9. Merelli B, Massi D, Cattaneo L et al. Targeting the PD1/PD-L1 axis in melanoma: biological rationale, clinical challenges and opportunities. Crit Rev Oncol Hematol 2014; 89: 140–165.
10. Taube JM, Anders RA, Young GD et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med 2012; 4: 127ra137.
11. Dong H, Strome SE, Salomao DR et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med 2002; 8: 793–800.
12. Therasse P, Arbuck SG, Eisenhauer EA et al. New guidelines to evaluate the response to treatment in solid tumors: European Organisation for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000; 92: 205–216.
13. Salama AK, Flaherty KT. BRAF in melanoma: current strategies and future directions. Clin Cancer Res 2013; 19: 4326–4334.
14. Wilmott JS, Long GV, Howle JR et al. Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. Clin Cancer Res 2012; 18: 1386–1394.
15. Hu-Lieskovan S, Robert L, Homet Moreno B, Ribas A. Combining targeted therapy with immunotherapy in BRAF-mutant melanoma: promise and challenges. J Clin Oncol 2014; 32: 2248–2254.
16. Parsa AT, Waldron JS, Panner A et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nat Med 2007; 13: 84–88.
17. Atefi M, Avramis E, Lassen A et al. Effects of MAPK and PI3K pathways on PD-L1 expression in melanoma. Clin Cancer Res 2014; 20: 3446–3457.
18. Madore J, Vilain RE, Menzies AM et al. PD-L1 expression in melanoma shows marked heterogeneity within and between patients: implications for anti-PD-1/PD-L1 clinical trials. Pigment Cell Melanoma Res 2015; 28: 245–253.
19. Long GV, Stroyakovskiy D, Gogas H et al. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. N Engl J Med 2014; 371: 1877–1888.
20. Robert C, Karaszewska B, Schachter J et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med 2015; 372: 30–39.
21. Larkin J, Ascierto PA, Dréno B et al. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. N Engl J Med 2014; 371: 1867–1876.
22. Topalian SL, Hodi FS, Brahmer JR et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012; 366: 2443–2454.
23. Tumeh PC, Harview CL, Yearley JH et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014; 515: 568–571.

Annals of Oncology 26: 1987–1993, 2015
doi:10.1093/annonc/mdv252
Published online 10 June 2015

Pazopanib in pretreated advanced neuroendocrine tumors: a phase II, open-label trial of the Spanish Task Force Group for Neuroendocrine Tumors (GETNE)†

E. Grande[1*], J. Capdevila[2], D. Castellano[3], A. Teulé[4], I. Durán[5], J. Fuster[6], I. Sevilla[7], P. Escudero[8], J. Sastre[9], J. García-Donas[10], O. Casanovas[11], J. Earl[12], L. Ortega[13], M. Apellaniz-Ruiz[14], C. Rodriguez-Antona[14], T. Alonso-Gordoa[1], J. J. Díez[15], A. Carrato[1] & R. García-Carbonero[5]

[1]Department of Medical Oncology, Ramón y Cajal University Hospital, Madrid; [2]Department of Medical Oncology, Vall d'Hebron University Hospital and Vall d'Hebron Institute of Oncology (VHIO), Universitat Autònoma de Barcelona, Barcelona; [3]Department of Medical Oncology, I + 12 Research Institute, 12 de Octubre University Hospital, Madrid; [4]Department of Medical Oncology, IDIBELL, Catalan Institute of Oncology L'Hospitalet, Barcelona; [5]Department of Medical Oncology, Instituto de Biomedicina de Sevilla (IBIS) [HUVR, CSIC, University of Seville], Virgen del Rocío University Hospital, Seville; [6]Department of Medical Oncology, Son Espases Hospital, Palma de Mallorca; [7]Department of Medical Oncology, Virgen de la Victoria University Hospital, Malaga; [8]Department of Medical Oncology, Clínico Lozano Blesa University Hospital, Zaragoza; [9]Department of Medical Oncology, Clínico San Carlos Hospital, Madrid; [10]Department of Medical Oncology, Centro Integral Oncológico Clara Campal, Madrid; [11]Tumor Angiogenesis Group, IDIBELL, Catalan Institute of Oncology L'Hospitalet, Barcelona; [12]Department of Medical Oncology Research Laboratory, Ramón y Cajal University Hospital, Madrid; [13]Department of Pathology, Clínico San Carlos Hospital, Madrid; [14]Hereditary Endocrine Cancer Group, Spanish National Cancer Research Center, ISCIII Center for Biomedical Research on Rare Disease (CIBERER) Madrid, Madrid; [15]Department of Endocrinology, Ramón y Cajal University Hospital, Madrid, Spain

Received 22 January 2015; revised 19 May 2015; accepted 26 May 2015

Background: The management of advanced neuroendocrine tumors (NETs) has recently changed. We assessed the activity of pazopanib after failure of other systemic treatments in advanced NETs.

