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MEDULLARY THYROID CANCER TREATED WITH VANDETANIB: PREDICTORS OF LONGER AND DURABLE RESPONSE

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ABSTRACT

Vandetanib is an important option to treat advanced metastatic medullary thyroid cancer. The aims of this study were to evaluate the predictors of both a longer response to vandetanib and of their outcome. Medical records of 79 medullary thyroid cancer patients treated with vandetanib in our center were analysed. Twenty-five patients were treated for <12 months, 54 were treated for ≥12 months and 24 of these latter were treated for ≥ 48 months (short, long and very long term treated). The median progression free survival of the long and very long term treated patients was significantly longer than in ZETA trial. When comparing the groups of short and long term treated patients the only significant difference was that these latter were less frequently previously treated with a tyrosine kinase inhibitor. However, the long term treated patients had a younger age, both at diagnosis and enrolment, that becomes statistically significant in the very long term treated patients. Inside the long term treated group, a younger age, the enrolment for symptoms and the development of adverse events were significantly correlated with a better outcome. The enrolment for symptoms remained the only statistically significant predictor of a good outcome in the very long term treated patients. In conclusion, an early treatment with vandetanib, when patients are younger, with a good ECOG performance status and a symptomatic disease, not necessarily progressing for RECIST, seem to be the best predictors of longer and durable response. Further studies are needed to confirm these evidences.

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Introduction

Medullary thyroid carcinoma (MTC) is a rare malignancy originating from the parafollicular C cells of the thyroid gland. MTC represent 5-10% of all thyroid cancers and it can be sporadic (75%) or inherited (25%). The pathogenesis of MTC is related to activating mutations of the rearranged during transfection (*RET*) gene that are somatic (45% of cases) in sporadic tumors and germline (98% of cases) in hereditary tumors (Romei, et al. 2015).

The 10-year overall survival rate in unselected patients with MTC is approximately 75%, but it decreases to approximately 40% in patients with locally or advanced MTC (Hundahl, et al. 1998; Lakhani, et al. 2007; Wells, et al. 2012). Any type of cytotoxic chemiotherapy have been demonstrated to have limited and transient antitumor activity in patients with unresectable or metastatic disease (Droz, et al. 1984; Orlandi, et al. 2001; Schlumberger, et al. 1995). Postoperative radiotherapy of the neck can determine locoregional disease control with limited morbidity (Schwartz, et al. 2008). Differently, the "target therapy" with tyrosine kinase inhibitors (TKIs) represents an important therapeutic option in advanced MTC cases. As demonstrated in preclinical studies, the ret kinase receptor, the vascular endothelial growth factor receptor (VEGFR) and the epidermal growth factor receptors (EGFR), whose signaling pathways contribute to the growth and invasiveness of MTC, are therapeutic targets for several TKIs (Carlomagno, et al. 2002; Poon, et al. 2001; Santoro and Carlomagno 2006; Wedge, et al. 2002).

Vandetanib is an oral agent that selectively targets *RET*, VEGFR and EGFR signaling pathways, as demonstrated in phase II and phase III clinical trials and it has been approved by Food and Drug Administration (FDA) in 2011 and European Medicines Agency (EMA) in 2013 for the treatment of patients with locally advanced or metastatic MTC (Wedge et al. 2002; Wells, et al. 2010; Wells et al. 2012).

Over the years, starting from the time of the phase III trial (ZETA study) (Wells et al. 2012), we had the opportunity to treat a rather big number of MTC patients with vandetanib and we

observed that there was a great variability in the length and duration of the response to the drug. The aim of this study was to find those factors that could predict a relatively long and durable response of the disease to vandetanib. To this purpose we analyzed the epidemiological, pathological, clinical and genetic features of patients with locally advanced or metastatic MTC treated with vandetanib in our center. In particular, we concentrated our attention on the subgroup of those patients who were treated for at least 12 months and longer, who have been considered as long term treated patients. A comparison between the short term (treated for <12 months) and the long term treated patients (≥ 12 months) has been also performed to verify if we could better predict the longer responsiveness to the drug. The analysis of a subgroup of very long term treated patients (≥ 48 months) was also carried out.

PATIENTS AND METHODS

Patients

We included all MTC patients treated with vandetanib and followed in our center both within AstraZeneca clinical trials and with the commercial drug after its approval. The AstraZeneca clinical trials that we could participate by enrolling patients were: AZ58 – An International, Phase III, Randomized, Double-Blinded, Placebo-Controlled, Multi-Center Study to Asses the Efficacy of ZD6474 (ZACTIMA™) versus Placebo in Subjects with Unresectable Locally Advanced or Metastatic Medullary Thyroid Cancer (D4200C00058) (Wells et al. 2012); AZ68 - A Phase II, Open-Label Study To Assess The Efficacy of ZD6474 (ZACTIMA™) 100 mg Monotherapy In Subjects with Locally Advanced or Metastatic Hereditary Medullary Thyroid Cancer (D4200C00068) (Robinson, et al. 2010); AZ88 – A Randomized, International, Open-Label, Multi-Centre, Phase III Study to Assess the Effect of a Patient Outreach Program on the Percentage of Time Patients with Locally Advanced or Metastatic Medullary Thyroid Cancer Experience Grade 2 or Higher Adverse Events during the First 12 Months of Treatment with Vandetanib (D4200C00088) (Bastholt, et al. 2016); AZ97 - An International, Randomized, Double-Blind, Two-

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Arm Study To Evaluate The Safety And Efficacy Of Vandetanib 150 And 300 mg/Day In patients With Unresectable Locally Advanced Or Metastatic Medullary Thyroid Carcinoma With Progressive Or Symptomatic Disease (D4200C00097) (Hu, et al. 2019); AZ104 – European, Observational, Prospective Study to Evaluate the Benefit/Risk of Vandetanib (CAPRELSA™) 300 mg in RET Mutation Negative and RET Mutation Positive Patients with Symptomatic, Aggressive, Sporadic, Unresectable, Locally Advanced/Metastatic Medullary Thyroid Cancer (MTC) (D4200C00104).

