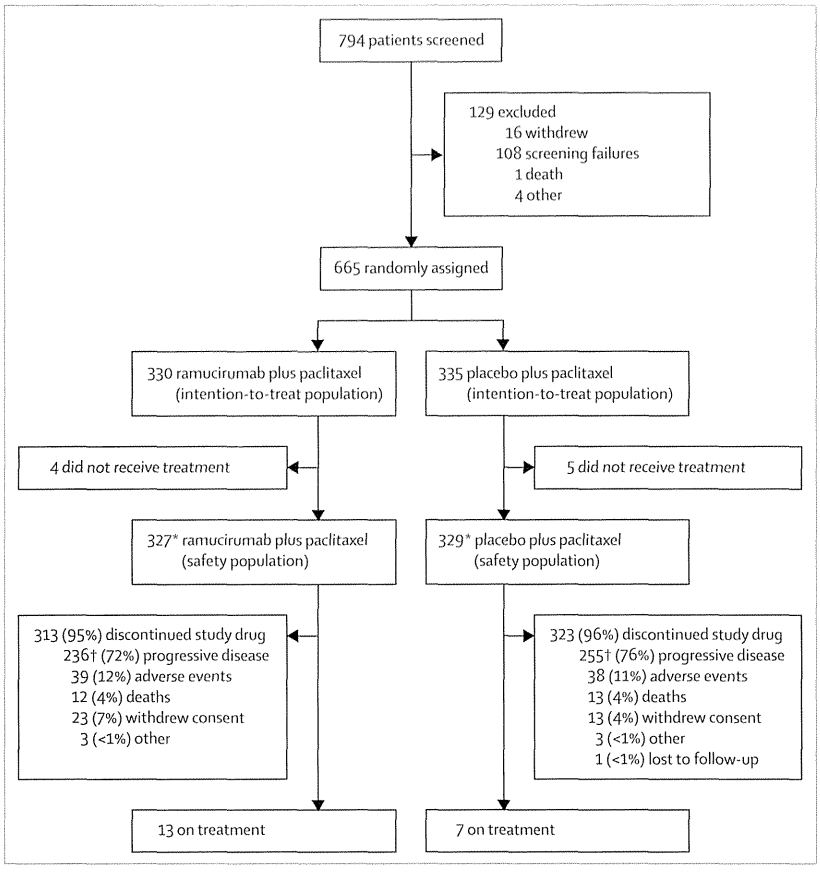

Figure 1: Trial profile
*One patient was randomly assigned to the placebo group, but received one dose of ramucirumab. †Radiographic progression or symptomatic deterioration.

response of complete response, partial response, or stable disease; patient-reported outcomes, as assessed using EORTC QLQ-C30 and EQ-5D-3L; immunogenicity of ramucirumab, where a treatment-emergent antibody-positive response was defined as a post-baseline positive response greater than four times increase in the antibody titre, or a missing baseline assessment and an on-treatment titre of at least 1:20; and safety. Disease progression and tumour response were assessed by investigators in accordance with RECIST 1.1.[18]

Statistical analysis

We calculated that to achieve 90% power to detect an overall difference in survival between the two treatment groups (hazard ratio [HR] 0·75; anticipated median overall survival 7·0 months in the placebo plus paclitaxel group *vs* 9·3 months in the ramucirumab plus paclitaxel group) with a one-sided α of 0·025 (two-sided 0·05), 510 deaths were needed, and 663 patients would need to be randomly assigned. The results presented here are based on a two-sided α of 0·05.

We used a log-rank test, stratified by geographic region, time to progression on first-line therapy, and disease measurability, to assess overall survival and progression-free survival. Estimations of time-to-event curves were generated with the Kaplan-Meier method. The HR was estimated with a stratified Cox proportional hazards model. We did a pre-planned multivariate analysis with a stepwise Cox regression model of predefined baseline characteristics to examine the effect of treatment on overall survival and progression-free survival after adjustment for significant prognostic factors. The proportion of patients achieving an objective response and disease control were compared between treatment groups with the Cochran-Mantel-Haenszel test, with adjustment for the randomisation strata. All analyses were done with SAS (version 9.2).

Efficacy analyses were based on the intention-to-treat population and predefined subgroups. The intention-to-treat population comprised all randomly assigned patients, irrespective of whether the patient received study medication. Safety analyses included all patients who received at least one dose of any study drug.

EORTC QLQ-C30 and EQ-5D data were scored according to developer guidelines and summarised descriptively for the intention-to-treat population.[22,23] The EQ-5D index score was calculated using the algorithm developed to represent UK population preferences for health states.[24]

This trial is registered with ClinicalTrials.gov, number NCT01170663.

Role of the funding source

The study funder provided the study drug and collaborated with investigators on protocol, study design, data gathering, analysis, and interpretation, and writing and preparation of this report. HW prepared the first draft in collaboration with the funder and other coauthors. HW had full access to all patient-level study data and all authors approved the submission for publication.

Results

Between Dec 23, 2010, and Sept 23, 2012, 665 (84%) of 794 screened patients were randomly assigned to receive ramucirumab plus paclitaxel (n=330) or placebo plus paclitaxel (n=335) at 170 centres in 27 countries in North and South America, Europe, Asia, and Australia (appendix). Figure 1 shows the trial profile. All patients were included in the efficacy analyses. As of data cutoff (July 12, 2013), with a median follow-up for overall survival of 7·9 months (IQR 4·2–13·0), 516 (78%) of 665 patients had died. 13 (4%) patients in the ramucirumab and paclitaxel group and seven (2%) in the placebo and paclitaxel group are still receiving treatment, and are in the extension phase of the study.