Methods: This was a multicenter, open-label, phase II study evaluating pazopanib as a single agent in advanced NETs (PAZONET study). The clinical benefit rate (CBR) at 6 months was the primary end point. Translational correlation of

*Correspondence to: Dr Enrique Grande, Department of Medical Oncology, Ramón y Cajal University Hospital, Carretera de Colmenar Viejo km. 9100, Madrid 28034, Spain. Tel: +34-91-336-8263; E-mail: egrande@oncologiahrc.com

†2011 ASCO Annual meeting: Poster Session; 2012 ASCO Annual meeting: Poster Session; 2013 ASCO Annual meeting: Poster Session; 2014 ASCO Annual meeting: Abstract; ESMO 2012 Congress Vienna: Oral presentation; European Cancer Congress 2013: Poster Session; Sociedad Española de Oncología Médica (SEOM) 2013: Oral presentation.

radiological response and progression-free survival (PFS) with circulating and tissue biomarkers was also evaluated.

Results: A total of 44 patients were enrolled. Twenty-five patients (59.5%) were progression-free at 6 months (4 partial responses, 21 stable diseases) with a median PFS of 9.5 months [95% confidence interval (CI) 4.8–14.1]. The CBR varied according to prior therapy received, with 73%, 60% and 25% in patients treated with prior multitarget inhibitors, prior mTOR inhibitors and both agents, respectively. A nonsignificant increase in PFS was observed in patients presenting lower baseline circulating tumor cell (CTC) counts (9.1 versus 5.8 months; $P = 0.22$) and in those with decreased levels of soluble-vascular endothelial growth factor receptor-2 (sVEGFR-2) (12.6 versus 9.1 months; $P = 0.067$). A trend toward reduced survival was documented in patients with *VEGFR3* rs307821 and rs307826 missense polymorphisms [hazard ratio (HR): 12.3; 95% CI 1.09–139.2; $P = 0.042$ and HR: 6.9; 95% CI 0.96–49.9; $P = 0.055$, respectively].

Conclusions: Pazopanib showed clinical activity in patients with advanced NETs regardless of previous treatments. Additionally, CTCs, soluble-s VEFGR-2 and VEGFR3 gene polymorphisms constitute potential biomarkers for selecting patients for pazopanib (NCT01280201).

Clinical trial number: NCT01280201.

Key words: pazopanib, gastroenteropancreatic tumors, bronchial carcinoids, thymic tumors, angiogenic markers, polymorphisms

introduction

Neuroendocrine tumors (NETs) comprise a heterogeneous group of malignant diseases, including gastroenteropancreatic (GEP) neoplasms and bronchial or thymic tumors. In this context, the incorporation of novel targeted agents, such as everolimus (mTOR inhibitor) or sunitinib (tirosine kinase inhibitor, TKI), but not sorafenib and/or bevacizumab [1], has led to a significant increase in progression-free survival (PFS) in patients with pancreatic NETs and also shows promising activity in nonpancreatic tumors [2–5]. However, while treatment options increase, there are little data regarding the activity of novel targeted agents after progression to others, and the optimal sequencing strategy are both a subject of debate [6].

Pazopanib is a multitargeted agent against vascular endothelial growth factor receptors (VEGFR-1, -2 and -3), platelet-derived growth factor receptor α and β (PDGFRα and β) and proto-oncogene c-Kit [7, 8]. Pazopanib has already shown clinical activity in metastatic GEP NECs with an objective response rate (ORR) of 18.9%, a disease control rate of 75.7% and a median PFS of 9.1 months [9]. The PAZONET study evaluated the efficacy and safety of pazopanib in patients with advanced NETs who might have progressed with at least one prior systemic approach (including novel targeted agents). The following molecular analyses were also carried out to assess their potential role as predictive biomarkers of pazopanib: (i) circulating tumor-related biomarkers [including circulating tumor cells (CTCs), circulating endothelial cells (CECs) and angiogenic factors]; (ii) immunohistochemical markers in tumor tissue; (iii) cytochrome P450 3A5 (CYP3A5) and *VEGFR3* single-nucleotide polymorphisms (SNPs) in blood samples.

methods

patients

Eligible patients were aged ≥18 years, had confirmed pathological diagnosis of moderately to well-differentiated (grade 1 and 2, according to the European Neuroendocrine Tumor Society) metastatic or locally advanced pancreatic islet cell tumors, gastrointestinal, bronchial or thymic NETs and were not candidates for surgery. Patients with both sporadic and/or inherited NETs were allowed, and those who might have documented disease progression with at least one prior systemic approach (including the use of novel targeted agents), according to the Response Evaluation Criteria in Solid Tumors (RECIST, version 1.0) [10] within the previous 12 months. Additional inclusion criteria were at least one measurable target lesion, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and adequate hematological, hepatic and renal function. Previous therapy with somatostatin analogs, interferon, chemotherapy agents, monoclonal antibodies against VEGF, TKIs and mTOR inhibitors were allowed.