Patients were all adults with histologically confirmed, unresectable, locally advanced or metastatic MTC. Patients enrolled in clinical trials have been requested to satisfy the enrolling criteria of the specific clinical trial. In some trials (i.e AZ58, AZ68, AZ88, AZ97, AZ104) patients must have radiographic disease progression per modified Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (Eisenhauer, et al. 2009) at screening. In other trials (i.e. AZ58, AZ88, AZ97, AZ104) patients could be also enrolled if a symptomatic metastatic disease was present. In the majority of trials prior systemic anticancer therapy within 4 weeks or significant cardiac, hematopoietic, hepatic or renal failure were considered exclusion criteria. Very similar inclusion criteria were used to start vandetanib after its approval although, in the real life, the clinical judgment became fundamental. For patients treated after the approval of the drug there was no limit on prior therapy, including exposure to other TKIs. The main exclusion criteria were the presence of the cardiac disorders (i.e. QTc longer than 480 ms) or important alterations in blood samples before starting the drug. All patients provided written informed consent to participate in clinical trials. Patients who were treated after the approval of the drug started vandetanib treatment either for the evidence of progression or for symptoms according to the clinical judgment. Treatment was withdrawn in the presence of severe adverse events or progression disease according to RECIST. The present observational study was approved by the local Ethic Committee (CEAVNO - Comitato Etico Area Vasta Nord Ovest). As policy of our University Hospital, at the

time of first entering in the Hospital, all patients give their signed approval to the use of the clinical and biochemical personal data for research and scientific purposes.

RET genetic analysis

In 72/79 patients tumor tissues were available for *RET* genetic analysis that was performed according to our standard protocols (Elisei, et al. 2019). The analysis was performed in 49 primary tissues, 14 lymphnode metastases and 9 distant metastases. In all cases the blood DNA extraction and the search of germline *RET* mutations was also performed to distinguish the somatic from the germline *RET* mutations.

Data Analysis

We collected all epidemiological, clinical, biochemical, electrocardiographic, radiological and pathological data of each patient both at the time of starting therapy and during follow-up. In particular patients were investigated after 1, 3, 6 and 12 weeks in the first 3 months of therapy and then every 12 weeks. The radiographic tumor assessment with a computed tomography (CT) scan was performed every 12 weeks using RECIST until progression or withdrawal of consent. The progressive disease (PD), the stable disease (SD) and the partial response (PR) were established according to RECIST. This strategy was followed both in the clinical trials and for patients treated after the approval of the drug.

To the purpose of this study, we arbitrarily distinguished two groups of patients according to the duration of the treatment: a group of short term treated patients (duration of treatment < 12 months) and long term treated patients (duration of treatment ≥ 12 months). The choice to put a line at 12 months to distinguish the two groups is arbitrary but conceivable with a real effectiveness of the drug in the clinical practice. We then concentrated our attention also in a subgroup of very long term treated patients who were those treated for ≥ 48 months.

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As previously said, the main objective of the present study was the identification of epidemiological, clinical, pathological and/or genetic factors of MTC patients that could predict a long and durable response to vandetanib of at least 12 months or more. In this analysis we included the objective response rate (ORR), the progression free-survival (PFS), the morphological (RECIST) and biochemical response (i.e. decreases in serum levels of calcitonin [Ct] (ELSA-hCt; Cis-Bio-International, Gif sur Yvette, France until September 2013 and with Immulite® 2000 Calcitonin, Siemens Health Care Diagnostics Products LTD, Llanberis, UK, thereafter September 2013) and carcinoembryonic antigen [CEA] (Elecsys CEA, Roche Diagnostics, Holliston, MA USA).

Safety and Tolerability

The safety and tolerability of the treatment were evaluated by monitoring Adverse Events (AEs) as indicated by the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) (U.S. Department of Healt and Human Services NIoH May 28,2009). Vandetanib dose reductions were made for grade 3 or 4 toxicities.

Statistical Analysis

Pearson's chi-squared test and Fisher's exact test were used to evaluate differences in counts and frequency between groups, as appropriate. Student's t-test and Mann-Whitney U test were used to assess differences between groups for continuous variables with Gaussian and skewed distributions, respectively. The Kaplan-Meier 'time-to-event' method was used to calculate curves of progression-free survival (PFS). Progression was defined according to RECIST criteria. Patients who did not show progressive disease (PD) were included in the analysis until they stopped the treatment. Statistical analyses were performed using SPSS (version 21; Armonk, NY: IBM Corp.). Alpha was set at 0.05. Data are presented as mean±SD or frequency (percentage).

RESULTS

Clinical, pathological, epidemiological and genetic features of MTC patients treated with vandetanib

Between February 2007, when we enrolled the first patient in the ZETA study, and December 2018 a total of 79 locally advanced or metastatic MTC patients, referred to our Center, started vandetanib therapy, 62 in the frame of a clinical trial and 17 after the approval of the commercial drug. Fifty-six/79 (71%) were males and 23/79 (29%) were females. The median age at the time of starting therapy was 56 years (range 20-79 years); the median age at the time of MTC diagnosis was 50 years (range 13-79). The mean time elapsing from the diagnosis and the start of therapy was 5.4 years. The median PFS of all enrolled patients was 47 months (95% CI: 25-91 months) that was longer than that estimated in patients treated with vandetanib in the ZETA trial (Fig. 1, panel A).

Fifty-four/79 (68.3%) were able to continue the vandetanib treatment for at least 12 months and represent our group of long term treated patients. The median follow up was 41 months (mean: 54.8 months, range 12-140 months). As shown in Table 1, 40/54 (74.1%) were males and 14/54 (25.9%) were females. The median age at the time of starting therapy was 51.5 years (range 20-76 years); the median age at the time of the MTC diagnosis was 45 years (range 13-73). The mean time elapsing from the diagnosis and the start of therapy was 5.4 years. The majority of patients (88.9%) had a metastatic disease (stage IVC) when vandetanib was started. Among them, 32/54 (59.3%) started the therapy for progressive disease, according to RECIST while 22/54 (40.7%) started vandetanib for symptomatic metastatic disease. The median PFS of this subgroup was 87 months (95% CI: 38-91 months) (Fig. 1, panel B) that is significantly longer than that of the entire group.

