Cabozantinib in Progressive Medullary Thyroid Cancer

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SUPPLEMENTARY METHODS

Inclusion and Exclusion Criteria

Eligible patients had a confirmed diagnosis of medullary thyroid cancer (MTC) that was unresectable, locally advanced, or metastatic, as well as disease that was measurable or nonmeasurable per modified Response Evaluation Criteria in Solid Tumors (mRECIST). All patients were ≥ 18 years old, had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, and had documented progressive disease at screening (using mRECIST) compared with a previous image taken within the prior 14 months. Additional parameters for study entry included adequate organ and marrow function as follows: neutrophil counts ≥ 1500/mm^3, platelets ≥ 100,000/mm^3, hemoglobin ≥ 9 g/dL, bilirubin ≤ 1.5 × upper limit of normal (ULN; criteria did not apply for patients with Gilbert's syndrome), serum creatinine ≤ 1.5 mg/dL, and alanine transferase and aspartate transferase ≤ 2.5 × ULN.

Patients were ineligible if they had received prior systemic antitumor therapy within 4 weeks of randomization (6 weeks for nitrosoureas or mitomycin C), received radiation to ≥ 25% of bone marrow, had previously been treated with cabozantinib, or had treatment with other investigational agents within 4 weeks of randomization. Patients with untreated or symptomatic brain metastases or spinal cord compression or with other diagnoses of malignancy (unless non-melanoma skin cancer, carcinoma in situ of the cervix, or a malignancy diagnosed ≥ 2 years previously) were ineligible. Patients were also ineligible if they had a history of clinically significant hematemesis or history of hemoptysis of > 2.5 mL of red blood; had a urine protein/creatinine ratio of ≥ 1; had serious intercurrent illness, such as hypertension, despite optimal treatment; unhealed wounds from recent surgery; or cardiac arrhythmias.

Determination of **RET** *Mutational Status*

A blood sample was collected predose cycle 1, day 1 (C1D1) from all patients to assess the mutational status of the rearranged during transfection (*RET)* gene. A tumor tissue sample (formalin-fixed paraffin-embedded archival tissue or frozen tumor tissue sample) was required of each patient enrolled. If documentation of the presence of a *RET* mutation in a patient's blood or tumor could be provided, a tumor tissue sample was not required.

DNA samples derived from blood and tumor tissue were analyzed for alterations in the sequence of the gene encoding RET. Genomic DNA was isolated from formalin-fixed paraffin-embedded biopsies as described.[1] For the separation of tumor and adjacent normal tissue, manual microdissection guided by a hematoxylin- and eosin-stained serial section was performed when necessary. DNA extraction from blood was performed using the QIAamp DNA mini kit (Qiagen, Valencia, CA). Genomic DNA was amplified by polymerase chain reaction (PCR) and sequenced using the Sanger method in most cases[2]; however, samples with lower percentage tumor cell content (< 40%) were evaluated with a highly parallel sequencing method (454 Life Sciences, Branford, CT) to increase sensitivity to approximately 5% mutant allele burden.

Of the 330 patients enrolled in the study, partial or complete *RET* sequence data from one or both sample types was obtained for 319 patients. For blood DNA samples, *RET* exons 5, 8, 10, 11, 13, 14, 15, and 16 (which cover the majority of the characterized *RET* mutations) were analyzed at a minimum. For tumor DNA samples, *RET* mutational hotspot exons 11 and 16 were analyzed initially, with additional exons analyzed subsequently if no mutations were identified in exons 11 and 16. Due to sample limitations, exons 5 and 8 were not routinely analyzed in tumor DNA samples. However, mutations in these exons are likely limited only to patients with familial