The study was approved by an independent ethics committee according to local laws and complied with the International Conference on Harmonization Good Clinical Practice guidelines and the Declaration of Helsinki. All patients granted informed consent in writing before study entry.

study design and treatment

PAZONET was a multicenter, open-label, phase II trial evaluating the efficacy and toxicity of pazopanib in advanced NETs. The patients received oral pazopanib 800 mg/day during a 28-day treatment cycle. They were allowed to receive concomitant treatment with somatostatin analogs at the investigator's discretion. Pazopanib dose reductions were allowed as follows: level 1: 600 mg and level 2: 400 mg. Study therapy was discontinued when clinical or radiological evidence of progressive disease was documented, when a participant experienced unacceptable adverse events (AEs), withdrew consent or per investigator's decision. This trial was registered at www.clinicaltrialsregister.eu with EudraCT number 2010-020749-28 and at ClinicalTrials.gov as NCT01280201.

assessments

Efficacy assessments were carried out at scheduled visits every 8 weeks, and safety was evaluated every 2 weeks up to week 24 and monthly thereafter. The primary end point of the study was the 6-month clinical benefit rate (CBR), which was defined as the percentage of patients achieving complete response, partial response (PR) or stable disease (SD) at month 6 after pazopanib was started. Tumor responses were assessed according to RECIST criteria v1.0.

Secondary efficacy end points included PFS, overall survival (OS), ORR and duration of response. All study end points were assessed according to the previous systemic therapy received, including four subgroups: mTOR inhibitors, TKIs, mTOR inhibitors plus TKI agents and no prior biological treatment. Additionally, all patients who received at least one dose of the study drug and had at least one follow-up assessment were evaluable for safety. AEs were

assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.0.

translational approach to biomarkers of response

The following translational analyses were carried out at baseline: CTC, CEC and soluble angiogenic markers; immunohistochemical assessment in tumor tissue and polymorphisms in selected genes.

The Veridex Cell Search System (Janssen Diagnostics LLC) was used to analyze CTC and CEC. These determinations were carried out centrally at the Ramón y Cajal University Hospital in Madrid. Serum VEGF-A and VEGFR-2 levels were determined by enzyme-linked immunosorbent assay in blood samples that were retrieved at baseline and after 12 weeks of treatment, and centrally analyzed at the Catalan Institute of Oncology in Barcelona.

The SNP analysis was carried out using genomic DNA isolated from blood samples. *VEGFR3* rs307821 (R1324L), *VEGFR3* rs307826 (T494A) and *CYP3A5* rs776747 (splicing defect) were genotyped using KBiosciences Competitive Allele Specific PCR (KASPar) SNP Genotyping Systems in collaboration with the Spanish National Cancer Research Centre (CNIO).

Immunohistochemical analyses included VEGF, VEGFR-1, VEGFR-2, CD31, Ki67, p53, p21ras, Cyclin D1, e-cadherin and hypoxia-inducible factor 1 (HIF-1) and were carried out centrally at Hospital Clínico San Carlos in Madrid. Tissue sections of 5 μm of paraffin-embedded tissue array were placed on glass slides after being deparaffinized in xylol and rehydrated in graded alcohol. Immunostaining was carried out with a Dako Techmate Horizon immunostaining machine.

statistical analysis

Qualitative variables were registered as the number and percentage of subjects in each category. The Kolmogorov–Smirnov bilateral test with a confidence level of 95% was carried out to assess the normality of distribution of quantitative variables. The mean and standard deviation was provided for normal variables, and median and interquartile range for those not adjusted to normal distribution. Comparisons between means were carried out using Student's *t*-test for normally distributed variables and the Mann–Whitney bilateral test for nonparametric variables.

PFS was computed and plotted using the Kaplan–Meier method and differences among study subgroups were assessed by the log-rank test. The PFS was calculated from the first administration of pazopanib to the first evidence of disease progression, death from any cause or up to the date of the last follow-up visit. The multivariate analysis for the hazard ratio (HR) measurement was estimated using Cox proportional hazards regression to evaluate the association between biomarkers and response to pazopanib and PFS. Differences in frequencies or proportions were estimated with the Pearson χ^2 test. *P* values of ≤0.05 were considered statistically significant. The statistical analysis was carried out using the SPSS software (IBM SPSS Statistics for Windows, Version 19.0; IBM Corp., Armonk, NY).

results

Between January 2011 and March 2012, a total of 44 patients were enrolled at 9 Spanish sites, belonging to GETNE. Two patients were excluded from the efficacy analysis due to lack of computed tomographic scan for tumor response evaluation. Baseline patient characteristics are summarized in Table 1. Regarding previous treatment, 9 patients (21.4%) had never received targeted therapy (7 were under somatostatin analogs alone and 2 in combination with chemotherapy), 10 (23.8%) had previously been treated with mTOR inhibitors, 15 (35.7%) received TKIs and 8 (19.1%) had previously received both mTOR inhibitors and other TKIs. During the study, 29 participants (68.2%) received concomitant treatment with somatostatin analogs (Table 1). Twelve of them had functional tumor and 17 were nonfunctional, but all of them were progressing on this therapy alone or combined with chemotherapy. Those functioning tumors were 1 pancreatic islet cell tumor, 10 gastrointestinal and 1 unknown, whereas 11 of the nonfunctioning tumors were pancreatic islet cell tumors, 4 gastrointestinal, 1 bronchial and another unknown.