The estimated ORR of this subgroup at the first morphological evaluation after 3 months of treatment was 26.0%. The best response was observed after 3 months of treatment in 50/54 (92.6%) patients. About the other 4 patients the best response was after 6 months in 3 patients and after 9 months in 1 patient (Fig. 2).

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Twenty-five/79 patients were treated with vandetanib for less than 12 months. All these patients discontinued vandetanib before 12 months of treatment due to either PD (n= 9) or an important adverse event (n=7) or patient death (n=6) or consent withdrawal (n=3) for the deterioration of their quality of life and these 25 patients represent our group of short term treated patients. As shown in Table 1, 16/25 (64.0%) were males and 9/25 (36.0%) were females. The median age at the time of starting therapy was 61 years (range 24-79 years); the median age at the time of the MTC diagnosis was 55 years (range 22-79). The mean time elapsing from the diagnosis and the start of therapy was 6 years. Among them, 17/25 (68.0%) were enrolled for progressive disease, according to RECIST while only 8/25 (32.0%) were enrolled for symptomatic metastatic disease (Table 1).

The *RET* genetic screening of the 54 long term treated patients showed that 8/54 (14.8%) and 46/54 (85.2%) cases were hereditary and sporadic, respectively. The study of *RET* somatic mutations was performed in a total of 43/46 (93.5%) sporadic cases and it was found that 41/43 (95.3%) patients were carrying a *RET* somatic mutation (Table 1). Among the 25 short term treated patients, *RET* screening showed that 4/25 (16.0%) and 21/25 (84.0%) cases were hereditary and sporadic, respectively. The study of *RET* somatic mutations was performed in a total of 17/21 (80.9%) sporadic cases and it was observed that 14/17 (82.3%) patients were positive for a *RET* somatic mutation (Table 1). Moreover, it was observed that the 3 out of 4 (75%) patients with *RET* Val804Met mutation, that is known to be associated with "in vitro" resistance to vandetanib (Carlomagno, et al. 2004), belonged to the long term treated patients and in particular two of them showed a PR and one showed a SD. Their specific median follow up was 22 months (range 12-66 months).

Efficacy of vandetanib in the long term treated patients

At the first CT scan evaluation, performed after 3 months of treatment, 14/54 (26.0%) patients had a partial response (PR), 39/54 (72.2%) patients showed a stable disease (SD); 1/54

(1.8%) showed a progressive disease (PD). In the longest term outcome, considering the last CT scan performed at the data cut-off, during the treatment, 29/54 (53.7%) showed a SD and 25/54 (46.3%) patients showed a progressive disease (PD) while any patient had a PR (Fig. 3). The duration and the type of long term response in each patient of this group of long term treated patients are shown in Fig. 4: after an initial response to treatment, either PR or SD, 24/54 (44.4%) patients started to progress (i.e., escape's phenomenon) after a median follow up of 25.5 months (range 12-91 months). Twenty-two/54 (40.7%) patients are still under therapy after a median follow up of 88 months (range 17-140 months) and the disease is still stable in 20/22 (90.9 %) of these patients. Two/22 (9.1%) patients still treated with vandetanib showed a PD at last CT scan. In one patient the progression was limited to liver metastases and these were treated with transarterial chemoembolisation (TACE) while the other patient showed the appearance of a brain micrometastasis that did not require a treatment but only radiological follow up. Eighteen/54 (33.3%) patients of this group of long term treated patients died during follow up after a median time survival of 25 months (mean 46.6 months, range 12-136 months).

During the follow up of this group we also observed a reduction in the Ct levels (median Ct at screening: 1820.5 pg/ml vs median Ct at data cut-off: 1112.5 pg/ml). At variance, CEA levels showed a tendency to a slight increase (median CEA at screening: 74 ng/ml vs median CEA at data cut-off: 98 ng/ml).

Safety and tolerability in the long and short term treated patients

The common AEs observed during the treatment are summarized in Table 2. As far as the group of long term treated patients was concerned, with the exception of hypothyroidism that was present in 100% of patients, any symptom was present in more than 48% of cases, being the cutaneous rash the most prevalent. In this group, seven patients required to discontinue the treatment because of a very severe AE (i.e., heart attack, stroke, orbital edema, hypertension, weight loss, asthenia, creatinine increase). One patient developed a QTc-prolongation but there was no

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reports of torsades de pointes. Other common AEs that were present in ≥20% of patients were diarrhea (39%), asthenia (35%), hypertension (28%) and nausea/anorexia (20%) (Table 2).

As far as the group of short term treated patients was concerned, with the exception of hypothyroidism that was present in 92%, the most prevalent AE was the cutaneous rash also in this group but its prevalence was of 28%. Also in this group, seven patients required to discontinue the treatment because of a very severe AE (i.e., creatinine increased, anorexia, neuropathy, weight loss, asthenia, diarrhea). Two patients developed a QTc-prolongation but there was no reports of torsades de pointes. Other common AEs that were present in ≥20% of patients were hypertension (24%), asthenia (20%) and nausea/anorexia (20%) (Table 2).

By comparing the AEs in the two groups of patients we could observe that they were in general much more prevalent in the group of long term treated patients with the hypothyroidism and the diarrhea that were significantly more prevalent ($p< 0.05$) in the long term treated group while, at variance, the weight loss and neuropathy were present only in 5.5% and 3.7% of them while they reached the prevalence of 16% and 8.0% in the short term treated patients (p= ns).

Predictors of longer responses to vandetanib

As shown in Table 1, the comparison of different clinical, epidemiological and pathological features between the two groups of short and long term treated patients did not show any statistically significant difference with the exception that the short term treated patients have been more frequently previously treated with other TKI ($p=0.04$). However, looking carefully at the results we could observe that the median age at diagnosis (55 vs 45 years, $p=0.08$) and at screening (61 vs 51.5 years, $p=0.054$) was greater in short term treated patients than in long term treated patients (Table 1) with a mean of 10 years of difference. A greater frequency of liver (72% vs 66.7%, $p=0.8$), lung (60% vs 51.9%, $p=0.63$) and in particular bone metastases (32% vs 18.5%, $p=0.25$) was found in short term treated patients with respect to long term treated patients.