Baseline characteristics of patients and their tumours were generally well balanced between the groups (table 1). 662 of 665 patients received previous treatment with platinum-based and fluoropyrimidine-based chemotherapy regimens, including regimens with an anthracycline (163 [25%]); of the remaining

	Ramucirumab plus paclitaxel (n=330)	Placebo plus paclitaxel (n=335)
Age (years)		
Median (range)	61 (25–83)	61 (24–84)
<65	204 (62%)	212 (63%)
≥65	126 (38%)	123 (37%)
Sex		
Male	229 (69%)	243 (73%)
Ethnic origin*		
White	208 (63%)	199 (59%)
Asian	110 (33%)	121 (36%)
Black or other	12 (4%)	15 (4%)
ECOG performance status		
0	117 (35%)	144 (43%)
1	213 (65%)	191 (57%)
Geographic region†		
1	198 (60%)	200 (60%)
2	23 (7%)	21 (6%)
3	109 (33%)	114 (34%)
Site of primary tumour		
Gastric	264 (80%)	264 (79%)
Gastro-oesophageal junction adenocarcinoma	66 (20%)	71 (21%)
Disease‡		
Measurable	267 (81%)	273 (81%)
Non-measurable	63 (19%)	62 (19%)
Time to progressive disease on first-line therapy‡		
<6 months	250 (76%)	256 (76%)
≥6 months	80 (24%)	79 (24%)
Disease progression		
During first-line therapy	227 (69%)	217 (65%)
Tumor grade		
Well differentiated	28 (8%)	22 (7%)
Moderately differentiated	96 (29%)	106 (32%)
Poorly differentiated	186 (56%)	186 (56%)
Unknown or missing	20 (6%)	21 (6%)
(Table 1 continues in next column)		
(Continued from previous column)		
Histological subtype (Lauren classification)		
Intestinal	145 (44%)	135 (40%)
Diffuse	115 (35%)	133 (40%)
Mixed	21 (6%)	14 (4%)
Unknown or not available	49 (15%)	53 (16%)
Primary tumour present	209 (63%)	209 (62%)
Number of metastatic sites		
0–2	209 (63%)	232 (69%)
≥3	121 (37%)	103 (31%)
Peritoneal metastases	163 (49%)	152 (45%)
Presence of ascites		
Yes	130 (39%)	107 (32%)
No	200 (61%)	228 (68%)
Weight loss (past 3 months)		
<10%	277 (84%)	286 (85%)
≥10%	53 (16%)	47 (14%)
Previous treatment		
Triplet: platinum and fluoropyrimidine with anthracycline	76 (23%)	87 (26%)
Doublet: platinum and fluoropyrimidine	253 (77%)	246 (73%)
HER2, EGFR, or other	31 (9%)	26 (8%)
Previous surgery for gastric cancer		
Yes	133 (40%)	126 (38%)
Total gastrectomy	52 (16%)	65 (19%)
Partial gastrectomy	80 (24%)	59 (18%)
Other	1 (<1%)	2 (<1%)

Data are number (%) unless otherwise indicated. ECOG=Eastern Cooperative Oncology Group. *By self-report. †Region 1: Europe, Israel, Australia, and the USA; region 2: Argentina, Brazil, Chile, and Mexico; and region 3: Japan, South Korea, Hong Kong, Singapore, and Taiwan. ‡As reported in the interactive voice response system.

Table 1: **Baseline characteristics of patients and their tumours in the intention-to-treat population**

three patients, who had protocol violations, two patients in the placebo plus paclitaxel group had received a platinum-based and fluoropyrimidine-based therapy in the neoadjuvant and adjuvant setting and a fluoropyrimidine-based therapy containing irinotecan and fluorouracil in the first-line setting before enrolling on this study, and one patient in the placebo plus paclitaxel group had received fluoropyrimidine monotherapy in the first-line setting and a fluoropyrimidine and platinum combination in the second-line setting. About two-thirds of the patients had disease progression while still on first-line therapy (table 1). Additionally, a large proportion of patients had other poor prognostic factors including poorly differentiated tumours, disease progression within 6 months after the start of the previous therapy, at least three metastatic sites, presence of primary tumour, peritoneal metastases, or presence of ascites (table 1).

There were 256 deaths in the ramucirumab plus paclitaxel group, and 260 in the placebo plus paclitaxel group (figure 2A). Overall survival with ramucirumab plus paclitaxel was significantly longer than with placebo plus paclitaxel (median 9·6 months [95% CI 8·5–10·8] *vs* 7·4 months [95% CI 6·3–8·4], stratified HR 0·807 [95% CI 0·678–0·962]; p=0·017; figure 2A). 6-month overall survival was 72% (95% CI 66–76) in the ramucirumab plus paclitaxel group, and 57% (95% CI 51–62) in the placebo plus paclitaxel group; 12-month overall survival was 40% (95% CI 35–45) and 30% (95% CI 25–35), respectively (figure 2A).

Overall survival was significantly increased in the ramucirumab plus paclitaxel group compared with the placebo and paclitaxel group (see figure 3 for subgroup analyses).