Table 1. Summary of demographic and baseline characteristics

Characteristics	All patients
Sex, *n* (%)	
Male	24 (54.5)
Female	20 (45.5)
Age (years)	
Mean	60.2
Range	38–81
ECOG, *n* (%)	
0	16 (36.4)
1	28 (63.6)
Tumor type, *n* (%)	
Pancreatic islet cell tumors	18 (40.9)
Gastrointestinal neuroendocrine tumors	15 (34.1)
Pulmonary carcinoid tumors	5 (11.4)
Thymic carcinoid tumors	3 (6.8)
Unknown primary origin tumors	3 (6.8)
Functionality, *n* (%)	
Functional	13 (29.5)
Nonfunctional	31 (70.5)
Histologic status of tumor, *n* (%)	
Well differentiated	30 (68.2)
Moderately differentiated	3 (6.8)
Poorly differentiated	2 (4.5)
Unknown	9 (20.5)
Ki67 index, *n* (%)	
≤2%	6 (13.6)
3%–10%	13 (29.5)
>10%	5 (11.4)
Unknown	20 (45.5)
Previous biologic treatment, *n* (%)	
Everolimus	11 (25)
Multitargeted agent	16 (36.4)
mTOR and multitargeted inhibitor	8 (18.2)
None	9 (20.5)
Previous chemotherapy	16 (38.1)
Previous somatostatin analogs	35
Concurrent somatostatin analogs	30 (68.2)

Baseline levels of Ki67 marker were only available in 24 study patients.
ECOG, Eastern Cooperative Oncology Group.

response

Twenty-five patients (59.5%) were progression-free at 6 months. CBR at 6 months was observed in 73.3% (11/15) of the patients previously treated only with TKIs, followed by 66.7% (6/9) of those without any previous therapy, 60.0% (6/10)

in the group that previously received mTOR inhibitors and in 25% (2/8) for those who received pazopanib after TKIs and mTOR inhibitors.

Overall, after a median follow-up of 17 months (range 10–23 months), a confirmed tumor response was observed in 4 patients [95% confidence interval (CI) 0.6–17.6] and 21 had SD (95% CI 32.9–62.5). The median duration of response was 11.3 months (95% CI 2.0–20.6), and the median OS in patients previously treated with targeted therapy was 24.1 months (95% CI 20.0–28.3). Additionally, the majority of patients experienced a reduction in target tumor size (Figure 1), with a decrease of >10% in the longest diameter of target lesions found in 32.5% ($P = 0.309$) of the patients irrespective of the prior treatment received.

progression-free survival

Thirty-six (85.7%) of the patients had an event (disease progression or death) during the study follow-up with a median PFS of 9.5 months (95% CI 4.8–14.1) (Figure 2A). According to prior systemic therapy, median PFS ranged from 12.4 months (95% CI 11.3–13.5) in patients previously treated with TKIs, to 9.5 months (95% CI 8.8–10.1) in those without any previous novel targeted agent treatment and 6.8 months (95% CI 0.0–15.3) in patients treated with prior mTOR inhibitors (Figure 2B). These differences were not statistically significant. In contrast, PFS was significantly shorter in patients who had previously received both TKIs and mTOR inhibitors (4.0 months; 95% CI 1.3–6.8; $P = 0.040$; Figure 2B). The results according to primary tumor origin showed a median PFS of 12.8 months (95% CI 11.0–14.6) for patients with pancreatic NETs, 10.0 months (95% CI 4.9–15.1) for gastrointestinal and 3.4 months (95% CI 0.0–7.0; $P = 0.005$) in lung and thymic NETs (Figure 2C). Median PFS for patients receiving pazopanib concomitantly with long-acting somatostatin analogs ($n = 29$) was significantly longer than in those treated with pazopanib alone ($n = 13$) (11.7 months; 95% CI 9.7–13.7 versus 4.2 months; 95% CI 3.3–5.1; $P = 0.043$; Figure 2D).

safety

Overall median length of exposure to pazopanib was 32 weeks (17–73), and 104 weeks (84–107) for the population still receiving the study treatment at cutoff time. The initially planned dose of 800 mg was reduced in nine patients (21.4%) during the study. Five patients discontinued pazopanib treatment due to toxicity (obstructive jaundice, cerebellar hematoma, hepatic toxicity, "acute coronary syndrome" and several AEs: ECOG grade 3 asthenia, grade 3 hyporexia, grade 2 mucositis and grade 2 nausea), one participant left the study due to investigator's criteria and one withdrew consent. A total of 17 patients (40.5%) died, 16 due to disease progression. One patient, after stopping the study medication with pazopanib and during the next treatment, was admitted for sepsis by Klebsiella associated with febrile neutropenia, and died after multiorgan failure; hence, the investigator did not associate the event with pazopanib.