Otherwise, the long term treated patients showed a greater frequency of lymph node metastases than short term treated patients (98.1% vs 88.0%, p=0.09) (Table 1).

Other comparisons showed that a greater number of short term treated patients were previously treated with re-surgery (68% vs 46.3%, p=0.09), chemiotherapy (24% vs 7.4%, p=0.06), radiotherapy (20% vs 20.4%, p=1), tyrosine kinase inhibitors (28% vs 9.3%, p=0.04) or other treatments (16% vs 5.6%, p=0.2) with respect to long term treated patients (Table 1).

A higher percentage of short term treated patients (68.0%) respect to long term treated patients (59.3%) were enrolled for the progression of the disease, according to RECIST (Table 1), although this difference did not reach the statistical significance (p=0.62).

RET somatic mutations were highly frequent in both groups and their distribution was similar in the short and long term treated patients and the *RET* Met918Thr mutation was the most, but equally, represented in both groups (Table 1).

No difference were observed between these two groups neither when the ECOG performance status nor when the tumor burden were compared.

Predictors of the better outcome in the long term treated patients group

We analyzed the predictors of the outcome of patients looking at both the radiological response (PR, SD and PD) and clinical response (disappearance of the initial symptoms associated to SD), at univariate analysis.

Sex and tumor stage did not correlate with the outcome of the long term treated MTC patients to vandetanib while patients with age $\leq$ 45 years at screening showed a better outcome than patients with age >45 years at screening (p=0.01) (Table 3). A positive correlation was also observed between the absence of RECIST progressive disease at screening (i.e., patients enrolled for symptoms) and response to treatment (p<0.0001) (Table 3) as well as between the presence of AEs, any type, and a better response to treatment (p=0.02) (Table 3). Unfortunately, the relatively low number of cases did not allow to perform a multivariate analysis.

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356 At variance, all the other examined features, including the presence of *RET* mutations, did
357 not show any predictive value of the outcome of the disease in this subgroup of long term treated
358 patients (Table 3).
359
360 **The subgroup of very long term treated patients**
361 Twenty-four patients belonging to the long term treated patients were able to continue the
362 vandetanib treatment for ≥ 48 months and represent our group of "very long" term treated patients.
363 The median follow up was 89.5 months (mean: 93.1 months, range 52-140 months). As shown in
364 Table 4, 19/24 (79.2%) were males and 5/24 (20.8%) were females. The median age at the time of
365 starting therapy was 47.5 years (range 20-75 years); the median age at the time of the MTC
366 diagnosis was 43 years (range 13-71). The mean time elapsing from the diagnosis and the start of
367 therapy was 5.7 years. Only 11/24 (45.8%) very long term treated patients started the therapy for
368 progressive disease, according to RECIST. *RET* M918T mutation was the most prevalent as in the
369 other groups. The majority of patients (87.5%) had a metastatic disease (stage IVC) when
370 vandetanib was started but the largest metastases were not bigger than 5 cm in 23/24 patients and
371 the ECOG performance status was 0 in 22/24 patients. The mean PFS of this subgroup was 120
372 months (95% CI: 105-135 months).
373 When we compared this subgroup of very long term treated patients (≥ 48 months) with the
374 short term treated patients we found that the very long term treated patients were significantly
375 younger than short term treated patients both at diagnosis of MTC and at screening for vandetanib
376 treatment ($p=0.03$) and ($p=0.02$), respectively. Moreover, they had a statistically significant better
377 ECOG performance status at screening than short term treated patients ($p<0.001$).
378 Also in this subgroup we then analyzed the predictors of the outcome of patients looking at
379 both the radiological response (PR, SD and PD) and clinical response (disappearance of initial
380 symptoms associated to SD), at univariate analysis (Table 5). Sex, tumor stage and age at screening
381 did not correlate with the outcome of the very long term treated MTC patients to vandetanib.

Moreover, the presence of AEs, any type, did not correlate with a better outcome (p=0.6) as well as the presence of liver metastases (p=0.9), lung metastases (p=0.6), lymph nodes metastases (p=0.6), bone metastases (p=0.4) and the presence of *RET* mutations (p=0.6). At variance, at the data cut off of the present study, we found a statistically significant correlation of a better outcome (SD *vs* PD) with both the enrollment for symptoms (p=0.006) and the ECOG performance status (p=0.004).

DISCUSSION

Medullary thyroid cancer is a relatively indolent tumor and the use of systemic therapies is limited to patients with progressive or symptomatic locally advanced or metastatic disease (Elisei and Matrone 2018). Cytotoxic chemotherapy, external beam radiation, hormone therapy, cytokines and radioiodine are associated with high toxicity and low efficacy (Brierley 2011; De Besi, et al. 1991; Droz et al. 1984). A new option of treatment is surely represented by TKIs (Valerio, et al. 2017; Viola, et al. 2016). Vandetanib is a TKI with antitumor activity against *RET*, VEGFR and EGFR signaling pathways that has been demonstrated to be able to prolong the PFS in patients with locally advanced or metastatic MTC in a phase III clinical trial (i.e., ZETA trial) (Wells et al. 2012).

In this study we have analyzed the presence of epidemiological, clinical, pathological and genetic factors that could predict either the duration of the response to vandetanib and the better outcome in the patients with locally advanced or metastatic MTC referred to our Center and treated with vandetanib, within AstraZeneca clinical trials or with vandetanib after its approval by FDA and EMA.

When we compared the short and the long term treated patients we did not find any epidemiological factors that could predict a longer response to vandetanib. However, a mean of 10 years younger age was observed in the group of longer with respect to shorter term treated patients, both at the time of the diagnosis and at the time of starting therapy and when we concentrated our attention on the subgroup of very long term treated patients the younger age, both at the time of MTC diagnosis and at the time of starting therapy, became statistically significant (p=0.03 and

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p=0.02, respectively). This finding suggests that, independently from the type of metastatic localization, that were not predictive of the response to the therapy in any group, the youngest patients have a greater probability to continue the therapy for more than 12 months and even for more than 48 months. Although in the ZETA trial several subgroup analyses have been performed (Wells et al. 2012), age was not taken into consideration and we cannot confirm our observation. However, it is plausible that younger patients, despite the same tumor burden than older, can better adapt to the therapy and its AEs thus allowing to continue the therapy for a longer period of time. The question of whether an earlier treatment, when patients are still young, could improve the outcome of the disease needs to be clarified.

