Original Research

Exploratory analysis of biomarkers associated with clinical outcomes from the study of lenvatinib in differentiated cancer of the thyroid

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Abstract *Background:* Lenvatinib significantly prolonged progression-free survival (PFS) versus placebo in the phase III Study of (E7080) LEnvatinib in differentiated Cancer of the Thyroid (SELECT) of patients with radioiodine-refractory differentiated thyroid cancer. This exploratory analysis investigated potential predictive biomarkers of lenvatinib efficacy and target engagement.

Patients and methods: Circulating cytokine/angiogenic factors (CAFs) in blood samples collected at baseline and throughout treatment were analysed from patients randomised to receive lenvatinib or placebo from August 5, 2011 to October 4, 2012. For CAF biomarker analyses, patients were dichotomised by baseline levels. Tumour tissues were analysed for *BRAF* and *NRAS/KRAS/HRAS* mutations.

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Results: Tumours and CAFs were analysed from 183/392 (47%) and 387/392 (99%) patients, respectively. Lenvatinib PFS benefit was maintained in all assessments. For lenvatinib-treated patients, interaction-term analyses revealed that low baseline Ang2 level was predictive of tumour shrinkage ($P_{interaction} = 0.016$) and PFS ($P_{interaction} = 0.018$). Vascular endothelial growth factor and fibroblast growth factor 23 (FGF23) were significantly upregulated with lenvatinib, and FGF23 upregulation on cycle 1/day 15 was associated with longer PFS. In mutation analyses, no significant differences in clinical outcomes were observed. $BRAF^{WT}$ may be a negative prognostic factor for PFS in placebo-treated patients with papillary thyroid cancer ($P = 0.019$).

Conclusion: The lenvatinib PFS benefit was maintained regardless of baseline CAF or *BRAF/RAS* status. Baseline Ang2 was predictive of PFS in a subgroup of lenvatinib-treated patients, indicating that Ang2 may be predictive of lenvatinib sensitivity. $BRAF^{WT}$ may be a poor prognostic factor in patients with radioiodine-refractory papillary thyroid cancer. Improved PFS associated with upregulated FGF23 suggests that lenvatinib-induced FGF receptor inhibition contributes to lenvatinib efficacy.

Trial registration ID of the main study, SELECT: ClinicalTrials.gov: NCT01321554.

1. Introduction

Tumour angiogenesis is essential to cancer cell survival, local tumour growth, and development of distant metastases [1]. Increased vascular endothelial growth factor (VEGF) expression is significantly associated with angiogenesis and advanced-stage thyroid cancer [2]; therefore, the use of VEGF receptor (VEGFR) signalling pathway inhibitors represented a rational and attractive approach to control malignant thyroid cancer [3]. However, other molecular drivers of tumour growth beyond VEGF-driven angiogenesis contribute to the pathogenesis of cancer, including fibroblast growth factor receptor (FGFR) signalling [4]. Such pathways may provide escape mechanisms to VEGF-targeted therapies and lead to the development of resistance; this phenomenon has driven the development of multitargeted kinase inhibitors, such as sorafenib and lenvatinib, which are approved for the treatment of radioiodine-refractory differentiated thyroid cancer (RR-DTC).

Lenvatinib is an oral multikinase inhibitor of VEGFR 1–3, FGFR 1–4, platelet-derived growth factor receptor–alpha, and RET and KIT proto-oncogenes [5–7]. Lenvatinib was approved for the treatment of locally recurrent or metastatic, progressive RR-DTC in the United States, Europe and Japan based on results from the phase III Study of (E7080) LEnvatinib in differentiated Cancer of the Thyroid (SELECT) trial [8]. SELECT was a global, randomised, double-blind, multicenter, phase III study that demonstrated significant improvements in progression-free survival (PFS) and objective response rate (ORR) among patients with RR-DTC treated with lenvatinib compared with placebo [8]. Median PFS was 18.3 months in lenvatinib-treated patients versus 3.6 months in placebo-treated patients (hazard ratio [HR]: 0.21; 99% confidence interval [CI]: 0.14–0.31; $P < 0.001$). ORR was 64.8% in lenvatinib-treated patients versus 1.5% in placebo-treated patients ($P < 0.001$).

To date, there are no established biomarkers that are prognostic (for disease progression) or predictive (for response to therapy) of benefit in RR-DTC or its treatments. Several candidates of interest have been proposed based on the mechanisms of action of lenvatinib and the biology of DTC. Activation of the *RAS/RAF* pathway has been reported to increase VEGF production in thyroid cancer; however, it is still unclear if *RAS/RAF* activation is associated with tumour cell sensitivity to anti-VEGF therapy in thyroid cancer or other cancers [9].

Another molecular driver of tumour growth in the pathogenesis of thyroid cancer is FGF/FGFR. Elevated expression of FGF2, FGFR1, FGFR3 and FGFR4 have been detected in human thyroid carcinoma compared with normal thyroid tissue [10,11]. The FGF/FGFR pathway is part of an escape mechanism to VEGF-targeted antiangiogenic therapies [4]. One member of the FGF ligand family is FGF23, which is secreted by osteocytes, and has a key role in phosphorus homoeostasis and vitamin D metabolism [12]. Elevation of FGF23 levels has been shown to be a surrogate marker of FGFR1 inhibition [13].