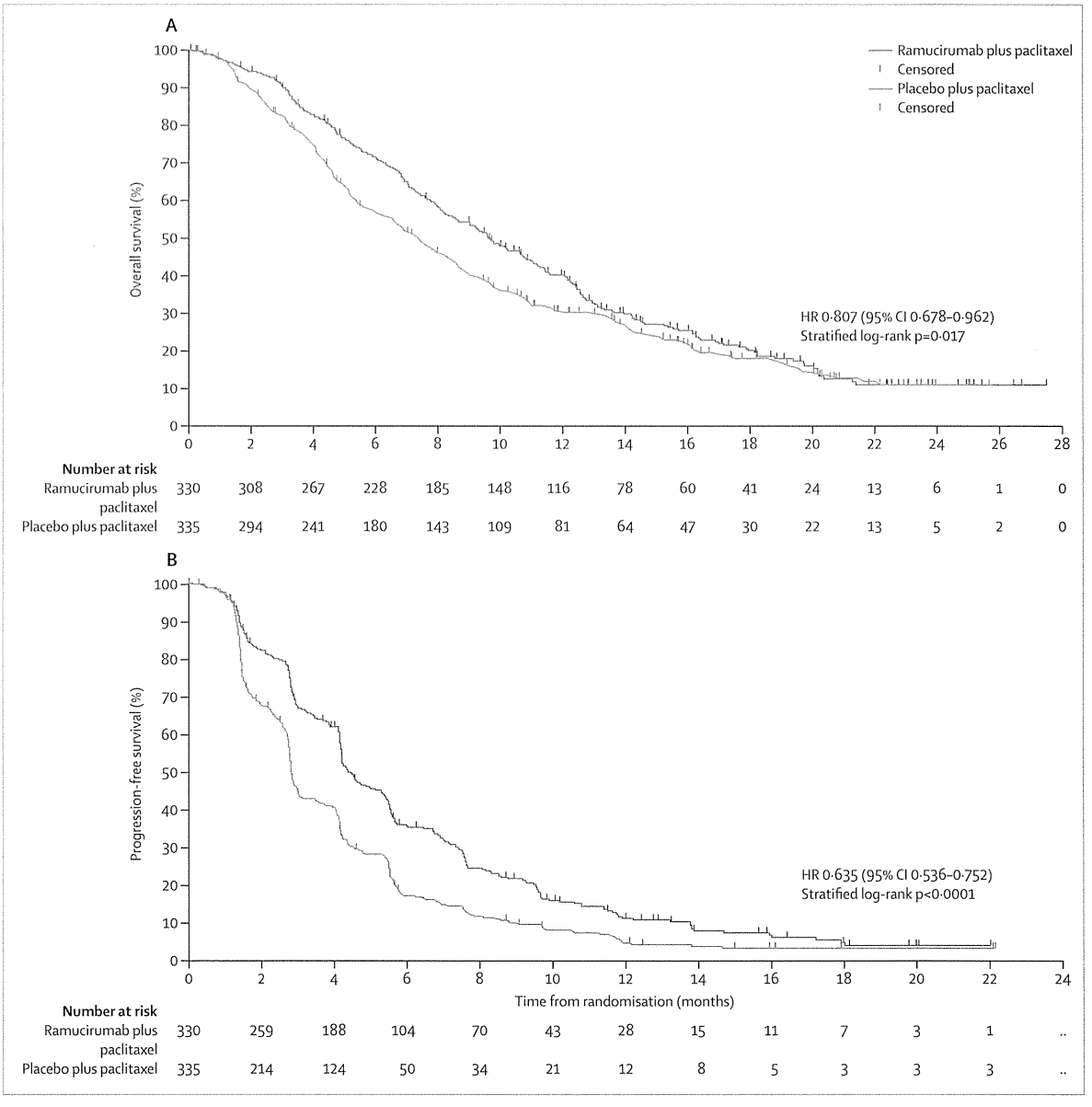

Figure 2: **Kaplan-Meier curves of overall survival (A) and progression-free survival (B)**
HR=hazard ratio.

We did a multivariate analysis using the stepwise Cox model with inclusion of all prespecified factors, and identified seven significant independent predictors for improved survival: region 3, ECOG performance status 0, weight loss of less 10%, up to two metastatic sites, absence of ascites, well or moderately differentiated tumour, and previous gastrectomy. After adjustment for these factors, the HR for overall survival with ramucirumab plus paclitaxel compared with placebo plus paclitaxel was 0·745 (95% CI 0·626–0·888 (p=0·0010; appendix). ECOG performance status, region, and presence of ascites were the strongest predictors for survival (appendix). Slight imbalances in ECOG performance status and ascites between the treatment groups (table 1 and appendix) might have contributed to the difference between the adjusted and unadjusted estimates.

Median progression-free survival with ramucirumab plus paclitaxel was significantly longer than with placebo plus paclitaxel (4·4 months [95% CI 4·2–5·3] *vs* 2·9 months [2·8–3·0]; stratified HR 0·635, [95% CI 0·536–0·752]; p<0·0001; figure 2B). 6-month progression-free survival was 36% (95% CI 31–41) in the ramucirumab plus paclitaxel group and 17% (13–22) in the placebo plus paclitaxel group; 9-month progression-free survival was 22% (95% CI 17–27) and 10% (7–14; figure 2B). Progression-free survival for the ramucirumab plus paclitaxel was