MTC, and no mutations were detected in these exons from the blood samples in this study. For a sample to be considered negative for *RET* mutation, the complete DNA sequence for exons 10, 11, 13, 14, 15, and 16 must have been obtained, and all *RET* sequences analyzed must have been clearly free of mutation. Blood or tumor samples that showed evidence for a *RET* sequence alteration were considered *RET* mutation–positive if the identified mutation is listed as being related to MTC or MEN 2 syndromes in the American Thyroid Association Medullary Thyroid Cancer Guideline publication.[3] Note that *RET* sequence alterations not described in this publication were classified in the "unknown" category, even though some of these are likely to be functional mutations. Also described as "unknown" was any sample lacking sufficient sequence coverage of *RET* and without an identified qualifying *RET* mutation. Most patients in the unknown category either had no tumor sample available at the time of analysis or had a tumor DNA sample that failed PCR amplification. In addition, a patient was classified as having sporadic MTC if his or her blood or tumor DNA sample qualified as *RET* mutation–negative as described in **Table S8**. *RET* mutation status, patients with the common *RET* M918T mutation, and MTC disease type (sporadic or hereditary) were evaluated in progression-free survival (PFS) and response rate subgroup analyses. Criteria used to define MTC disease type, as well as *RET* and *RET* M918T mutation status, are listed in **Table S9**. Of note, the assessment of *RAS* mutation status is ongoing.

Tumor Markers: Calcitonin and CEA

The tumor markers calcitonin and carcinoembryonic antigen (CEA) were evaluated from serum samples at baseline, and every 12 weeks after initiation of treatment, to coincide with radiologic tumor assessments. Tumor markers were evaluated at additional time points at the discretion of the investigator. All serum calcitonin and CEA assessments were performed by central

laboratory (Covance, Indianapolis, IN). CEA determinations were performed using a microparticle enzyme immunoassay system (AxSYM Analyzer System, Abbott Laboratories, Abbott Park, IL). For calcitonin, two different assay formats were employed over the duration of the study. Initially, a radioimmunoassay (RIA) format was used (Beckman Coulter # DSL1200). Approximately midway through the study, the assay was changed to a chemiluminescence format (Siemens Immulite, # L2KCL2) due to discontinuation of the RIA format assay by the manufacturer. For the RIA assay, the lower and upper limits of quantitation were 65 pg/mL (19 pmol/L) and 315 ng/mL (91350 pmol/L), respectively. For the chemiluminescence assay, the lower and upper limits of quantitation were 2.4 pg/mL (0.59 pmol/L) and 200 ng/mL (57890 pmol/L), respectively. The upper limits of quantitation were achieved through dilution of the sample 600-fold or 100-fold, respectively, for the RIA and chemiluminescence assays. Sixty-five patients have calcitonin data using only the RIA method, and 145 patients have data using only the chemiluminescence method. There are 117 patients with data generated from both assays. Validation studies indicate that calcitonin data from the two assay formats are not directly comparable. Thus, for patients with baseline data using the RIA method, any subsequent data points obtained using the chemiluminescence method have been removed from the analysis for determination of biochemical response. A total of 45 patients were nonevaluable for calcitonin biochemical response due to this change in assay format (11 placebo [9.9%] and 34 cabozantinib [15.5%]). A total of 36 patients were nonevaluable for calcitonin change at week 12 (9 placebo [8.8%] and 27 cabozantinib [12.3%]).

All patients who had adequate baseline and week-12 tumor marker data were included in the week-12 response analysis. Patients were typically excluded based on a lack of a week-12 sample or a mismatch between baseline and week-12 calcitonin assay formats. Tumor marker

response at week-12 was calculated as percent change compared to baseline: ([week-12 value - baseline value]/baseline value) * 100. A complete analysis of the calcitonin and CEA data is intended for publication in a separate manuscript.

Statistical Analysis

For censored patients, the censoring date is the date of the most-recent adequate IRC tumor assessment that occurred prior to the date of the cause (e.g. subsequent therapy) of censoring. Patients with no tumor assessments performed after randomization who lived at least 26 weeks after randomization are censored at the date of randomization. For censored patients, the duration of PFS is calculated as the time since randomization until the date of censoring. Patients with no tumor assessments performed after randomization who died within 26 weeks of randomization are counted as events at the date of death. In this fashion, all patients contribute to the PFS analysis.