Another interesting finding was that a bigger number of cases treated for symptoms were present in the subgroup of long term treated patients. Although in the ZETA trial many patients were enrolled for symptoms and randomized either in the drug or in the placebo arm, so far, it has been unrevealed if there was a difference in their PFS (Wells et al. 2012). As matter of fact, the real benefit to start the therapy only at the time when the disease is progressing according to RECIST, as requested in clinical trials (Elisei, et al. 2013; Wells et al. 2012), is not proven. The major reason for this indication derived from the evidence that in many cases, even if advanced and multimetastatic, MTC growth is very slow and patients have a good quality of life that could be affected by the use of the drug (Bergholm, et al. 1997; Elisei and Matrone 2018; Hadoux, et al. 2016) and for this reason there is a tendency to delay as much as possible the start of the drug. Moreover, vandetanib and TKI in general, have a cytostatic, more than a cytotoxic, action thus they should be used to block the tumoral growth and if there is no growth there is no rationale to use the drug (Viola et al. 2016). However, vandetanib action is likely more than a cytostatic action as demonstrated by the cases with hypercortisolism due to ectopic ACTH and successfully treated with the drug (Baudry, et al. 2013; Pitoia, et al. 2015). The advantage to use vandetanib in patients with symptomatic disease, even if not progressing, seems to be evident especially when considering that the better outcome of the very long responders significantly (p=0.006) correlated with the enrolment for symptoms.

A positive correlation was found in our series between the presence of AEs and response to treatment in the long term treated patients ($p< 0.0001$). This finding is new for vandetanib but is not new for other TKI. There are reports about sorafenib treatment in hepatocellular carcinoma that show that the development of AEs could be a marker of treatment efficacy (Granito, et al. 2016; Lee, et al. 2019). This observation should induce to strive to continue the therapy despite the AEs perhaps by reducing the daily dose and find a compromise between the continuation of the therapy and the tolerability of the AEs.

As far as the PFS of our patients was concerned, we found that when we considered all patients together this parameters was slightly longer than that of patients treated with the drug in the ZETA trial (47 vs 30.5 months), but, if we considered only the subgroup of those who were able to maintain the therapy for at least 12 months, the PFS almost doubled from 47 to 87 months and became 120 months in the very long term treated patients. Moreover, the best response ORR in the majority of patients of the long term treated patients was at 3 months. These two findings suggest that the disease of long term treated patients, after a good response in terms of maximum shrinkage within the first 3 months of therapy, thereafter become stable for a long time, even up to more than 7 years. According to these results, the escape phenomenon can arrive much later than expected or even never, and patients can find a good balance between the daily dose and the block of the growing disease. These hypotheses can also fit with the evidence that serum Ct values, that is mainly related to the cancer activity, declined during the follow up while CEA, that is more related to the tumor burden, remained stable over the years. Moreover, there are a few studies that show an increased value of tumor markers in patients who have morphologic response of the disease. These results suggest that the mechanisms that control tumor growth and markers secretion, sometimes can present fluctuations during follow-up and can be dissociated in patients treated with TKI (Hajje, et al. 2013; Schlumberger, et al. 2009).

In our patients, *RET* mutations gene were presents in 100% of patients with hereditary form and in almost 90% of sporadic cases. Among all *RET* mutations, the somatic *RET*

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Met918Thr mutation was the most represented and it is known that this mutation is associated with a more aggressive phenotypes of MTC (Elisei, et al. 2008; Mian, et al. 2011). In our series, the presence of *RET* mutations was not a predictor of longer and/or better response to vandetanib, as already demonstrated in other studies performed with either vandetanib (Wells et al. 2010; Wells et al. 2012) or cabozantinib (Elisei et al. 2013). However, the very high prevalence of *RET* mutated cases, as expected in advanced cases (Romei, et al. 2016) and the correspondent low prevalence of non mutated cases probably would require a much bigger number of cases to reach the statistical power. It was also demonstrated in "in vitro" studies that the presence of *RET* Val804Met mutation determines a selective resistance to various TKIs including vandetanib (Carlomagno et al. 2004). This mutation requires an increase of vandetanib concentration necessary to obtain the 50% of activity receptor reduction (IC50 = 5 uM). In our study, it was observed a full resistance to vandetanib in only one patient who presented the *RET* V804M mutation. This patient did not respond to vandetanib and discontinued the treatment after only four months. At variance, the other three patients, who were carrying this *RET* mutation, showed an initial SD in one case and a PR in two cases. The possible explanation of this significant response, despite the partial resistance induced by V804M mutation, is that the drug is a multikinase inhibitor and it can likely act also through mechanisms of action independently from *RET* inhibition such as VEGFR inhibition (Ciardiello, et al. 2004). Thus, also in the presence of this *RET* mutation, it is appropriate to start treatment with vandetanib, especially if no other therapeutic options are available.

It is worth to note that in our study almost 100% of patients showed an increase of TSH that require the increase of LT4 daily dose. This evidence suggests that TSH must be monitored very frequently especially in the beginning of the treatment to allow a prompt correction of hypothyroidism that can be part of the very common symptoms of asthenia and fatigue. The majority of patients experienced at least one of the other AEs, but AEs were generally manageable with dose interruption or reduction associated with symptomatic therapies. In our series, the

permanent discontinuation of vandetanib was needed only in 7/54 (13%) of patients and this data are in agreement with the data shown in the ZETA study (Wells et al. 2012). On this regard, it is important to say that over the years the ability of doctors and nurses in managing the AEs improved a lot and they are now able to better manage these AEs with the complicity of the patient who is invited to refer all the AEs as soon as possible.

