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RESEARCH LETTER

Preliminary data of VEGF-A and VEGFR-2 polymorphisms as predictive factors of radiological response and clinical outcome in iodine-refractory differentiated thyroid cancer treated with sorafenib

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Introduction

Tyrosine-kinase inhibitors (TKIs) have revolutioned the management of iodine-refractory differentiated thyroid cancer (DTC), previously considered as an orphan disease [1, 2]. Basing on results from the international phase III trial DECISION, which reported significant improvement of median progression-free survival (PFS) in the treatment group, as compared with placebo (10.8 vs. 5.8 months; HR 0.58, 95% CI 0.45–0.75, $p < 0.0001$) [3], sorafenib was the first TKI to be approved by the regulatory agencies. To date, sorafenib should still be considered as the first-line therapeutic option for progressive iodine-refractory DTC, as lenvatinib, another TKI showing higher efficacy in terms of both objective response and PFS in a recent phase III trial [4], proved to be active independently from previous TKIs administration. Considering that the DECISION cohort only included *naive* patients and therefore activity of sorafenib in TKIs pre-treated subjects is theoretically unmet, the best evidence-based approach is to preserve lenvatinib as salvage option after sorafenib failure. Importantly, a post-hoc analysis of the DECISION trial showed that the actual disease control rate obtained through sorafenib administration, with inclusion of partial response (PR) and stable disease (SD) lasting at least 6 months, was only 54.1%, meaning that nearly half of patients do not experience significant benefit from the treatment. This has been also confirmed by some studies reporting data from "real-life" clinical practice [5, 6]. Therefore, the identification of predictive factors of response is mandatory in order to avoid useless drug administration leading to toxicities and expensive costs for public health. Although use of sorafenib in iodine-refractory DTC was initially emphasized due to its inhibitory activity on both wild type and mutant BRAF [7], which represents the most frequent DTC-related molecular alteration [8], the exploratory biomarker analysis reported by the DECISION trial failed to demonstrate any predictive role for the BRAF mutation [3]. Nevertheless, it is important to consider that sorafenib anti-cancer effect relies not only on the inhibition of tumor cells proliferation through the RAF kinases blockage, but also on the inhibition of tumor vascularization through the blockage of angiogenesis controlling tyrosine-kinase receptors (VEGFR-2, VEGFR-3, PDGFR-β, and Ftl-3). This anti-angiogenic effect was even stronger, as compared with the anti-proliferative activity [9]. Angiogenesis, including that related to cancer, is mainly determined by genetic background, rather than environmental exposure [10]. Particularly, a set of single nucleotide polymorphisms (SNPs) of the VEGF-A and VEGFR-2 genes, representing the most important angiogenic regulators [11], have demonstrated functional

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implications through the modulation of gene expression/post-trascriptional regulation and ligand affinity, respectively [12, 13], and could theoretically represent predictors of response to anti-angiogenic therapies, such as TKIs. We present a preliminary single center study about the possible role of germline VEGF-A and VEGFR-2 SNPs in predicting objective response and clinical outcome in iodine-refractory DTC patients treated with sorafenib.

Patients and methods

After written informed consent, blood samples were obtained from consecutive DTC patients meeting criteria for iodine-refractory disease [14], who were subjected to sorafenib. Sorafenib was administered only in case of documented progressive disease (PD), as defined by RECIST [15]. Clinical, biochemical, and instrumental follow-up was performed according to the current international guidelines [14], starting from the beginning of the treatment. DNA was extracted and purified from peripheral blood according to the manufacturer protocol using a QIAamp tissue kit (Qiagen, Hilden, Germany). DNA concentration was determined by means of NanoDrop® (Wilmington, DE) ND-1000 Spectrophotometer and samples were diluted to 10 ng/µl. SNP genotyping was carried out according to the TaqMan® protocol (Applied biosystems StepOnePlus™). Basing on available evidence about functional implications (namely the possible impact on VEGF-A production/VEGFR-2 ligand affinity) [12, 13, 16–18] and preceded SNPs association studies addressing the correlation with cancer prognosis [19–27], 6 SNPs were selected: −2578 C>A (rs699947), −460 T>C (rs833061), +405 G>C (rs2010963), and +936 C>T (rs3025039) for the VEGF-A gene; +1192 C>T (rs2305948) and +1719 T>A (rs1870377) for the VEGFR-2 gene. Approval by the institutional review board of the University Federico II was obtained.