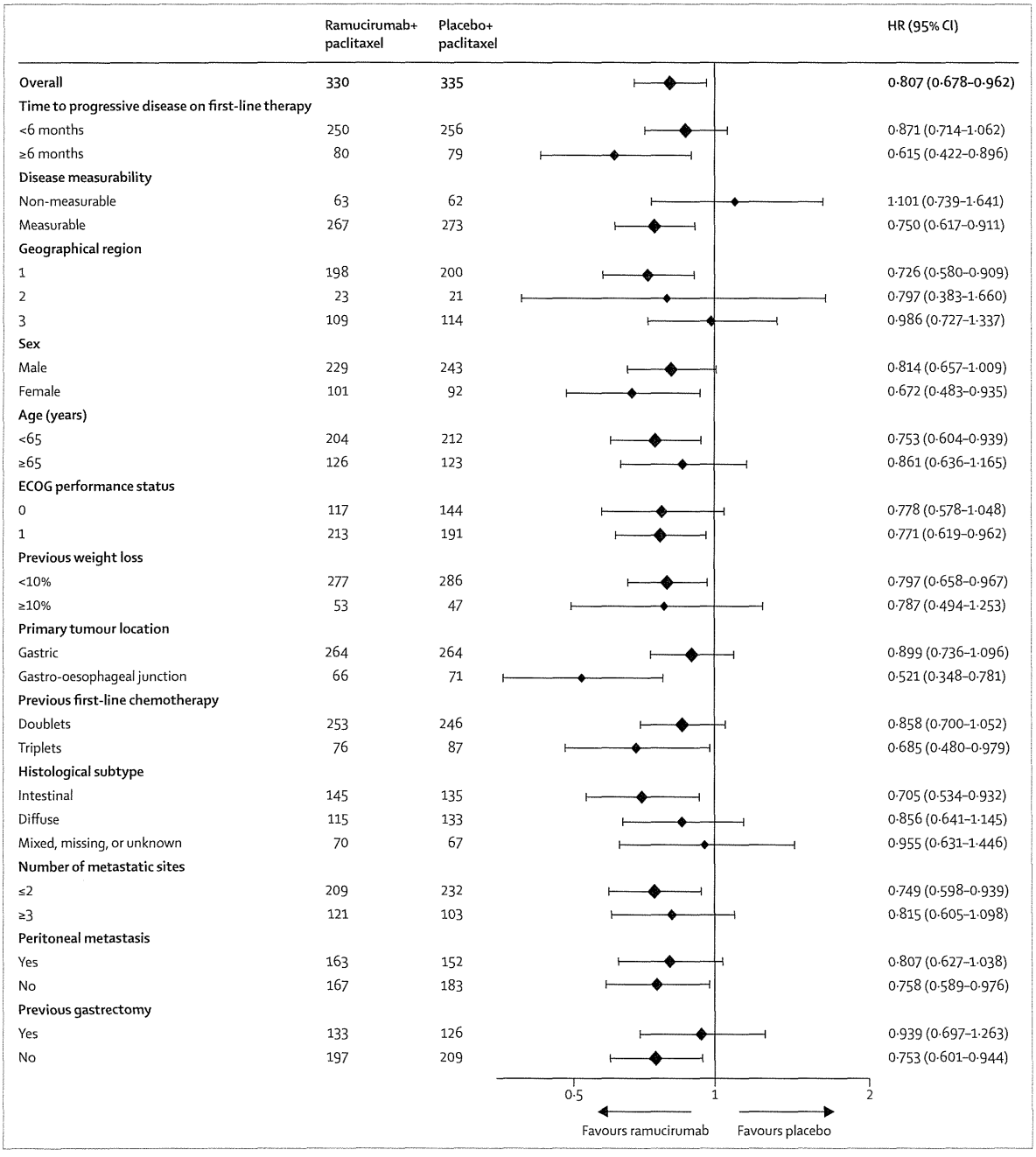

Figure 3: **Forest plot for subgroup univariate analyses of overall survival**
Data are stratified HR (95% CI). The size of the diamonds is proportional to the size of the subgroup. Geographic regions are defined as region 1: Europe, Israel, Australia, and the USA; region 2: Argentina, Brazil, Chile, and Mexico; and region 3: Japan, South Korea, Hong Kong, Singapore, and Taiwan. ECOG=Eastern Cooperative Oncology Group. HR=hazard ratio.

longer than for the placebo plus paclitaxel group in most subgroups (figure 4). Progression-free survival was significantly increased in the ramucirumab plus paclitaxel group compared with the placebo plus paclitaxel group after adjustment for significant baseline factors (adjusted HR 0·599; [95% CI 0·506–0·708]; p<0·0001; appendix).

A significantly greater proportion of patients achieved an objective response in the ramucirumab plus paclitaxel group than in the placebo plus paclitaxel group (92 [28%, 95% CI 23–33] of 330 *vs* 54 [16%, 13–20] of 335, respectively; p=0·0001, table 2). A significantly greater proportion of patients also achieved disease control in the ramucirumab plus paclitaxel group than in the placebo plus paclitaxel group (264 [80%, 95% CI 75–84] *vs* 213 [64%, 58–69], respectively; p<0·0001). The median duration of response was longer in the ramucirumab