Angiopoietin-2 (Ang2), a relatively novel regulator of angiogenesis that acts through the TEK tyrosine kinase, endothelial (Tie2) receptor, has been identified as a potential prognostic biomarker for some types of cancer, including hepatocellular carcinoma and melanoma, gastric, breast, bladder and prostate cancers [14]. The potential role of Ang2 as a predictive biomarker has not yet been comprehensively tested in multitargeted tyrosine kinase inhibitors of VEGFR-2 in RR-

DTC, although studies in VEGF-targeted therapies have been conducted for other cancers. In one such study, Ang2 was reported to be a predictive biomarker for bevacizumab in patients with pancreatic cancer [15]. Exploratory analyses from previous phase II trials of lenvatinib in medullary and differentiated thyroid cancers had identified associations between baseline Ang2 levels and patient outcomes, including ORR, PFS and overall survival [16,17].

Therefore, *BRAF, RAS,* VEGFR-2, FGF23, Ang2 and Tie2 were chosen for further investigation based on these mechanistic rationales as well as prior studies suggestive of possible predictive or prognostic roles for these markers in phase II trials of lenvatinib in thyroid cancer [16,17]. This exploratory analysis investigated potential baseline prognostic and predictive biomarkers of lenvatinib efficacy and target engagement from the phase III SELECT trial in patients with RR-DTC to understand the efficacy of lenvatinib and the nature of RR-DTC.

2. Materials and methods

The SELECT protocol and this study were approved by the relevant Institutional Review Boards, in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki. All patients provided written informed consent.

2.1. Patients and study design

SELECT (NCT01321554) methodology has been published previously [8]. Briefly, patients had measurable, pathologically confirmed DTC, evidence of radioiodine-refractory disease and independently reviewed radiologic (IRR) evidence of progression within the previous 13 months. From August 5, 2011 to October 4, 2012, eligible patients were randomised 2:1 to receive oral lenvatinib (24 mg once daily) or placebo in continuous 28-day cycles. Patients were treated until IRR-verified disease progression according to Response Evaluation Criteria in Solid Tumours, version 1.1. The primary data cutoff date was November 15, 2013.

2.2. Procedures

Patients' blood samples were collected at baseline, cycle 1 day 15, day 1 of subsequent cycles, and at the end of study treatment. Serum levels of circulating cytokine/angiogenic factor (CAF) were measured using commercially available enzyme-linked immunosorbent assays (see Appendix A and Tables A. 1A and A. 1B).

Archival tumour-sample submission in SELECT was optional and tumour samples were obtained from 220/392 patients enrolled in SELECT. Of these patients, 190 had sufficient DNA for analysis and 183 passed the $500\times$ depth of coverage threshold to be included in the correlative analyses. All samples were tested as a batch analysis at the end of the study. Further details on methods used are available in Appendix A and Table A. 2.

2.3. Statistical analysis

CAF and thyroglobulin levels were examined for potential correlations with maximum tumour shrinkage (MTS; defined as maximum percentage change from baseline in sum of diameters of target lesions), ORR and PFS. For analyses of baseline levels of biomarker CAFs, patients were dichotomised into low (first quartile) or high group (all other quartiles), based on visual analysis of the Kaplan–Meier curves of baseline Ang2 quartiles, which showed greater PFS ratio in the first quartile of the lenvatinib treatment arm. Changes in biomarker CAF levels were plotted for both placebo and lenvatinib groups through cycle 9 day 1 of study treatment and at treatment end. In analyses of pharmacodynamics associations with PFS, patients were also dichotomised: low ($\leq$median ratio to baseline) versus high ($>$median ratio to baseline).

Survival end-points were assessed using Kaplan–Meier estimates, and the influence of gene mutations and circulating CAFs on PFS was analysed using Cox proportional hazards model and log-rank tests. Multivariate analysis of baseline patient characteristics (age group, sex, region, race, ethnicity, baseline thyroid-stimulating hormone levels, height, weight, Eastern Cooperative Oncology Group performance status, prior VEGF therapy, histology and histology subtype) was conducted to confirm findings and adjust for population bias.

3. Results

The overall SELECT population comprised 392 patients: 261 were assigned to lenvatinib and 131 to placebo. Baseline tumour and blood samples were analysed from 183/392 (47%) and 387/392 (99%) patients, respectively. Archival tumour samples consisted of primary tumours (126/183; 69%), lymph node metastases (26/183; 14%) and non-lymph node metastases (31/183; 17%). Patient demographics, baseline characteristics and the primary end-point of PFS were generally similar between the blood biomarker, tumour biomarker and overall study populations (Table 1).