A tabulation of events and reasons for censoring for the primary PFS analysis is shown in **Table S1**. The primary analysis was prespecified to include 138 events, but includes an additional event as the 139th event occurred on the same date as the 138th event.

The planned sensitivity analyses for PFS used the same statistical methods as described for the primary analysis but used alternative definitions for events and censoring:

- "uniform dates" analysis based on the date of radiographic progression as determined by the independent radiology review committee (IRC) at the scheduled tumor assessment rather than the date progression was recorded (designated PFS2);

- analysis based on investigator assessment of radiographic progression (designated PFS3);

- "investigator claims" analysis based on investigator assessment of radiographic progression, clinical deterioration, and initiation of subsequent systemic cancer therapy (designated PFS4).

Results for these analyses are shown in **Table S3.**

For the secondary efficacy end point of overall survival (OS), the study was designed to have 80% power to detect a hazard ratio (HR) of 0.667 using the log-rank test and a two-sided significance level of 4%; 217 deaths are required. The planned sample size was chosen to increase power to evaluate differences in OS. An interim analysis of OS was planned to occur at the time of the primary analysis, with a criterion for stopping the study early due to rejection of the null hypothesis at the interim analysis, based on a significance level determined from an alpha-spending function. The protocol did not include criteria for stopping the study early for futility. Type I error in the interim OS analysis was controlled by implementing a Lan-DeMets O'Brien Fleming alpha spending function. The multiplicity issue resulting from the analyses of one primary efficacy end point (PFS) and two key secondary efficacy end points (objective response rate and OS), as well as the performance of one interim analysis of OS, was addressed by employing a fixed-sequence testing procedure, dividing the alpha between the key secondary end points, and implementing an alpha spending function.

Study Oversight

An independent unblinded data monitoring committee provided oversight for safety evaluation throughout the study.

SUPPLEMENTARY TABLES AND FIGURES

Table S1. Event and Censoring Status in the Primary PFS Analysis

	Cabozantinib (n, %)	Placebo (n, %)
Randomized	219 (100)	111 (100)
Event	79 (36)	60 (54)
Radiographic PD	58 (26)	50 (45)
Death	21 (10)	10 (9)
Censored	140 (64)	51 (46)
No event observed by data cutoff	80 (37)	12 (11)
Received subsequent anticancer therapy	21 (10)	19 (17)
No postbaseline tumor assessments	37 (17)	20 (18)
Missed assessments before event	2 (1)	0 (0)

Abbreviations: PD, progressive disease; PFS, progression-free survival.

Table S2. Summary of Last Protocol-Specified Dose Levels (Safety Population Still On Treatment at Data Cutoff Date of 15 June 2011)

	Cabozantinib	Placebo
N	98	15
Mean (SD)	106.1 (34.82)	175.0 (0.00)
Median	100.0	175.0
Range	75 – 175	175 – 175

Abbreviation: SD, standard deviation.

Table S3. Sensitivity Analyses of PFS through the Date of the 138th Event (ITT Population)

PFS Analysis	XL184 Median Duration (months)	Placebo Median Duration (months)	Stratified HR	95% CI	Stratified log-rank *P*-value
IRC Uniform dates, PFS2*	11.1	5.4	0.29	0.20 to 0.42	<.0001
Investigator-documented radiographic, PFS3†	13.8	3.1	0.29	0.21 to 0.42	<.0001
Investigator claims, PFS4‡	11.2	3.0	0.32	0.23 to 0.43	<.0001

Note: 139 events occurred by the date of the 138th event.

Abbreviations: CI, confidence interval; HR, hazard ratio; IRC, independent radiology review committee; ITT, intention-to-treat; PD, progressive disease; PFS progression-free survival.

*PFS2 analysis: Date of radiographic progression as determined by the IRC at the scheduled tumor assessment rather than the date progression was recorded.