Twenty-eight patients with grade 3 or 4 AEs were reported and 9 events were associated with pazopanib therapy as per the investigator's criteria. Overall, 2 grade 4 and 35 grade 3 AEs were registered (Table 2). The most commonly reported grade 3 or 4 AEs were: hepatotoxicity (8%), asthenia (7%), diarrhea (4%) and hypertension (4%).

translational approach to potential biomarkers of response

The predictive value of the different biomarkers included in the study was evaluated using multivariate analysis (supplementary Table S1, available at *Annals of Oncology* online). Patients with no baseline CTCs (count of 0) showed an improved response [odds ratio (OR): 6.2; 95% CI 0.45–86.5; $P = 0.17$] and longer median PFS (9.1 versus 5.8 months; OR: 0.40; 95% CI 0.09–1.73; $P = 0.22$; supplementary Figure S3, available at *Annals of Oncology* online) than the patients with detectable baseline CTC (presence of circulating CTC in blood), although the differences were not statistically significant. Similarly, patients with CEC counts < median showed a trend toward a longer PFS (HR: 0.58; 95% CI 0.17–1.98; $P = 0.22$). In contrast, higher baseline levels of VEGF-A were associated with nonsignificant greater odds of achieving a PR

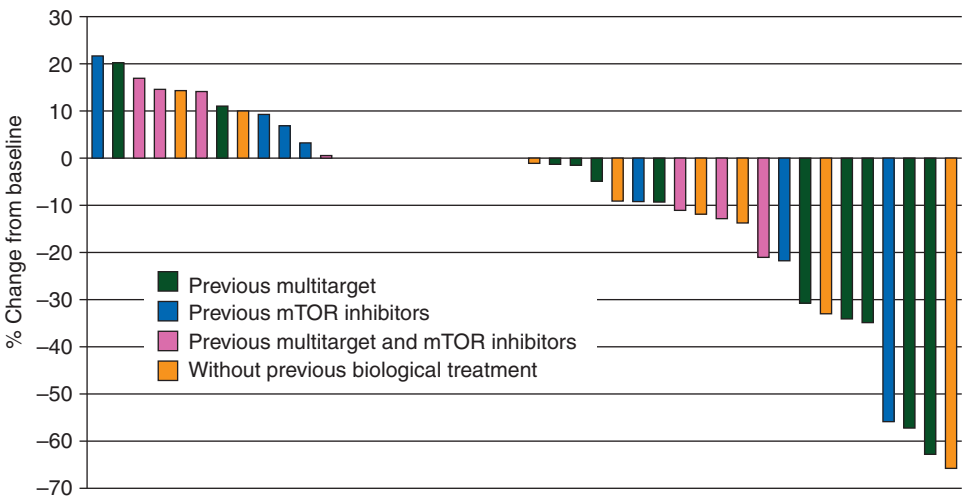

Figure 1. Maximum percentage change from baseline in target tumor measurement for each patient ($N = 42$).