In this exploratory analysis, 26/123 (21.1%) patients in the lenvatinib arm and 19/59 (32.2%) in the placebo arm had tumours with a *BRAF* mutation, whereas 34/122 (27.9%) patients in the lenvatinib arm and 7/60 (11.7%) patients in the placebo arm had tumours with a *RAS* mutation (Table A. 3). Mutations in *BRAF* and *RAS* were nearly exclusive; only 3 patients had both *BRAF* and *KRAS* mutations (Fig. A. 1). Importantly, the lenvatinib PFS benefit was observed for all

Table 1
Patient characteristics by analysis population.

Parameter	ITT population (n = 392)	Tumour biomarker population (n = 183)	Blood biomarker population (n = 387)
Age, years, mean (range)	61.9 (21–89)	61.3 (21–85)	61.9 (21–89)
Female, n (%)	192 (49)	80 (44)	189 (49)
ECOG performance status, n (%)			
0–1	377 (96)	178 (97)	372 (96)
2–3	15 (4)	5 (3)	15 (4)
Histology, n (%)			
Follicular, all	133 (34)	60 (33)	132 (34)
Hürthle cell	58 (15)	25 (14)	58 (15)
Papillary, all	259 (66)	123 (67)	255 (66)
Poorly differentiated	47 (12)	19 (10)	47 (12)
PFS HR (95% CI)	0.20 (0.15–0.27)	0.19 (0.12–0.28)	0.20 (0.15–0.26)
P-value	2.08×10^{-34}	9.06×10^{-35}	6.48×10^{-19}

CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; ITT, intent-to-treat; PFS, progression-free survival.

subgroups regardless of tumour *BRAF* or *RAS* mutation status (Fig. 1). In contrast with our previous study in patients from the phase II trial of lenvatinib in RR-DTC [18], *RAS* mutations in the present analysis were neither predictive of lenvatinib response nor prognostic of PFS (Fig. 2A). Although the *BRAF* mutation also was not predictive of lenvatinib response, it may be a prognostic factor for better PFS among patients with papillary thyroid cancer (PTC) who had developed progressive RR-PTC (Fig. 2B); the significant association between *BRAF* mutation and PFS was maintained in both univariate ($P = 0.031$) and multivariate ($P = 0.0083$) analyses in the placebo arm. These analyses were performed only for patients with PTC

because multiple studies, including the current study, have demonstrated that *BRAF* mutations are extremely rare in follicular thyroid cancer.

Baseline Ang2 levels correlated significantly with PFS ($P = 0.0067$; Table 2) in the placebo arm, and with MTS, ORR and PFS in the lenvatinib arm ($P < 0.0001$ for each; Table 2; Fig. A. 2A and Fig. A. 2B). Baseline VEGF levels correlated significantly with MTS, ORR and PFS in the placebo arm ($P = 0.044$, $P = 0.038$, and $P = 0.037$, respectively; Table 2), and with MTS ($P = 0.0082$) and with ORR ($P = 0.0009$; Table 2) in the lenvatinib arm. Low baseline Ang2 level was a predictive biomarker of MTS for lenvatinib-treated patients ($P_{interaction} = 0.016$; Fig. A. 3). Both univariate and multivariate analyses revealed a significant separation in the PFS Kaplan–Meier curves of lenvatinib-treated patients with high versus low levels of baseline Ang2, favouring patients with low baseline Ang2 levels; and low baseline Ang2 levels were a positive predictive factor for lenvatinib PFS ($P_{interaction} = 0.018$; Fig. 2C). Although ORR correlated significantly with baseline Ang2 and VEGF levels, neither were predictive of ORR to lenvatinib (Fig. A. 4A and Fig. A. 4B). The baseline level of VEGF or Tie2 was not shown to be prognostic or predictive of lenvatinib PFS (Fig. 2D and E). Univariate and multivariate analyses also suggested that high baseline thyroglobulin levels may be a prognostic factor for poorer PFS in RR-DTC ($P = 0.027$ and $P = 0.051$, respectively) because a clear separation in PFS curves was observed between placebo-treated patients with high versus low baseline thyroglobulin levels; this separation was absent among patients who received lenvatinib (Fig. 2F).