†PFS3 analysis: Progression events based on investigator assessment of radiographic progression. Clinical deterioration was not considered PD.

‡PFS4 analysis: Progression events included investigator assessment of radiographic progression, clinical deterioration, and initiation of subsequent systemic cancer therapy.

Table S4. Distribution by Time Between Historical Reference Scan and Screening Scan (ITT Population)*

	Treatment, n (%)	
Time (months)	Cabozantinib, N = 219	Placebo, N = 111
0–1	6 (2.7)	4 (3.6)
1–2	14 (6.4)	1 (0.9)
2–3	13 (5.9)	7 (6.3)
3–4	8 (3.7)	6 (5.4)
4–5	16 (7.3)	7 (6.3)
5–6	17 (7.8)	9 (8.1)
6–7	19 (8.7)	9 (8.1)
7–8	13 (5.9)	7 (6.3)
8–9	18 (8.2)	8 (7.2)
9–10	19 (8.7)	9 (8.1)
10–11	20 (9.1)	11 (9.9)
11–12	16 (7.3)	16 (14.4)
12–13	18 (8.2)	7 (6.3)
13–14	19 (8.7)	8 (7.2)
14–15	0	1 (0.9)
≥ 15	1 (0.5)	0

Abbreviation: ITT, intention-to-treat.

* The 14 month window for documentation of radiographic progression was chosen to select for a progressive (ie non-indolent) population with a high need for treatment. Those patients whose disease course mandated less frequent imaging were judged by the study steering committee to be more likely to have indolent disease.

Table S5. Current Extent of Metastatic Disease at Enrollment (ITT Population)

	Treatment, n (%)	
Site of Metastasis*	Cabozantinib, N = 219	Placebo, N = 111
Lymph nodes	175 (79.9)	86 (77.5)
Cervical	111 (50.7)	65 (58.6)
Mediastinum	130 (59.4)	60 (54.1)
Other	58 (26.5)	31 (27.9)
Liver	152 (69.4)	67 (60.4)
Lung	116 (53.0)	64 (57.7)
Bone	112 (51.1)	56 (50.5)
Neck	37 (16.9)	12 (10.8)
Other	24 (11.0)	20 (18.0)
Brain	5 (2.3)	2 (1.8)
Pelvis	5 (2.3)	5 (4.5)

Abbreviation: ITT, intention-to-treat.
*12.7% of study population (12.8% cabozantinib/12.6% placebo) had metastasis at only one organ site

Table S6. Tumor Response in Patients with Measurable Disease at Baseline*

	Cabozantinib	Placebo
Patients with measurable disease, N	208	104
Best overall response[†], n (%)		
Confirmed CR	0	0
Confirmed PR	58 (27.9)	0
SD	100 (48.1)	52 (50.0)
PD	18 (8.7)	35 (33.7)
Unable to evaluate	5 (2.4)	1 (1.0)
Missing[‡]	27 (13.0)	16 (15.4)
ORR[†§], n (%)	58 (27.9)	0
Disease stabilization rate[†,¶], n (%)	115 (55.3)	14 (13.5)
SD[†], n (%)		
Patients with SD or better at 12 weeks after randomization	120 (57.7)	39 (37.5)
Patients with SD or better at 24 weeks after randomization	91 (43.8)	11 (10.6)
Patients with SD or better at 48 weeks after randomization	36 (17.3)	3 (2.9)

Abbreviations: CR, complete response; IRC, independent radiology review committee; mRECIST, modified Response Evaluation Criteria in Solid Tumors; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease. [*]Table does not include 11 patients in the cabozantinib arm and 7 patients in the placebo arm without measureable disease at baseline.

[†]Best overall response determined by IRC using mRECIST criteria.

[‡]Missing = no qualifying postbaseline assessment for overall response.
[§]ORR is defined as the proportion of patients with measurable disease achieving best overall response of confirmed CR or confirmed PR.
[¶]Disease stabilization rate is defined as the proportion of patients with measurable disease achieving best overall response of confirmed CR, confirmed PR, or SD on or after the Week 24 tumor assessment without prior PD or receipt of subsequent therapy.