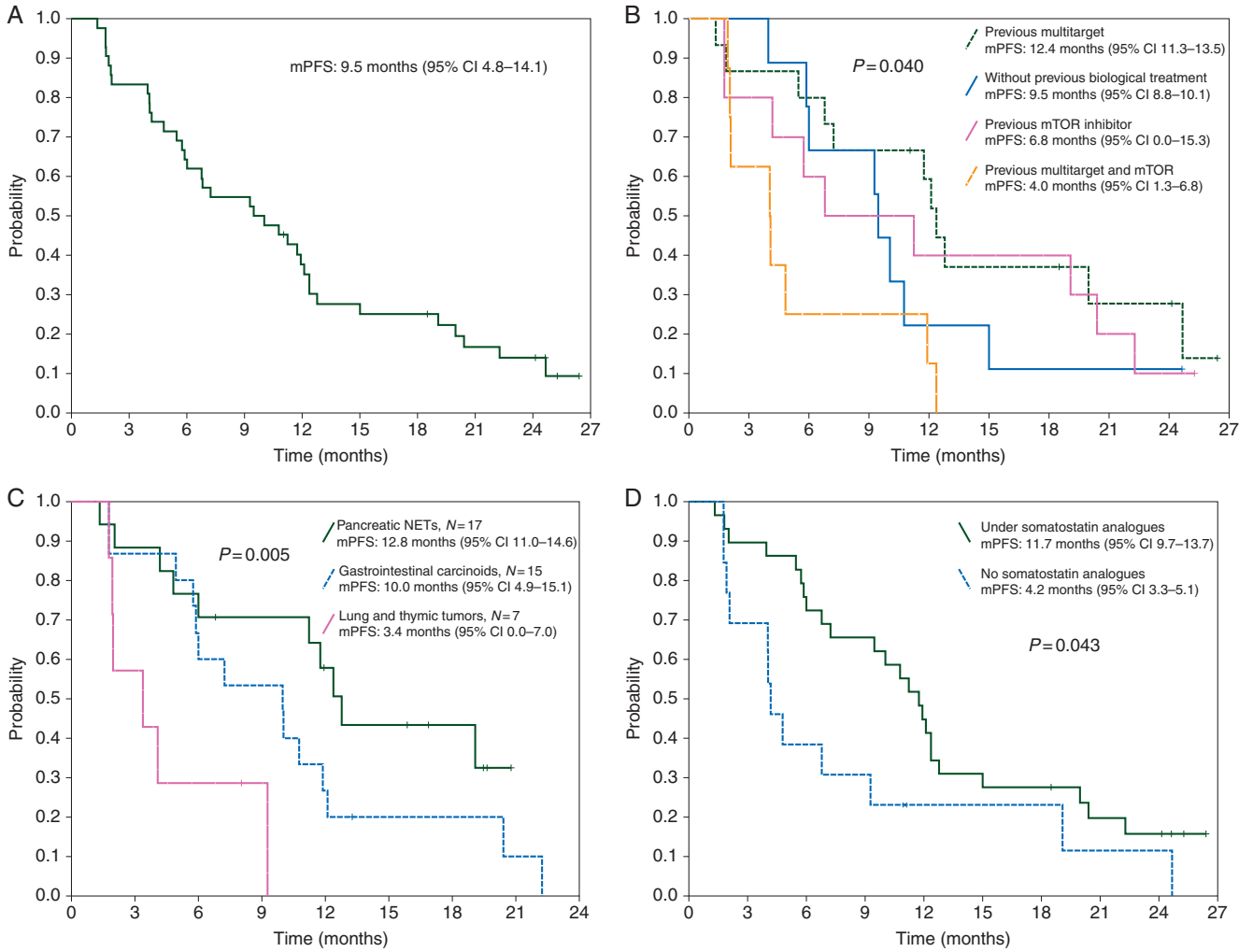

Figure 2. Kaplan–Meier analysis of progression-free survival in the overall population (A), according to previous chemotherapy (B), primary tumor origin at baseline (C) and concurrently use of somatostatin analogs or not (D). (A) Progression-free survival for all patients ($N = 42$). (B) Progression-free survival according to previous therapy ($N = 42$). (C) Progression-free survival according to primary tumor origin ($N = 39$). Other patients with primary tumor origin in the lung and thymus. (D) Progression-free survival according to concurrent treatment with somatostatin analogs ($N = 42$).

(OR: 6.8; 95% CI 0.98–47.2; $P = 0.053$), but shorter PFS (OR: 0.71; 95% CI 0.28–1.76; $P = 0.46$). Finally, patients with different VEGFR-2 levels had similar PR rate (OR: 1.45; 95% CI 0.16–12.8; $P = 0.74$), whereas PFS was slightly longer when VEGFR-2 levels were above the median (OR: 0.36; 95% CI 0.01–1.32; $P = 0.12$). Immunohistochemical results showed no statistically significant correlation with clinical outcomes for VEGFR-1, HIF-1 or VEGF. Patients with immunoexpression of VEGFR-2 over and below the median showed a PFS of 12.6 and 9.1 months, respectively ($P = 0.067$: supplementary Figure S4, available at *Annals of Oncology* online). This association was statistically significant among patients who had not received prior antiangiogenic therapy ($n = 13$), whose PFS was 20.1 versus 9.1 months, respectively ($P = 0.028$), but not in the subset of patients with previous antiangiogenic therapy ($n = 14$; $P = 0.93$).

Two *VEGFR3* missense polymorphisms, *VEGFR3* rs307821 (R1324L) and *VEGFR3* rs307826 (T494A), showed a trend toward reduced PFS in GEP NETs (HR: 12.3; 95% CI 1.09–139·2; $P = 0.042$ and HR: 6.9; 95% CI 0.96–49.9; $P = 0.055$; supplementary Figure S5, available at *Annals of Oncology* online); while no association was found between *CYP3A5* rs776746 and pazopanib toxicity or response.

discussion

The results from the proof-of-concept PAZONET study showed that pazopanib may have certain activity in previously treated, advanced NETs including patients who received mTOR inhibitors and other multitargeted agents. Around two thirds of the patients had a CBR at 6 months with a median PFS of 9.5 months. Additionally, 5 patients were under treatment with pazopanib at cutoff time after more than 26 months. These results, although preliminary and limited due to the small sample, are in line with the activity observed in the only study available with pazopanib in NET patients naïve of prior therapy with novel targeted agents or less heavily pretreated [9, 11]. Of note, pazopanib activity seemed to be similar in patients who had never received prior targeted agents and in those previously