In our pharmacodynamic analysis of changes in serum biomarker levels with treatments, thyroglobulin
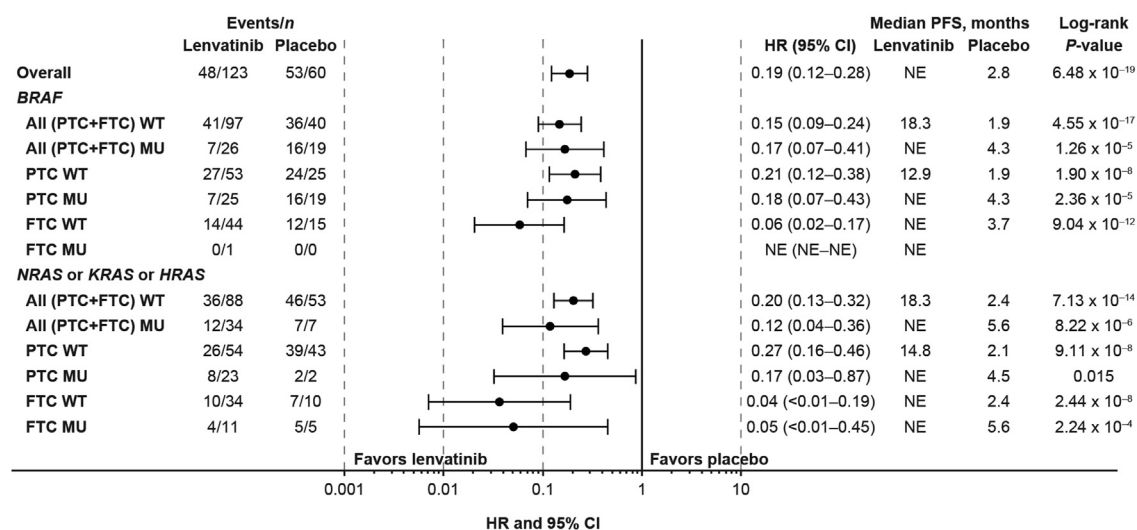

Fig. 1. Forest plot of progression-free survival stratified by tumour mutation status. HRs and 95% CIs of PFS in patients in the lenvatinib or placebo arm by *BRAF* or *RAS* tumour mutation status. CI, confidence interval; FTC, follicular thyroid cancer; HR, hazard ratio; MU, mutant; NE, not evaluable; PFS, progression-free survival; PTC, papillary thyroid cancer; WT, wild type.
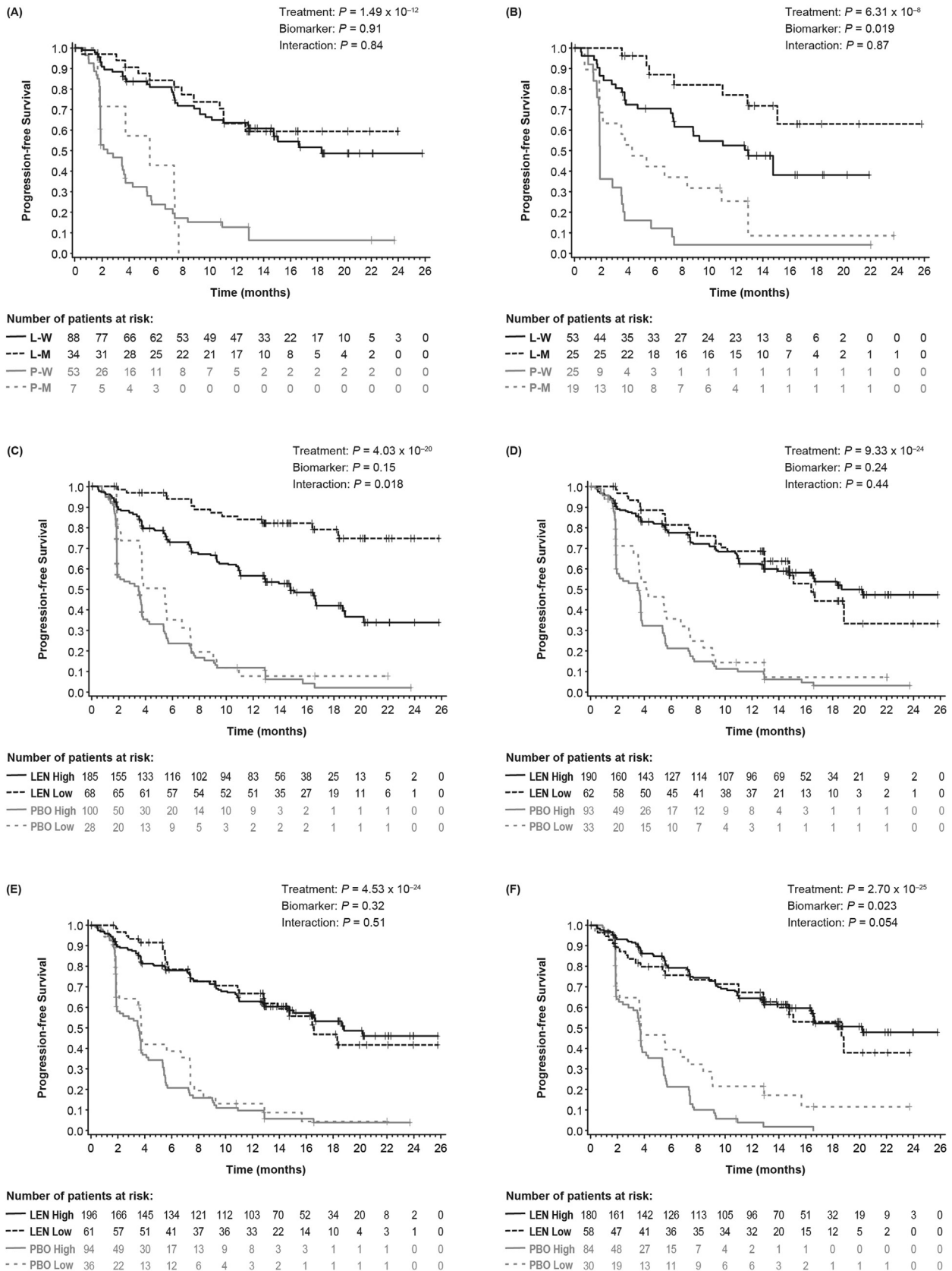
Fig. 2. Kaplan−Meier estimate of progression-free survival stratified by tumour mutation status or by low versus high levels of baseline biomarkers. Kaplan−Meier estimate of progression-free survival in patients in the lenvatinib or placebo arm by (A) *RAS* or (B) *BRAF* tumour mutation status, (C) Ang2, (D) VEGF, (E) Tie2 and (F) thyroglobulin levels stratified by low (first quartile) versus high (all other quartiles) baseline levels. Patients with both PTC and FTC were included in assessment of *RAS* mutation status, whereas only patients with PTC were included in assessment of *BRAF* mutation status. Ang2, angiopoietin-2; FTC, follicular thyroid cancer; L/LEN, lenvatinib; M, mutant; P/PBO, placebo; PTC, papillary thyroid cancer; Tie2, TEK tyrosine kinase, endothelial; VEGF, vascular endothelial growth factor; W, wild type.