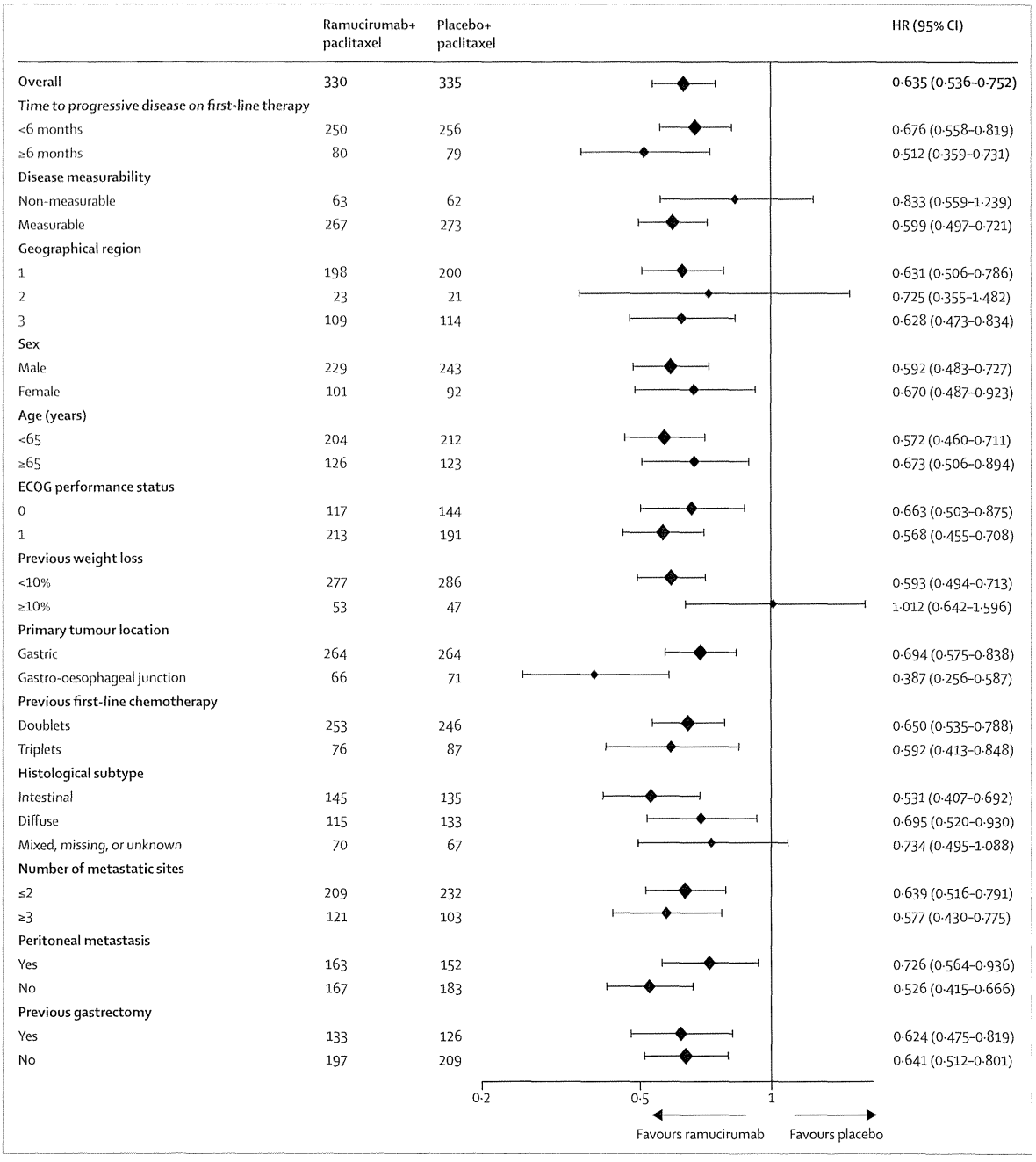

Figure 4: **Forest plots for subgroup univariate analyses of progression-free survival**
Data are stratified HR (95% CI). The size of the diamonds is proportional to the size of the subgroup. Geographic regions are defined as region 1: Europe, Israel, Australia, and the USA; region 2: Argentina, Brazil, Chile, and Mexico; and region 3: Japan, South Korea, Hong Kong, Singapore, and Taiwan. ECOG=Eastern Cooperative Oncology Group. HR=hazard ratio.

plus paclitaxel group than in the placebo plus paclitaxel group (4·4 months [IQR 2·8–7·5], *vs* 2·8 months [1·4–4·4], respectively).

Table 3 summarises overall survival, progression-free survival, and the proportion of patients achieving an objective response by geographic regions, comparing Asian with non-Asian patients. Overall survival in the ramucirumab plus paclitaxel group compared with placebo plus paclitaxel was not significantly increased for patients in region 3 compared with those in regions 1 and 2.

Baseline and end-of-treatment results for global quality of life from the QLQ-C30 and index scores from the EQ-5D-3L were similar in the treatment groups (table 4). Further details for quality of life will be published separately.

Median duration of treatment with ramucirumab was 18·0 weeks (IQR 10·0–31·1) in the ramucirumab plus paclitaxel group, and 12·0 weeks (6·4–20·0) with placebo in the placebo plus paclitaxel group. Median relative dose intensity of ramucirumab was similar to placebo (99% [IQR 94–101] *vs* 100% [97–101]) and was similar for paclitaxel in both groups (88% [IQR 72–97] *vs* 93% [85–99]). Dose reductions of ramucirumab occurred in 16 (5%) of 327 patients in the ramucirumab plus paclitaxel group, and of placebo in three (<1%) of 329 patients in the placebo plus paclitaxel group. Paclitaxel dose reductions occurred in 78 (24%) patients in the ramucirumab plus paclitaxel group, and in 24 (7%) patients in the placebo plus paclitaxel group. Median cumulative doses and number of infusions are provided in the appendix.