In conclusion, with this study we found that: a) the median PFS of our group was significantly longer than that of ZETA trial; b) the younger age of patients both at the time of diagnosis and at the starting time of therapy can predict a longer and more durable response to vandetanib; c) the same observation was for patients with metastatic MTC but treated with vandetanib for symptoms; d) the ECOG performance status at the time of starting therapy was significantly lower in the very long term treated patients; e) the development of at least one AE is also correlated with a better outcome of long term treated patients. No other clinical or pathological features, including the distribution of *RET* mutations, were different among the groups. Further bigger and possibly multicentric studies to verify if an early treatment with vandetanib, when patients are younger, still with a good ECOG performance status and with a symptomatic disease, not necessarily progressing for RECIST, should better clarify the right time to start the drug and obtain a better outcome and, hopefully, a long life PFS.

Declaration of interest

E.R. has been consultant for Astrazeneca and Sanofi-Genzyme for the vandetanib development. However, these commitments did not have any influence on this study that has been developed independently and there was no conflict of interest in writing the paper. All the other authors declare to have not conflict of interest.

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514

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518 **Legend of figures**

519

520 **Fig. 1:** panel A) Kaplan-Meier curves of progression-free survival of all our patients overlying
521 those of ZETA study; panel B) Kaplan-Meier curves of progression-free survival of the group of
522 long term treated patients overlying those of ZETA study. Progression was defined according to
523 RECIST criteria. Patients who did not show progression disease were included in the analysis until
524 they stopped the treatment.

525

526 **Fig. 2:** Morphological response (RECIST) at first CT scan after 3 months of vandetanib treatment:
527 these responses corresponded to the best objective response (ORR) in all cases but 4 cases
528 (indicated with dots) that reached the best ORR after 6 or 9 months.

529

530 **Fig. 3:** Vandetanib treatment efficacy per RECIST: comparison of results after 3 months and at the
531 data cut off.

532

533 **Fig. 4:** Morphological response to vandetanib in the long-term follow up: each bar represents one
534 patient with his/her own specific response to treatment (PR and/or SD) and progression of the
535 disease (PD), if happened, during all period of treatment and the indication if they are still under
536 therapy.

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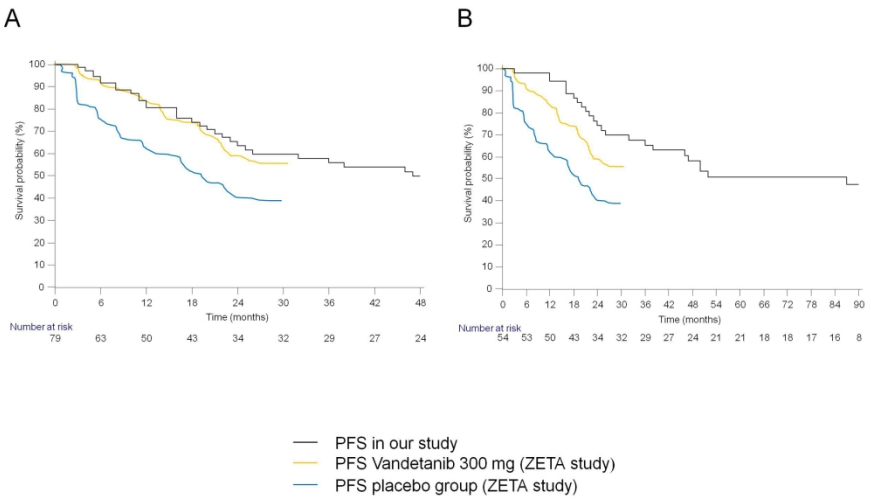

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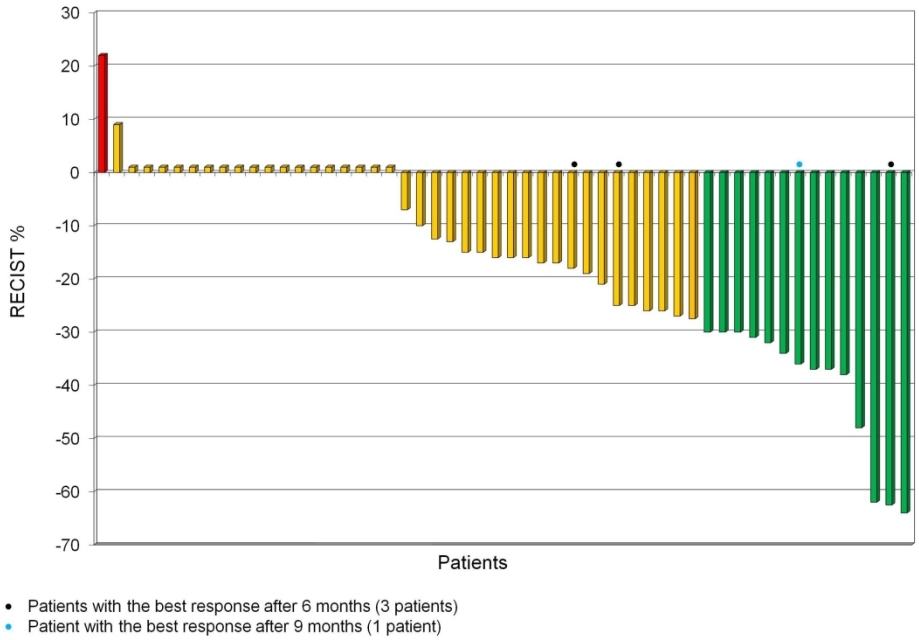

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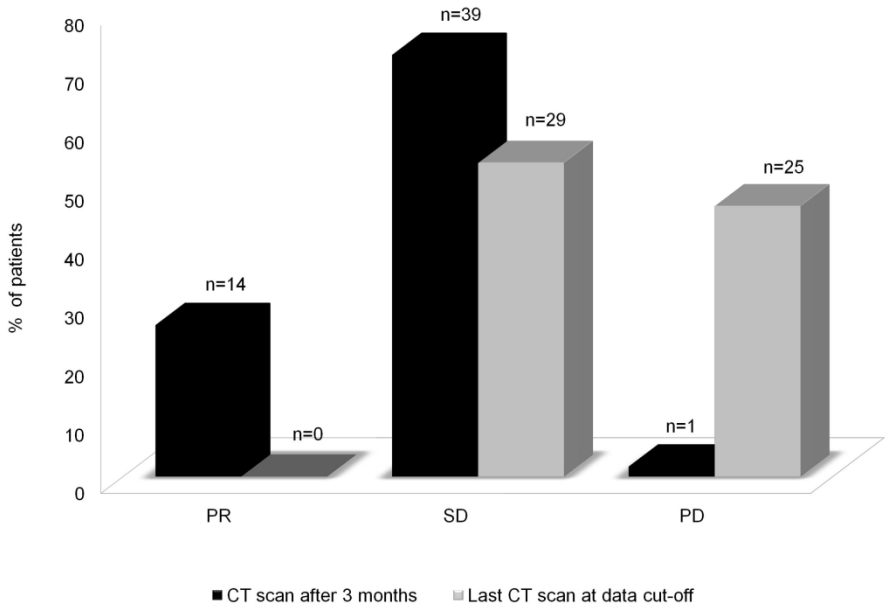