Table 2. Summary of adverse events occurring in >10% of the patients

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4	Total	
					N	%
Asthenia	12	18	7	0	37	84.1
Diarrhea	12	14	4	0	30	68.2
Abdominal pain	17	5	1	0	23	52.3
Pain	12	7	2	0	21	47.7
Nausea	9	9	0	0	18	40.9
Hypertension	9	5	4	0	18	40.9
Hepatotoxicity	5	3	7	1	16	36.4
Vomiting	11	3	1	0	15	34.1
Hand–foot syndrome	11	2	0	0	13	29.5
Mucositis	10	3	0	0	13	29.5
Anorexia	7	4	2	0	13	29.5
Hair depigmentation	9	2	0	0	11	25.0
Hyperglycemia	5	2	2	1	10	22.7
Edema	7	2	0	0	9	20.5
Hyporexia	4	3	1	0	8	18.2
Headache	5	3	0	0	8	18.2
Rash	2	4	1	0	7	15.9
Dizziness	6	1	0	0	7	15.9
Fever	6	1	0	0	7	15.9
High LDH	6	1	0	0	7	15.9
Somnolence	4	2	0	0	6	13.6
Insomnia	5	0	0	0	5	11.4
Erythema	4	1	0	0	5	11.4
Anemia	1	1	3	0	5	11.4
High ALP	4	1	0	0	5	11.4
High GGT	4	1	0	0	5	11.4

Adverse events assessed according to the NCI CTCAE, version 4.0.
ALP, alkaline phosphatase; GGT, γ-glutamyl transpeptidase; LDH, lactate dehydrogenase.

treated with a TKI or a mTOR inhibitor. Indeed, the CBR and PFS were similar irrespective of the kind of targeted agent previously received, with the exception of the group of patients who had received both TKI and mTOR inhibitors sequentially, in whom clinical outcomes may be poorer. Despite some evidence of resistance to a third-line therapy with targeted agents, our results showed a trend to non-cross-resistance between different multitargeted therapies, and open the concept of sequencing as a strategy for the treatment of patients with metastatic or advanced NETs. Nevertheless, further investigations to understand mechanisms of escape and acquired resistance to the different available chemotherapeutics are needed [12]. Our data should be interpreted with caution as an exploratory analysis, because the study population was heterogeneous with different grades of NETs involved. Additionally, there was no central pathological and radiological evaluation. Further studies including more restricted criteria are needed.

Another interesting, but preliminary finding of our study is that soluble biomarkers may predict response of individual patients to pazopanib. Here, we carried out a comprehensive translational exploratory analysis including CTC and proangiogenic factors or SNPs. There was a trend toward an association between the presence of CTC at baseline and worse clinical outcome. However, this was not statistically significant, probably due to the limited sample size, which hampered the analysis, but the magnitude of differences in PR and the benefit found in PFS reinforces this result. At the time of publication, only Khan et al. had evaluated CTC in NET population (although systemic anticancer therapy was not allowed) and concluded that the presence of CTC was able to define a subgroup of patients with poor prognosis [13]. The PAZONET trial is the first study to correlate the activity of a novel targeted agent to the CTC count in the field of NETs, suggesting the presence of a subgroup of patients that could obtain greater benefit with pazopanib therapy. Unfortunately, we cannot address whether CTCs are a prognostic or predictive biomarker in the absence of an adequate control population with no pazopanib treatment.

Our results suggest that specific polymorphisms in *VEGFR3* may define a subset of patients with decreased pazopanib response, especially in gastrointestinal NETs. These results are consistent with prior observations in advanced renal cell carcinoma patients treated with sunitinib [14], and VEGFR3 single SNPs rs307821 (R1324L) and *VEGFR3* rs307826 (T494A) could confer resistance to tyrosine kinase inhibitors, regardless of tumor type.

The activity of pazopanib also seems to be greater in patients with pancreatic NETs than in those with gastrointestinal or other primary NET locations. The addition of a long-acting somatostatin analog seems to be synergistic and significantly increased PFS. However, insufficient sample size, together with the heterogeneous origin of tumors and potential bias in the patients with better overall status, who could be more likely to receive combination therapy, needs to be considered. This finding needs to be validated in a prospective randomized trial with larger samples. However, no correlation between functional tumor status, Ki67 and ≥2 prior lines of treatment was observed (supplementary Figures S1 and S2, available at *Annals of Oncology* online).

Limitations to the study include small sample size, which decreased the statistical power of the analysis, heterogeneous location and degree of differentiation of primary tumors, and lack of an appropriate control group. Additionally, response was evaluated by the investigator and not by an independent committee.