Disease progression was the most common reason for treatment discontinuation in both treatment groups (236 [72%] of 330 in the ramucirumab plus paclitaxel group *vs* 255 [76%] of 335 in the placebo plus paclitaxel group); 39 (12%) and 38 (11%) patients, respectively, discontinued treatment because of adverse events (appendix). One patient (who was randomly assigned to placebo but received ramucirumab) was unmasked to the investigator before surgery because of the occurrence of serious adverse events (sepsis and intestinal occlusion). After the discontinuation of study drug, the number of patients receiving systemic anti-neoplastic treatment was similar in both groups (appendix). Of note, a higher percentage of patients in region 3 received treatment after discontinuation of the study drug than in regions 1 or 2 (appendix).

Nine randomly assigned patients did not receive study medication and were excluded from the safety analyses (figure 1). Hence, 656 patients were included in the safety analyses. The patient who was randomly assigned to the placebo plus paclitaxel group but erroneously received ramucirumab instead of placebo discontinued treatment after one infusion. This patient is included in the intention-to-treat population (as randomly assigned) in the placebo plus paclitaxel group and is included in the safety population (as treated) in the ramucirumab plus paclitaxel group. Consequently, the safety population consisted of 327 patients in the ramucirumab plus paclitaxel group and 329 patients in the placebo plus paclitaxel group.

The incidence of grade 3 or 4 adverse events was higher in the ramucirumab plus paclitaxel group, including grade 3 or 4 neutropenia, leucopenia, and grade 3 hypertension, abdominal pain, and fatigue (table 5). All grade 3–5 adverse events are listed in the appendix. Although the incidence of grade 3 or 4 neutropenia was higher in the ramucirumab plus paclitaxel group, the incidence of grade 3 or greater febrile neutropenia was similar in both groups (ten [3%] *vs* eight [2%]). Neuropathy, of all grades, was more common in the ramucirumab plus paclitaxel group than in the placebo plus paclitaxel group (table 5), and was associated with a higher cumulative paclitaxel dose (appendix).

	Ramucirumab plus paclitaxel (N=330)	Placebo plus paclitaxel (N=335)
Best overall response		
Complete response	2 (<1%)	1 (<1%)
Partial response	90 (27%)	53 (16%)
Stable disease	172 (52%)	159 (47%)
Progressive disease	43 (13%)	83 (25%)
Not evaluable or not assessed	23 (7%)	39 (12%)

Data are number (%) or number (%; 95% CI), unless otherwise indicated.

Table 2: Best overall response

	Ramucirumab plus paclitaxel	Placebo plus paclitaxel	Hazard ratio (95% CI)	Odds ratio (95% CI)
Median overall survival				
Regions 1 (n=398) and 2 (n=44)	8·5 months (7·4–9·8)	5·9 months (5·2–7·1)	0·732 (0·591–0·907)	
Region 3 (n=223)	12·1 months (10·0–13·3)	10·5 months (7·8–14·1)	0·986 (0·727–1·337)	
Median progression-free survival				
Region 1 (n=398) and 2 (n=44)	4·2 months (3·9–4·9)	2·9 months (2·6–3·5)	0·639 (0·518–0·788)	
Region 3 (n=223)	5·5 months (4·2–5·7)	2·8 months (2·8–4·1)	0·628 (0·473–0·834)	
Proportion of patients achieving an objective response				
Regions 1 (n=398) and 2 (n=44)	55 (25%)	31 (14%)		2·087 (1·278–3·409)
Region 3 (n=223)	37 (34%)	23 (20%)		2·235 (1·177–4·244)

Data are median (95% CI) or number (%), unless otherwise indicated. Region 1=Europe, Israel, Australia, USA. Region 2=Argentina, Brazil, Chile, and Mexico. A pooled analysis is presented for regions 1 and 2 because of the similarity of the patient populations in these two regions and the small sample size in region 2. Region 3=Japan, South Korea, Hong Kong, Singapore, and Taiwan.

Table 3: Efficacy by geographic region

	Ramucirumab plus paclitaxel (n=330)	Placebo plus paclitaxel (n=335)
EORTC QLQ-C30		
Patients completing baseline QLQ-C30	322 (98%)	328 (98%)
Baseline global quality-of-life score*	61·5 (22·0)	58·0 (22·0)
Patients completing baseline plus post-baseline QLQ-C30	287 (87%)	273 (81%)
Patients completing end-of-treatment QLQ-C30	211 (64%)	204 (61%)
End-of-treatment global quality-of-life score*	49·0 (23·0)	48·3 (23·9)
EQ-5D		
Patients completing baseline EQ-5D	323 (98%)	328 (98%)
Baseline index score†	0·75 (0·22)	0·75 (0·24)
Patients completing baseline plus post-baseline EQ-5D	287 (87%)	274 (82%)
Patients completing end-of-treatment EQ-5D	211 (64%)	206 (61%)
End-of-treatment index score†	0·61 (0·32)	0·60 (0·35)

Data are number (%) or mean (SD). EORTC QLQ-C30=European Organisation for Research and Treatment of Cancer quality-of-life questionnaire. EQ-5D=EuroQol five-dimension health questionnaire. *Based on a 100-point scale, with a higher score representing better quality of life. †Based on a -0·59 to 1 scale, with 1 representing perfect health.