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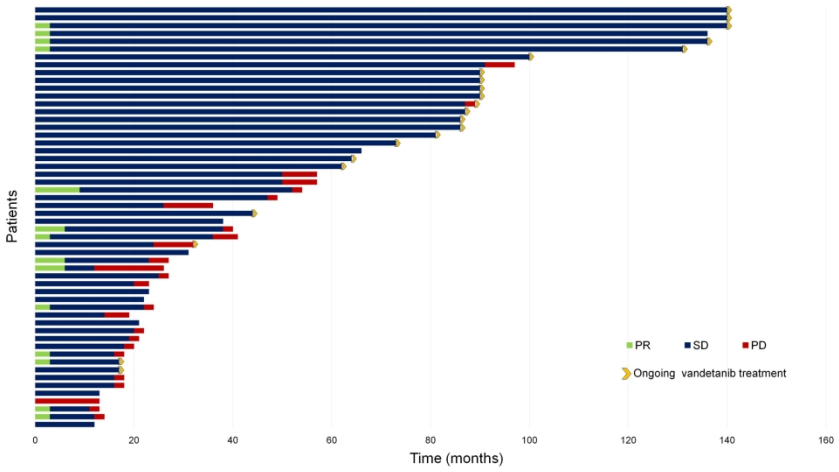

Fig. 4: Morphological response to vandetanib in the long-term follow up: each bar represents one patient with his/her own specific response to treatment (PR and/or SD) and progression of the disease (PD), if happened, during all period of treatment and the indication if they are still under therapy.

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Table 1: Epidemiological, pathological, clinical and genetic features of patients, either long or short term treated with vandetanib

	Long term treated pts (n=54)		Short term treated pts (n=25)		p-value#
Characteristics	**No.**	**%**	**No.**	**%**	
Sex					
Male	40	74.1	16	64.0	0.43
Female	14	25.9	9	36.0	
Age at diagnosis, years					
Mean	46.3		52.2		0.08
Median	45		55		
Range	13-73		22-79		
Age at screening, years					
Mean	51.9		58.3		0.054
Median	51.5		61.0		
Range	20-76		24-79		
Extent of disease at screening					
Locally advanced	6	11.1	3	12.0	1
Distant metastases	48	88.9	22	88.0	
Stage at screening					
III	1	1.8	1	4.0	0.84
IVA	5	9.3	2	8.0	
IVC	48	88.9	22	88.0	
Progressive disease at screening	32	59.3	17	68.0	0.62
Sites of metastases					
Lymph nodes	53	98.1	22	88.0	0.09
Liver	36	66.7	18	72.0	0.8
Lung	28	51.9	15	60.0	0.63
Bone	10	18.5	8	32.0	0.25
Prior therapy for thyroid cancer					
Surgery (Thyroidectomy)	54	100	25	100	1
Re-Surgery	25	46.3	17	68.0	0.09
Radiotherapy	11	20.4	5	20.0	1
Chemiotherapy	4	7.4	6	24.0	0.06
Tyrosine kinase inhibitors	5	9.3	7	28.0	**0.04**
Other	3	5.6	4	16.0	0.2
Sporadic Medullary Thyroid Cancer	46	85.2	21	84.0	1
RET codon mutation in sporadic medullary thyroid cancer*					
620	1	2.4	0	0	1
634	0	0	1	5.9	1
804	2	4.6	0	0	1
883	1	2.4	0	0	1
891	1	2.4	0	0	1
918	32	74.4	12	70.6	0.47
Del ex 11	2	4.6	0	0	1
Del ex 15	2	4.6	1	5.9	1
Negative	2	4.6	3	17.6	1
RET codon mutation in hereditary medullary thyroid cancer*					
618	0	0	1	25.0	1
634	3	37.5	2	50.0	1
709	1	12.5	0	0	1
768	1	12.5	0	0	1
871	1	12.5	0	0	1
918	1	12.5	0	0	1
804	1	12.5	1	25.0	1
ECOG at screening					
0	43	79.6	9	36.0	
1	8	14.8	10	40.0	0.40
2	3	5.6	6	24.0	
The largest tumor metastasis size (cm) at screening					
0-5	49	90.7	20	80.0	0.15
5-10	4	7.4	4	16.0	
>10	1	1.9	1	4.0	

#Statistical significance was calculated via chi-squared test, Fisher's exact test, Student's t-test or Mann-Whitney U test as appropriate.

* In 3 cases in long term treated group and in 4 cases in short term treated group data are not available.

^ Frequency were rounded to the nearest decimal digit.