Overall, these results corroborate previous data regarding the safety and efficacy of pazopanib against metastatic/advanced NETs. Furthermore, pazopanib activity in patients in whom previous biological treatment failed should be studied further with a sequencing drug strategy. In this respect, a phase III study using pazopanib as sequential therapy in a specific population with progressive pancreatic NETs is planned.

acknowledgements

The authors acknowledge the entire GSK team and the trial's CRO (MFAR—Marketing Farmacéutico & Investigación Clínica) for their contribution to the success of the study. They also thank the investigators and the patients, together with their families, who made this work possible. Scientific editorial assistance was provided by Irantzu Izco-Basurko.

funding

The sponsor of the study was the Spanish Task Force Group for Neuroendocrine Tumors (GETNE), which was responsible for the study design, data collection, analysis and interpretation, as well as

drafting the report and the decision to submit the paper for publication. The PAZONET study was funded by GlaxoSmithKline, which did not participate in the aforementioned roles. The funding for our study has not any grant number.

disclosure

AC: Consulting or advisory role (Company: Bayer, Merck, MSD, Lilly, Roche). OC: An Immediate family member was employed by a for-profit health care company (Esteve, Salvat Biotech). Honoraria (Company: Teva, Pfizer). Consulting or advisory role (Company: Boehringer Ingelheim). Patents, Royalties, Other Intellectual Property (IDIBELL). Travel, Accommodations, Expenses. EG: Consulting or advisory role (Company: GSK). Research funding (company: GSK). JS: Consulting or advisory role (Company: Merck, Amgen, Bayer, Roche, Lilly, Celgene). Research funding (company: Merck). AT: Consulting or advisory role (Company: Ipsen, Novartis). Speakers' Bureau (Company: Ipsen, Novartis, Pfizer). RG-C: Honoraria (Company: Pfizer, Lilly, Roche, Boerhinger, Amgen, Merck, Novartis) Recipient: An Immediate Family Member. Consulting or Advisory Role (Company: Pfizer, Lilly, Roche, Boerhinger) Recipient: An Immediate Family Member. Research Funding (Company: IPSEN). Travel, Accommodation, Expenses (Company: IPSEN, Novartis, Roche, Merck, Amgen, Sanofi, Pfizer). (Company: Lilly, Roche, Boerhinger, Pfizer) Recipient: An Immediate Family Member. CR-A: Research Funding (Company: Pfizer, Novartis, Bayer). IS: Consulting or Advisory Role (Company: IPSEN, Pfizer, Novartis). Speakers' Bureau (Company: Novartis). Travel, Accommodation, Expenses (Company: Novartis). All remaining authors have declared no conflicts of interest.

references

1. Castellano D, Capdevila J, Sastre J et al. Sorafenib and bevacizumab combination targeted therapy in advanced neuroendocrine tumour: a phase II study of Spanish Neuroendocrine Tumour Group (GETNE0801). Eur J Cancer 2013; 49(18): 3780–3787.
2. Yao JC, Shah MH, Ito T et al. Everolimus for advanced pancreatic neuroendocrine tumors. N Engl J Med 2011; 364: 514–523.
3. Raymond E, Dahan L, Raoul JL et al. Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. N Engl J Med 2011; 364: 501–513.
4. Pavel ME, Hainsworth JD, Baudin E et al. Everolimus plus octreotide long-acting repeatable for the treatment of advanced neuroendocrine tumours associated with carcinoid syndrome (RADIANT-2): a randomised, placebo-controlled, phase 3 study. Lancet 2011; 378(9808): 2005–2012.
5. Castellano D, Grande E, Barriuso J. Advances in pancreatic neuroendocrine tumor treatment. N Engl J Med 2011; 364(19): 1872–1873.
6. Salazar R, Wiedenmann B, Rindi G et al. ENETS 2011 Consensus Guidelines for the Management of Patients with digestive Neuroendocrine Tumors: an update. Neuroendocrinology 2012; 95: 71–73.
7. Kumar R, Crouthamel MC, Rominger DH et al. Myelosuppression and kinase selectivity of multikinase angiogenesis inhibitors. Br J Cancer 2009; 101: 1717–1723.
8. Hamberg P, Verweij J, Sleijfer S. (Pre-)clinical pharmacology and activity of pazopanib, a novel multikinase angiogenesis inhibitor. Oncologist 2010; 15: 539–547.
9. Ahn HK, Choi JY, Kim KM et al. Phase II study of pazopanib monotherapy in metastatic gastroenteropancreatic neuroendocrine tumours. Br J Cancer 2013; 109(6): 1414–1419.
10. Therasse P, Arbuck SG, Eisenhauer EA et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000; 92: 205–216.
11. Phan A, Yao JC, Fogelman DR et al. A prospective, multi-institutional phase II study of GW786034 (pazopanib) and depot octreotide (sandostatin LAR) in advanced low-grade neuroendocrine carcinoma (LGNEC). J Clin Oncol 2010; 28 (15 suppl): abstr 4001.
12. Raymond E, García-Carbonero R, Wiedenmann B et al. Systemic therapeutic strategies for GEP-NETS: what can we expect in the future? Cancer Metastasis Rev 2014; 33(1): 367–372.
13. Khan MS, Kirkwood A, Tsigani T et al. Circulating tumor cells as prognostic markers in neuroendocrine tumors. J Clin Oncol 2013; 31(3): 365–372.
14. García-Donas J, Esteban E, Leandro-García LJ et al. Single nucleotide polymorphism associations with response and toxic effects in patients with advanced renal-cell carcinoma treated with first-line sunitinib: a multicenter, observational, prospective study. Lancet Oncol 2011; 12: 1143–1150.