Table 4: Quality of life QLQ-C30 and EQ-5D mean scores at baseline and end of treatment

	Ramucirumab plus paclitaxel (n=327)				Placebo plus paclitaxel (n=329)			
	Grades 1–2	Grade 3	Grade 4	Grade 5	Grades 1–2	Grade 3	Grade 4	Grade 5
Any patients with a treatment-emergent adverse event	57 (17%)	155 (47%)	73 (22%)	39 (12%)	116 (35%)	128 (39%)	27 (8%)	51 (16%)
Non-haematological adverse events								
Fatigue*	147 (45%)	39 (12%)	0	0	126 (38%)	18 (5%)	0	0
Neuropathy*	123 (38%)	27 (8%)	0	0	104 (32%)	15 (5%)	0	0
Decreased appetite	121 (37%)	10 (3%)	0	0	92 (28%)	13 (4%)	0	0
Abdominal pain*	98 (30%)	20 (6%)	0	0	87 (26%)	10 (3%)	1 (<1%)	0
Nausea	109 (33%)	5 (2%)	1 (<1%)	0	100 (30%)	8 (2%)	0	0
Alopecia	107 (33%)	0	0	0	126 (38%)	1 (<1%)	0	0
Diarrhoea	94 (29%)	12 (4%)	0	0	71 (22%)	4 (1%)	1 (<1%)	0
Epistaxis	100 (31%)	0	0	0	23 (7%)	0	0	0
Vomiting	78 (24%)	9 (3%)	1 (<1%)	0	56 (17%)	12 (4%)	0	0
Peripheral oedema	77 (24%)	5 (2%)	0	0	43 (13%)	2 (<1%)	0	0
Hypertension	32 (10%)	46 (14%)	0	0	8 (2%)	8 (2%)	0	0
Constipation	70 (21%)	0	0	0	69 (21%)	2 (<1%)	0	0
Stomatitis	62 (19%)	2 (<1%)	0	0	22 (7%)	2 (<1%)	0	0
Pyrexia	56 (17%)	3 (<1%)	0	0	36 (11%)	1 (<1%)	0	0
Proteinuria	50 (15%)	4 (1%)	0	0	20 (6%)	0	0	0
Malignant neoplasm progression	5 (2%)	16 (5%)	4 (1%)	27 (8%)	1 (<1%)	24 (7%)	1 (<1%)	34 (10%)
Weight decreased	39 (12%)	6 (2%)	0	0	45 (14%)	4 (1%)	0	0
Dyspnoea	34 (10%)	8 (2%)	0	0	29 (9%)	2 (<1%)	0	0
Rash*	42 (13%)	0	0	0	31 (9%)	0	0	0
Cough	40 (12%)	0	0	0	25 (8%)	0	0	0
Back pain	35 (11%)	4 (1%)	0	0	35 (11%)	5 (2%)	0	0
Hypoalbuminaemia*	32 (10%)	4 (1%)	0	0	13 (4%)	2 (<1%)	0	1 (<1%)
Myalgia	34 (10%)	0	0	0	32 (10%)	1 (<1%)	0	0
Ascites	21 (6%)	11 (3%)	1 (<1%)	0	14 (4%)	13 (4%)	0	0
Headache	32 (10%)	0	0	0	21 (6%)	1 (<1%)	0	0
Haematological adverse events								
Neutropenia*	45 (14%)	71 (22%)	62 (19%)	0	40 (12%)	51 (16%)	11 (3%)	0
Anaemia*	84 (26%)	30 (9%)	0	0	85 (26%)	31 (9%)	3 (<1%)	0
Leucopenia*	54 (17%)	52 (16%)	5 (2%)	0	47 (14%)	19 (6%)	3 (<1%)	0
Thrombocytopenia*	38 (12%)	5 (2%)	0	0	14 (4%)	6 (2%)	0	0

Data are number (%), unless otherwise stated. *Consolidated adverse event category comprising synonymous MEdDRA preferred terms.

Table 5: Treatment-emergent adverse events occurring in at least 10% of patients on ramucirumab plus paclitaxel, irrespective of causality

Grade 3 adverse events that were potentially associated with the VEGF pathway—and thus were of special interest—that were more common in the ramucirumab plus paclitaxel group included hypertension, proteinuria, and bleeding or haemorrhage (table 6). The incidences of grade 4 and 5 adverse events of special interest were low in both groups, with no grade 4 or 5 hypertension, a similar incidence of gastrointestinal haemorrhage, and a higher incidence of gastrointestinal perforation in the ramucirumab plus paclitaxel group than the placebo plus paclitaxel group (table 6).

Similar numbers of patients had at least one serious adverse event (153 [47%] of 327 in the ramucirumab plus paclitaxel group *vs* 139 [42%] of 329 in the placebo plus paclitaxel group), or treatment-emergent adverse event leading to death (39 [12%] *vs* 51 [16%], respectively). Six (2%) patients in the ramucirumab plus paclitaxel group had adverse events leading to death with a causal relation to any study drug, which were septic shock; malabsorption; gastrointestinal haemorrhage; death of unknown origin; pulmonary embolism; and sepsis. Five (2%) patients in the placebo plus paclitaxel group had adverse events leading to death with a causal relation to any study drug, which were acute renal failure; cardiac failure; febrile neutropenia, septic shock, and pulmonary embolism; pulmonary embolism; and cerebral haemorrhage.

Serum samples for detection of anti-ramucirumab antibodies were available for 320 (98%) of 327 patients receiving ramucirumab plus paclitaxel and 323 (98%) of 329 patients receiving placebo plus paclitaxel. Five (2%) patients receiving ramucirumab plus paclitaxel and one

(<1%) patient receiving placebo plus paclitaxel had a positive response. No patients developed neutralising antibodies.