Table 2
Correlation between baseline serum biomarker levels and clinical outcomes.

Baseline	Treatment	Clinical outcome (*P*-value)		
		MTS[a]	ORR[b]	PFS[c]
Tie2	Placebo	0.067	0.33	0.030
	Lenvatinib	0.65	0.99	0.038
Ang2	Placebo	0.068	0.27	0.0067
	Lenvatinib	8.09×10^{-7}	6.66×10^{-6}	1.10×10^{-6}
VEGF	Placebo	0.044	0.038	0.037
	Lenvatinib	0.0082	8.64×10^{-4}	0.23
Tg	Placebo	0.13	0.18	0.022
	Lenvatinib	0.53	0.87	0.23

Ang2, angiopoietin-2; MTS, maximum tumour shrinkage; ORR, objective response rate; PFS, progression-free survival; Tie2, TEK tyrosine kinase, endothelial; Tg, thyroglobulin; VEGF, vascular endothelial growth factor.
[a] Spearman rank correlation test.
[b] Wilcoxon signed-rank test.
[c] Univariate Cox proportional hazard model.

levels decreased significantly in patients treated with lenvatinib, but increased in placebo recipients at each treatment cycle tested (Fig. 3A). When this analysis was subdivided by lenvatinib responders (complete response or partial response) and non-responders (stable disease [SD] or progressive disease [PD]), significantly lower levels of thyroglobulin were seen in lenvatinib responders, but decreased thyroglobulin levels also were observed in patients with SD or PD (Fig. 3B). Levels of both VEGF and FGF23 increased significantly with lenvatinib treatment at all time points tested and at the end of treatment (Fig. 3C and D). Patients who experienced a greater increase in FGF23 levels (>median) with lenvatinib treatment had longer PFS than patients whose FGF23 increase was more modest (≤median; Fig. A. 5A). However, there was no correlation between the degree of increase in VEGF levels and PFS (Fig. A. 5B). In additional exploratory analyses, positive correlations were found between serum levels of FGF23 and phosphate, as expected (Table A. 4).

4. Discussion

This exploratory biomarker analysis of SELECT demonstrated that the PFS benefit of lenvatinib compared with placebo was maintained regardless of baseline circulating-serum biomarker levels or *BRAF/RAS* mutational status, indicating that lenvatinib is an option for all eligible patients. It is important to clarify that this does not indicate that lenvatinib would not be beneficial for those patients with high levels of baseline Ang2, because this subgroup of patients still had an impressive HR (0.24) in SELECT. Rather, these results suggest that a potential strategy for improved efficacy could be explored in these patients (e.g. the combination of lenvatinib with inhibitors targeting the Ang2/Tie2 signalling pathway). The Ang2/Tie2 signalling pathway

is considered a potential target to improve anti-angiogenic therapy; however, the role of Ang2 in DTC has not yet been established [19]. This study provides new insight to improve clinical outcome over VEGF-targeted therapy alone.

Changes in baseline levels of VEGF and FGF23 are pharmacodynamic biomarkers for the inhibition of VEGF and FGF signalling pathways [13,20]. Along with VEGF levels, FGF23 levels increased consistently in the lenvatinib arm, suggestive of lenvatinib-mediated VEGFR and FGFR target engagement and signalling inhibition in these patients with RR-DTC from SELECT. To our knowledge, this is the first report of a tyrosine-kinase-inhibitor−mediated increase in FGF23 levels in a phase III study. In addition, the observed PFS improvement in patients with greater increases in FGF23 levels while receiving lenvatinib is suggestive of the essential role of FGFR inhibition on lenvatinib efficacy in patients with RR-DTC. Positive correlations between serum phosphate and FGF23 levels were expected and observed, reflecting the role of FGF23 in the regulation of phosphorus homoeostasis, and potentially confounding our interpretations of the FGF23 findings. Further exploration of the changes in serum phosphorus level and its relationship with FGF23 levels is warranted. Overall, these results indicate that inhibition against the FGFR signalling pathways (in addition to VEGFR) represents a promising and important approach to controlling RR-DTC.