Table S7. Percent-Patient Incidence of Laboratory Abnormalities Occurring at a Higher Incidence in Cabozantinib-Treated Patients. [Between Arm Difference of ≥ 5% (All Grades) or ≥ 2% (Grades 3-4)]

	Cabozantinib (N=214)		Placebo (N=109)	
	All Grades	Grade 3-4	All Grades	Grade 3-4
Chemistries				
Increased AST	86	3	35	2
Increased ALT	86	6	41	2
Increased ALP	52	3	35	3
Hypocalcemia	52	12	27	3
Hypophosphatemia	28	3	10	1
Hyperbilirubinemia	25	2	14	5
Hypomagnesemia	19	1	4	0
Hypokalemia	18	4	9	3
Hyponatremia	10	2	5	0
Hematologic				

Lymphopenia	53	16	51	11
Neutropenia	35	3	15	2
Thrombocytopenia	35	0	4	3

ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase

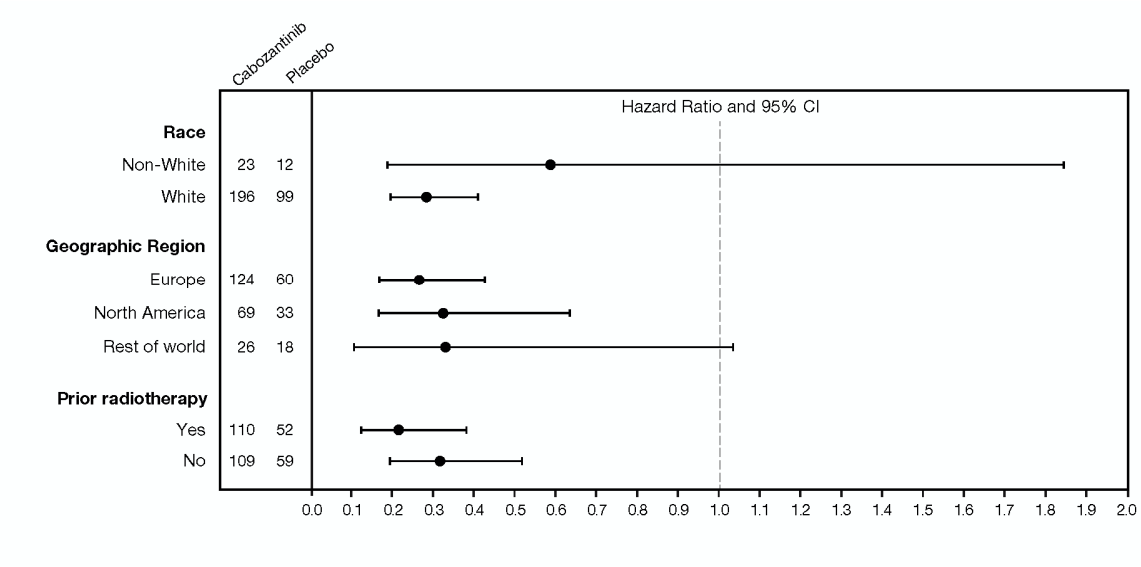
Table S8. *RET* Genotyping Subgroup Definitions

Genotyping category	Definition
RET mutation status–positive	*RET* mutation (as defined in Kloos et al.[3]) identified in blood or tumor DNA sample or as documented by pathology report from a previous analysis.
RET mutation status–negative	DNA sequence available from exons 10, 11, 13, 14, 15, and 16 of tumor sample with no *RET* mutations identified.
RET mutation status unknown	Either insufficient DNA sequence information to assign *RET* mutation–negative status and no *RET* mutation identified, or patient harbors a *RET* mutation of unknown significance.
MTC disease type hereditary	*RET* mutation (as defined in Kloos et al.[3]) identified in blood DNA sample or as documented by pathology report from a previous analysis.
MTC disease type sporadic	DNA sequence available from exons 10, 11, 13, 14, 15, or 16 of blood sample with no *RET* mutations identified.
MTC disease type unknown	Insufficient DNA sequence information from blood sample to assign *RET* mutation status and tumor sample cannot be classified as *RET* mutation–negative.