Discussion

Ramucirumab plus paclitaxel significantly increased overall survival compared with placebo plus paclitaxel in patients with advanced gastric or gastro-oesophageal junction adenocarcinoma that had progressed after first-line chemotherapy. Patients treated with ramucirumab plus paclitaxel also had significantly longer progression-free survival, and a higher proportion of patients achieving an overall response and disease control than did those treated with placebo plus paclitaxel. Increased proportions of patients achieving an overall response has been noted previously in trials of anti-angiogenic drugs in combination with chemotherapy, especially when there seems to be an improvement in survival.[25] Results of a preplanned subgroup analysis showed a difference in treatment effect for ramucirumab plus paclitaxel on survival between non-Asian (region 1 and 2) and Asian (region 3) regions. We could speculate that the higher use of post-study discontinuation treatment in Asia (almost 70%) than in the non-Asian regions (almost 40%) attenuated the survival benefit in this region.

Fatigue, diarrhoea, and abdominal pain were some of the most frequently reported non-haematological toxicities in both groups, and were more common in the ramucirumab plus paclitaxel group than the placebo plus paclitaxel group. These events are common in patients with gastric cancer; incidences reported in our trial are in the range of what was previously reported in large phase 3 gastric cancer trials.[9,26–28] Peripheral neuropathy, a typical side-effect of taxanes, was more common in the ramucirumab plus paclitaxel group and, as expected, was associated with a higher cumulative paclitaxel dose. Neutropenia was one of the most frequently reported haematological toxicities in both groups, and had a similar incidence to that reported in other trials of the same paclitaxel dose and schedule.[12,13,17,29] Although severe neutropenia was more frequently reported for ramucirumab plus paclitaxel, the incidence of febrile neutropenia was low and similar in the groups. As expected, hypertension, proteinuria, bleeding (mainly grade 1 or 2 epistaxis), and gastrointestinal perforations, adverse events associated with most anti-angiogenic treatments, were more common in the ramucirumab plus paclitaxel group. Grade 3 hypertension was controlled with antihypertensive medication; grade 4 or greater hypertension was not noted in this study. The incidences of grade 3 bleeding (mainly grade 3 gastrointestinal haemorrhage) and grade 3 proteinuria were higher in the ramucirumab plus paclitaxel group (table 6). However, nephrotic syndrome was not reported, and grade 4 or 5 bleeding events occurred with a similar incidence in both groups. Grade 3 or greater gastrointestinal perforation was reported only in the ramucirumab plus paclitaxel group (four patients, including one death). Importantly, the overall higher rate of grade 3 or 4 adverse events in the ramucirumab plus paclitaxel group did not result in a higher number of patients discontinuing, or a higher number of deaths, than in the placebo plus paclitaxel group. We also showed that quality of life was maintained during treatment with ramucirumab.

	Ramucirumab plus paclitaxel (n=327)				Placebo plus paclitaxel (n=329)			
	Grades 1–2	Grade 3	Grade 4	Grade 5	Grades 1–2	Grade 3	Grade 4	Grade 5
Bleeding or haemorrhage	123 (38%)	12 (4%)	1 (<1%)	1 (<1%)	51 (16%)	4 (1%)	2 (<1%)	2 (<1%)
Proteinuria	51 (16%)	4 (1%)	0	0	20 (6%)	0	0	0
Liver injury or failure	39 (12%)	12 (4%)	3 (<1%)	0	28 (9%)	11 (3%)	2 (<1%)	0
Hypertension	34 (10%)	48 (15%)	0	0	10 (3%)	9 (3%)	0	0
Gastrointestinal haemorrhage†	21 (6%)	10 (3%)	1 (<1%)	1 (<1%)	15 (5%)	3 (<1%)	1 (<1%)	1 (<1%)
Infusion-related reaction	17 (5%)	2 (<1%)	0	0	12 (4%)	0	0	0
Renal failure	16 (5%)	4 (1%)	2 (<1%)	0	11 (3%)	0	1 (<1%)	2 (<1%)
Congestive heart failure	6 (2%)	2 (<1%)	0	0	2 (<1%)	1 (<1%)	0	1 (<1%)
Venous thromboembolic events	5 (2%)	7 (2%)	0	1 (<1%)	7 (2%)	8 (2%)	1 (<1%)	2 (<1%)
Arterial thromboembolic events	3 (<1%)	1 (<1%)	2 (<1%)	0	2 (<1%)	2 (<1%)	0	1 (<1%)
Gastrointestinal perforation	0	1 (<1%)	2 (<1%)	1 (<1%)	1 (<1%)	0	0	0

*Pooled adverse-event terms. †Events pooled as gastrointestinal haemorrhage are also pooled as bleeding or haemorrhage.

Table 6: Adverse events of special interest*

To our knowledge, RAINBOW is the largest trial in second-line gastric cancer, and the first report of a survival benefit with a VEGFR-2 targeted antibody in combination with chemotherapy. REGARD, a randomised phase 3 trial that compared ramucirumab as a single agent with best supportive care, showed a significant median survival benefit of 1·4 months, favouring ramucirumab treatment (median survival 5·2 months [IQR 2·3–9·9]) versus best supportive care (3·8 months [IQR 1·7–7·1]).[30] Other recently published randomised second-line gastric cancer trials showed an increase in median survival of about 1·5 months with single-agent chemotherapy (docetaxel or irinotecan) relative to supportive care.[3–5] By contrast, a trial of everolimus versus placebo in the second-line setting did not significantly extend overall survival,[27] and trials of anti-EGFR therapy in the first-line setting adding panitumumab[31] or cetuximab[28] to chemotherapy have also not significantly extended overall survival or progression-free survival, respectively.

The increased overall survival for patients treated with ramucirumab plus paclitaxel compared with