The similar PFS HRs and 95% CIs between the blood biomarker, tumour biomarker and overall study populations illustrate that the biomarker study populations in this investigation were a robust representation of the overall SELECT population. Surprisingly, although neither *BRAF* nor *RAS* mutations were predictive nor prognostic biomarkers in the overall population of patients with DTC, *BRAF*^WT was a prognostic factor for poorer PFS in metastatic progressive RR-PTC with placebo in this analysis. Our observation that the prognosis for patients with RR-PTC and *BRAF*^WT is worse in the absence of therapy strengthens the argument for prompt initiation of VEGFR-targeted treatment, especially because *BRAF* inhibitors are not a therapeutic option for this subset of patients. This finding was also seen in the phase III study of sorafenib in locally advanced metastatic patients with radioactive iodine refractory thyroid cancer (Study of Sorafenib in Locally Advanced or Metastatic Patients with RAI-Refractory Thyroid Cancer, DECISION), and suggests that in metastatic progressive RR-PTC, *BRAF*^WT may be prognostic for poorer PFS in the placebo arm [21]. Our findings also raise questions of possible coexisting mutations, as well as changes or evolution in mutational profile from the initial tumour to the radioiodine-refractory metastatic disease. This supports the importance of obtaining genomic data from tumour
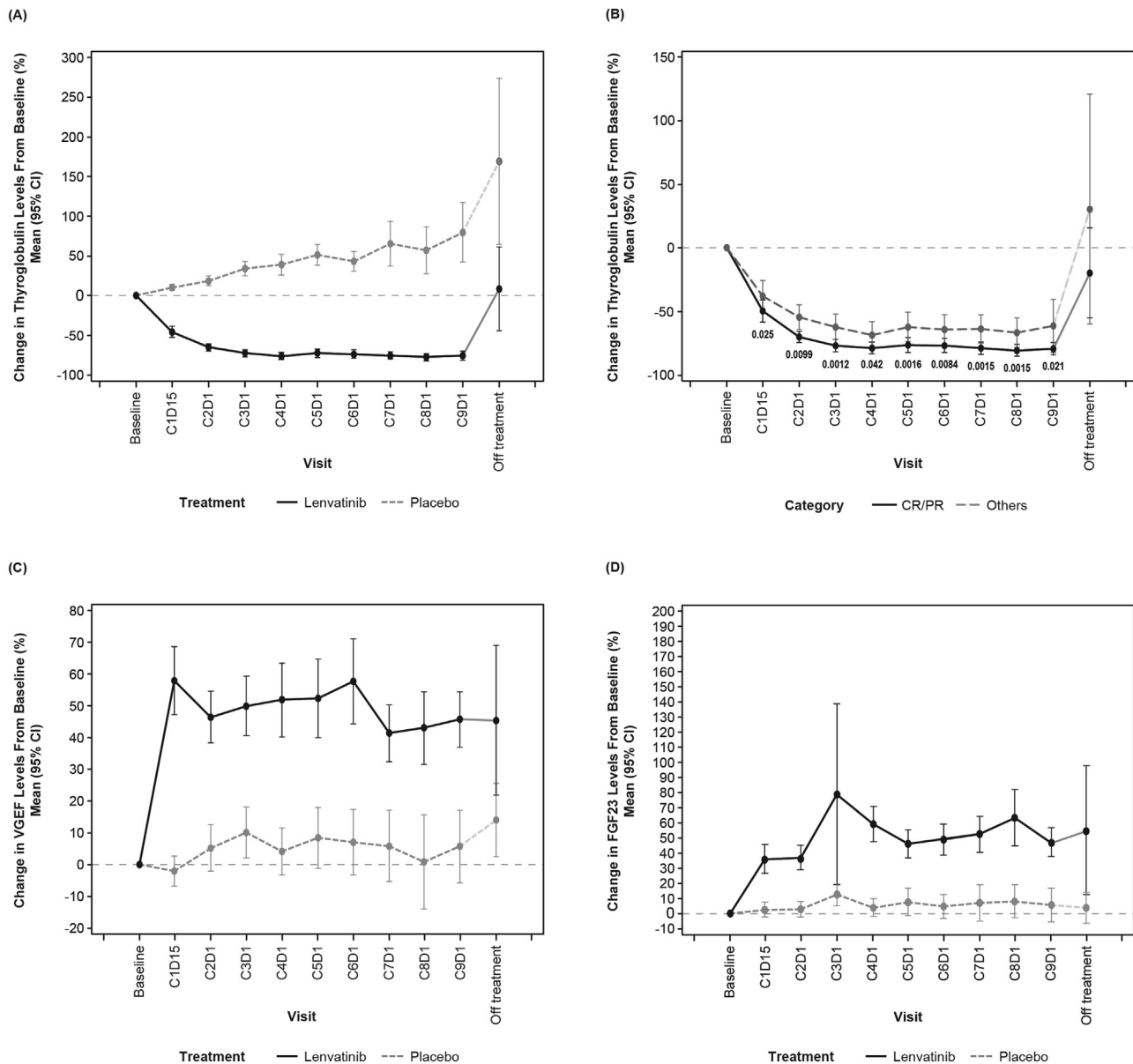
Fig. 3. Changes in (A) biomarker levels for thyroglobulin by treatment arm: placebo versus lenvatinib, (B) thyroglobulin levels by response: complete/partial responders versus patients with stable/progressive disease (lenvatinib arm only), (C) VEGF and (D) FGF23 levels by treatment arm: lenvatinib versus placebo. Cx Dx, cycle # day # of treatment; CI, confidence interval; CR, complete response; FGF, fibroblast growth factor; PR, partial response; VEGF, vascular endothelial growth factor.