RET M918T mutation status–positive	*RET* M918T mutation identified in blood or tumor DNA sample or as documented by pathology report from a previous analysis.
RET M918T mutation status–negative	DNA sequence available from exon 16 of the tumor sample with no evidence of *RET* M918T.
RET M918T mutation status unknown	Patient lacks *RET* M918T mutation in the blood sample and lacks *RET* exon 16 data from the tumor sample.

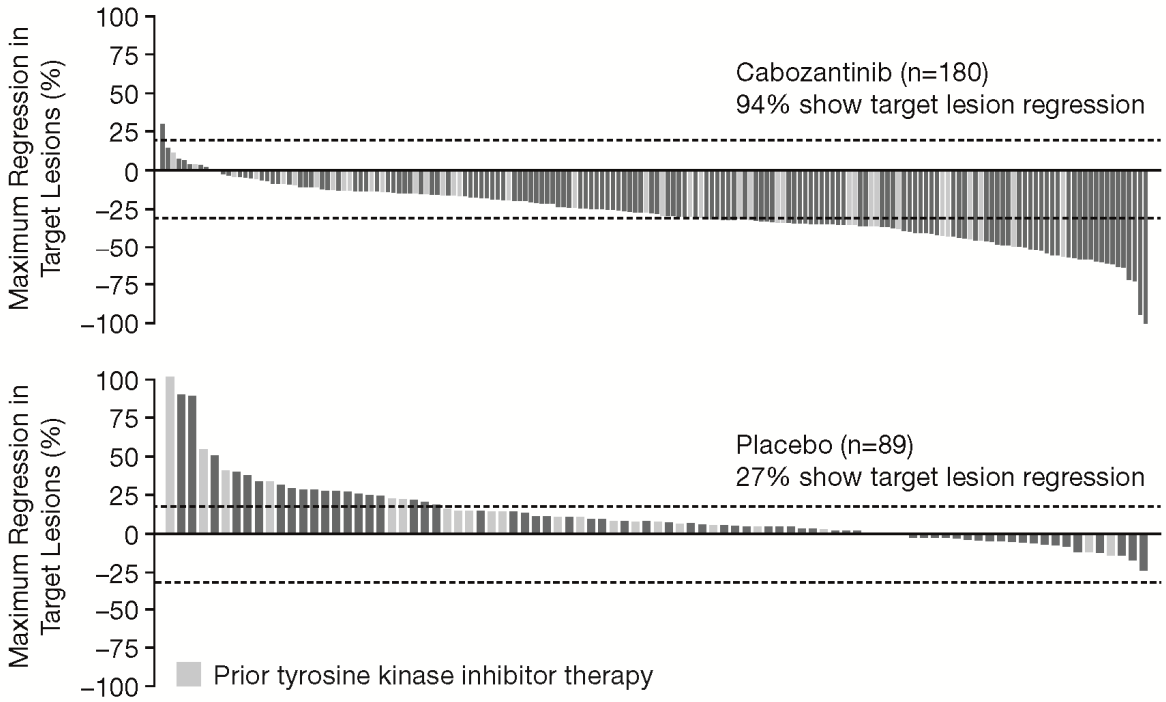
Abbreviations: MTC, medullary thyroid cancer; RET, rearranged during transfection; SNPs, single nucleotide polymorphisms.

Fig S1. Additional PFS Subgroup Analyses



CI, confidence interval; PFS, progression-free survival.

Fig S2. Maximum Regression in Target Lesions From Baseline (IRC-Determined, ITT Population With Measurable Disease and ≥ 1 Postbaseline Scan)



IRC, independent radiology review committee; ITT, intention-to-treat; TKI, tyrosine kinase. inhibitor

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