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Table 2: Common Adverse Events related to vandetanib treatment in long and short term treated patients

	Long term treated patients by Event Grade						Short term treated patients by Event Grade					
	Total		Grades 1 or 2*		Grades 3 or 4*		Total		Grades 1 or 2*		Grades 3 or 4*	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Hypothyroidism^	54/54	100	54	100	0	0	23/25	92.0	23	100	0	0
Rash	26/54	48.1	22	84.6	4	15.4	7/25	28.0	5	71.4	2	28.6
Diarrhea^	21/54	38.9	20	95.2	1	4.8	3/25	12.0	2	66.7	1	33.3
Asthenia	19/54	35.2	17	89.5	2	10.5	5/25	20.0	4	80.0	1	20.0
Hypertension	15/54	27.8	11	73.3	4	26.7	6/25	24.0	6	100	0	0
Biochemical alteration*	12/54	22.2	12	100	0	0	3/25	12.0	3	100	0	0
Dysgeusia	7/54	12.9	7	100	0	0	2/25	8.0	2	100	0	0
Corneal alterations	3/54	5.5	3	100	0	0	2/25	8.0	2	100	0	0
Oral mycosis	2/54	3.7	2	100	0	0	0/25	0	0	0	0	0
Neuropathy	2/54	3.7	2	100	0	0	2/25	8.0	0	0	2	100
Headache	2/54	3.7	1	50.0	1	50.0	1/25	4.0	1	100	0	0
Weight loss	3/54	5.5	2	66.7	1	33.3	4/25	16.0	4	100	0	0
Nausea/Anorexia	11/54	20.3	7	63.6	4	36.4	5/25	20.0	2	40	3	60
Pancreatitis	1/54	1.8	0	0	1	100	1/25	4.0	0	0	1	100
Orbital edema	1/54	1.8	0	0	1	100	0/25	0	0	0	0	0
QTc prolungation	1/54	1.8	1	100	0	0	2/25	8.0	2	100	0	0
Heart attack	1/54	1.8	0	0	1	100	0/25	0	0	0	0	0
Embolism	1/54	1.8	0	0	1	100	0/25	0	0	0	0	0
TIA	1/54	1.8	0	0	1	100	0/25	0	0	0	0	0

* In long term treated patients in 4 cases creatinine increased; in 2 cases proteinuria increased; in 4 cases AST, ALT or γGT alterations; in 3 cases electrolytes alterations; in 1 case thrombocytopenia.

* In short term treated patients in 2 cases creatinine increased and in 1 case CPK increased.

^ $p < 0.05$ between long and short term treated patients by chi-squared test or Fisher's exact test as appropriate.

Table 3: Correlation between epidemiological, pathological, clinical and genetic features with the outcome of the long term treated patients at the data cut-off of this study

No. of patients 54	PD	SD	p-value[#]
M **F**	19 6	21 8	0.7
Age at diagnosis: **≤ 45** **> 45**	 10 15	 18 11	0.1
Age at screening: **≤ 45** **> 45**	 4 21	 14 15	**0.01**
Stage at diagnosis°: **II** **III** **IVA** **IVB** **IVC**	 0 7 8 0 8	 1 6 14 1 4	0.4
Stage at screening: **III** **IVA** **IVC**	 0 2 23	 1 3 25	0.6
Progressive disease at screening: **Yes** **No**	 22 3	 10 19	**<0.0001**
RET gene mutation*	24/51	25/51	0.1
Liver metastases	18	18	0.4
Lung metastases	12	16	0.6
Lymph node metastases	25	28	0.3
Bone metastases	6	4	0.3
Adverse Events	19	28	**0.02**
ECOG at screening **0** **1** **2**	 17 5 3	 26 3 0	0.08
The largest tumor metastasis size (cm) at screenig **0-5** **5-10** **>10**	 20 4 1	 29 0 0	0.05

[#] Statistical significance was calculated via chi-squared test or Fisher's exact test as appropriate.
°In 5 cases the data were not available
*In 3 cases the data were not available

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Table 4 : Epidemiological, pathological, clinical and genetic features of very long term treated patients

Very long term treated pts (n=24)		
Characteristics	**No.**	**%**
Sex		
Male	19	79.2
Female	5	20.8
Age at diagnosis, years		
Mean	43.3	
Median	43	
Range	13-71	
Age at screening, years		
Mean	48.9	
Median	47.5	
Range	20-75	
Extent of disease at screening		
Locally advanced	3	12.5
Distant metastases	21	87.5
Stage at screening		
III	1	4.2
IVA	2	8.3
IVC	21	87.5
Progressive disease at screening	11	45.8
Sites of metastases		
Lymph nodes	23	95.8
Liver	15	62.5
Lung	12	50.0
Bone	2	8.3
Prior therapy for thyroid cancer		
Surgery (Thyroidectomy)	24	100
Re-Surgery	10	41.7
Radiotherapy	2	8.3
Chemiotherapy	1	4.2
Tyrosine kinase inhibitors	0	0
Other	1	4.2
Sporadic Medullary Thyroid Cancer	16	66.7
RET codon mutation in sporadic medullary thyroid cancer*		
883	1	4.3
918	12	52.2
Del ex 15	1	4.3
Negative	1	4.3
RET codon mutation in hereditary medullary thyroid cancer*		
634	3	13
709	1	4.3
768	1	4.3
871	1	4.3
918	1	4.3
804	1	4.3
ECOG at screening		
0	22	91.7
1	2	8.3
2	0	0
The largest metastasis size (cm) at screening		
0-5	23	95.8
5-10	1	4.2
>10	0	0

* In 1 case data is not available.

^ Frequency were rounded to the nearest decimal digit.

Table 5: Correlation between the epidemiological, pathological, clinical and genetic features with the outcome of the very long term treated patients, at the data cut-off of this study.

No. of patients 24	PD	SD	p-value#
M	4	15	0.9
F	1	4	
Age at diagnosis:			
≤ 45	2	12	0.3
>45	3	7	
Age at screening:			
≤ 45	1	10	0.2
> 45	4	9	
Stage at diagnosis°:			
II	0	1	0.9
III	1	3	
IVA	2	10	
IVB	0	1	
IVC	1	3	
Stage at screening:			
III	0	1	0.5
IVA	1	1	
IVC	4	17	
Progressive disease at screening:			
Yes	5	6	**0.006**
No	0	13	
RET gene mutation*	5/23	17/23	0.6
Liver metastases	3	12	0.9
Lung metastases	3	9	0.6
Lymph node metastases	5	18	0.6
Bone metastases	0	2	0.4
Adverse Events	5	18	0.6
ECOG at screening			
0	3	19	**0.004**
1	2	0	
2	0	0	
The largest tumor metastasis size (cm) at screening			
0-5	4	19	0.05
5-10	1	0	
>10	0	0	

Statistical significance was calculated via chi-squared test or Fisher's exact test as appropriate

°In 2 cases the data were not available

*In 1 case the data was not available