specimens closer to the time of study therapy [22]. Of note, no central review of histology was performed in SELECT, and poorly differentiated carcinomas (which have low frequency of *BRAF*-mutated genes and higher frequency of *RAS*-mutated genes [23,24]) were classified as PTC, which may also explain our results. It is also important to note that the low frequency of *BRAF*-mutant tumours in this study limits our conclusions. Finally, *TERT* promoter mutations (which were not tested in the current study) in synergy with *BRAF* mutations have recently emerged as a novel genetic background in aggressive forms of thyroid cancer, and

should be investigated in further studies of RR-DTC biomarkers [25,26].

Our analysis indicates that a rapid decrease in thyroglobulin levels occurs with lenvatinib treatment, consistent with the rapid tumour response observed with lenvatinib [8]. In addition, the magnitude of change in thyroglobulin levels appear to be associated with objective responses; however, a decrease in thyroglobulin levels also was observed in patients with SD or PD. Therefore, although thyroglobulin is possibly a prognostic marker, it is an unreliable biomarker of lenvatinib response, underlining the importance of regular tumour

assessments to monitor patients on therapy (as opposed to reliance on thyroglobulin alone).

This study had several limitations, the foremost being the exploratory nature of the analysis, which, for example, led to imbalances in the distribution of *RAS* mutants in the lenvatinib versus placebo arms. In addition, it should be noted that at the time of analysis, many patients were continuing treatment with lenvatinib; therefore, future correlative analyses with a more mature data set may provide more robust observations, particularly with regard to overall survival outcomes. Additionally, only 183 (46.7%) of SELECT patient tumours could be analysed for *RAS* and *BRAF* mutations, and only 57/183 (31.3%) tumours were from metastatic sites.

In conclusion, all lenvatinib-treated patients in the SELECT trial, regardless of baseline circulating-serum biomarker levels or *BRAF/RAS* mutational status, derived a PFS benefit compared with placebo. In patients with RR-DTC treated with lenvatinib, FGFR inhibition in addition to VEGFR blockade is likely to play a role in improving PFS. Further study of new treatments or combination therapies to further improve PFS in patients with $BRAF^{WT}$ RR-PTC or RR-DTC with high baseline Ang2 levels is warranted.

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Conflict of interest statement

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejca.2017.01.013.

References

[1] Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996;86:353–64.

[2] Erdem H, Gundogdu C, Sipal S. Correlation of E-cadherin, VEGF, COX-2 expression to prognostic parameters in papillary thyroid carcinoma. Exp Mol Pathol 2011;90:312–7.

[3] Anderson RT, Linnehan JE, Tongbram V, Keating K, Wirth LJ. Clinical, safety, and economic evidence in radioactive iodine-refractory differentiated thyroid cancer: a systematic literature review. Thyroid 2013;23:392–407.

[4] Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer 2008;8:592–603.

[5] Okamoto K, Kodama K, Takase K, Sugi NH, Yamamoto Y, Iwata M, et al. Antitumor activities of the targeted multi-tyrosine kinase inhibitor lenvatinib (E7080) against RET gene fusion-driven tumor models. Cancer Lett 2013;340:97–103.

[6] Yamamoto Y, Matsui J, Matsushima T, Obaishi H, Miyazaki K, Nakamura K, et al. Lenvatinib, an angiogenesis inhibitor targeting VEGFR/FGFR, shows broad antitumor activity in human tumor xenograft models associated with microvessel density and pericyte coverage. Vasc Cell 2014;6:18.

[7] Tohyama O, Matsui J, Kodama K, Hata-Sugi N, Kimura T, Okamoto K, et al. Antitumor activity of lenvatinib (e7080): an angiogenesis inhibitor that targets multiple receptor tyrosine kinases in preclinical human thyroid cancer models. J Thyroid Res 2014;2014:638747.

[8] Schlumberger M, Tahara M, Wirth LJ, Robinson B, Brose MS, Elisei R, et al. Lenvatinib versus placebo in radioiodine-refractory thyroid cancer. N Engl J Med 2015;372:621–30.

[9] Kranenburg O, Gebbink MF, Voest EE. Stimulation of angiogenesis by Ras proteins. Biochim Biophys Acta 2004;1654:23–37.

[10] Boelaert K, McCabe CJ, Tannahill LA, Gittoes NJ, Holder RL, Watkinson JC, et al. Pituitary tumor transforming gene and fibroblast growth factor-2 expression: potential prognostic indicators in differentiated thyroid cancer. J Clin Endocrinol Metab 2003;88:2341–7.

[11] St Bernard R, Zheng L, Liu W, Winer D, Asa SL, Ezzat S. Fibroblast growth factor receptors as molecular targets in thyroid carcinoma. Endocrinology 2005;146:1145−53.

[12] Wöhrle S, Henninger C, Bonny O, Thuery A, Beluch N, Hynes NE, et al. Pharmacological inhibition of fibroblast growth factor (FGF) receptor signaling ameliorates FGF23-mediated hypophosphatemic rickets. J Bone Miner Res 2013; 28:899−911.

[13] Kim KB, Chesney J, Robinson D, Gardner H, Shi MM, Kirkwood JM. Phase I/II and pharmacodynamic study of dovitinib (TKI258), an inhibitor of fibroblast growth factor receptors and VEGF receptors, in patients with advanced melanoma. Clin Cancer Res 2011;17:7451−61.

[14] Secord AA, Nixon AB, Hurwitz HI. The search for biomarkers to direct antiangiogenic treatment in epithelial ovarian cancer. Gynecol Oncol 2014;135:349−58.

[15] Nixon AB, Pang H, Starr MD, Friedman PN, Bertagnolli MM, Kindler HL, et al. Prognostic and predictive blood-based biomarkers in patients with advanced pancreatic cancer: results from CALGB80303 (Alliance). Clin Cancer Res 2013;19:6957−66.

[16] Cabanillas ME, Schlumberger M, Jarzab B, Martins RG, Pacini F, Robinson B, et al. A phase 2 trial of lenvatinib (E7080) in advanced, progressive, radioiodine-refractory, differentiated thyroid cancer: a clinical outcomes and biomarker assessment. Cancer 2015;121:2749−56.

[17] Schlumberger M, Jarzab B, Cabanillas ME, Robinson B, Pacini F, Ball DW, et al. A phase II trial of the multitargeted tyrosine kinase inhibitor lenvatinib (E7080) in advanced medullary thyroid cancer. Clin Cancer Res 2016;22:44−53.

[18] Ball DW, Sherman SI, Jarzab B, Cabanillas ME, Martins R, Shah MH, et al. Lenvatinib treatment of advanced RAI-refractory differentiated thyroid cancer (DTC): cytokine and angiogenic factor (CAF) profiling in combination with tumor

genetic analysis to identify markers associated with response [abstract]. J Clin Oncol 2012;30(15). Abstract 5518.

[19] Gerald D, Chintharlapalli S, Augustin HG, Benjamin LE. Angiopoietin-2: an attractive target for improved antiangiogenic tumor therapy. Cancer Res 2013;73:1649−57.

[20] Bass MB, Sherman SI, Schlumberger MJ, Davis MT, Kivman L, Khoo HM, et al. Biomarkers as predictors of response to treatment with motesanib in patients with progressive advanced thyroid cancer. J Clin Endocrinol Metab 2010;95:5018−27.

[21] Brose MS, Nutting CM, Jarzab B, Elisei R, Siena S, Bastholt L, et al. Sorafenib in radioactive iodine-refractory, locally advanced or metastatic differentiated thyroid cancer: a randomised, double-blind, phase 3 trial. Lancet 2014;384:319−28.

[22] Bible KC, Cote GJ, Demeure MJ, Elisei R, Jhiang S, Ringel MD, et al. Correlative studies in clinical trials: a position statement from the International Thyroid Oncology Group. J Clin Endocrinol Metab 2015;100:4387−95.

[23] Volante M, Landolfi S, Chiusa L, Palestini N, Motta M, Codegone A, et al. Poorly differentiated carcinomas of the thyroid with trabecular, insular, and solid patterns: a clinicopathologic study of 183 patients. Cancer 2004;100:950−7.

[24] Volante M, Rapa I, Gandhi M, Bussolati G, Giachino D, Papotti M, et al. RAS mutations are the predominant molecular alteration in poorly differentiated thyroid carcinomas and bear prognostic impact. J Clin Endocrinol Metab 2009;94:4735−41.

[25] Liu X, Qu S, Liu R, Sheng C, Shi X, Zhu G, et al. TERT promoter mutations and their association with BRAF V600E mutation and aggressive clinicopathological characteristics of thyroid cancer. J Clin Endocrinol Metab 2014;99:E1130−6.

[26] Xing M, Liu R, Liu X, Murugan AK, Zhu G, Zeiger MA, et al. BRAF V600E and TERT promoter mutations cooperatively identify the most aggressive papillary thyroid cancer with highest recurrence. J Clin Oncol 2014;32:2718−26.