Statistical analysis

Chi-square test was applied for assessing Hardy–Weinberg equilibrium (HWE). The degree of linkage disequilibrium (LD) was calculated by coefficient of correlation of r^2. Each SNP was analyzed as a three-group categorical variable in accordance to the reference model (homozygous common variant versus heterozygous versus homozygous minor variant) and by grouping in accordance to the dominant (homozygous common variant versus heterozygous + homozygous minor variant) and recessive (homozygous common variant + heterozygous versus homozygous minor variant) models. In case of minor homozygous genotype frequency ≤10%, analyses were performed exclusively by means of dominant model. Analysis of RECIST response was performed by comparing the rate of PR between groups using the chi-square test. Odds ratios (OR) with 95% confidence interval (CI) were calculated. Survival analyses for PFS (defined as the length of time after beginning the treatment and the occurrence of RECIST progression or the death of the patient) were performed according to the Kaplan–Meier method, and the log-rank test was used to search for differences between groups. All tests were two sided, and results were considered as statistically significant for p-values less than 0.05.

Results

Seventeen patients were enrolled (baseline features and RECIST response were reported in Table 1). Median age was 58 years. All patients showed acceptable performance status with Eastern Cooperative Oncology Group (ECOG) ≤2. Cervical lymph nodes and chest (lung and/or mediastinic lymph nodes) were involved in all cases, whereas liver and bone metastases were present in 7 (41%) and 4 (24%) subjects, respectively. Median follow-up was 17 months. No patients died during follow-up. RECIST response was

Table 1 Baseline features and best RECIST response to sorafenib

N of patients	17
Gender M/F N(%)	4(24)/13(76)
Median age	58
Tumor histotype	*N* (%)
Papillary	6 (35)
Follicular	11 (65)
ECOG status	*N* (%)
0	4 (24)
1	7 (41)
2	6 (35)
3	0 (0)
4	0 (0)
Site of metastasis	*N* (%)
Cervical lymph nodes	17 (100)
Lung	14 (82)
Mediastinic lymph-nodes	8 (47)
Liver	7 (41)
Bone	4 (24)
Previous chemotherapy *N* (%)	0 (0)
External beam irradiation *N* (%)	5 (29)
Previous treatment with TK-inhibitors *N* (%)	2 (12)
Recist response *N* (%)	
PR	6 (35)
SD	8 (47)
PD	3 (18)

N number, % proportion, *PR* partial response, *SD* stable disease, *PD* progressive disease

PR in 6 (35%) patients, SD in 8 (47%) subjects, and PD in 3 cases (18%). Median PFS was 12 months. SNPs were successfully genotyped in all patients and did not deviate from HWE (genotypes frequencies and HWE results were reported in Supplemental File 1). Significant results were found for the 2 VEGF-A SNPs −2578 C>A and −460 T>C, and for the VEGFR-2 SNP +1719 T>A. Since the −2578 C>A and −460 T>C VEGF-A SNPs were in complete LD ($r^2 = 1$), we considered their combination as a unique genotype. Patients with the AA/CC genotype showed significantly higher rate of PR (75 vs. 15.4%; $p = 0.022$; OR 16.5 95% CI 1.08–250.18) (Fig. 1a) and significantly improved median PFS (25 vs. 10 months; $p = 0.006$) (Fig. 1c), as compared with the CC/TT + CA/TC group. Regarding the VEGFR-2 SNP +1719 T>A, patients with the AA + AT genotype showed significantly higher rate of PR (57.1% vs. 10%; $p = 0.036$; OR 12 95% CI 0.93–153.89) (Fig. 1b) and significantly improved median PFS (22 vs. 7 months; $p < 0.001$) (Fig. 1d), as compared with the TT group.

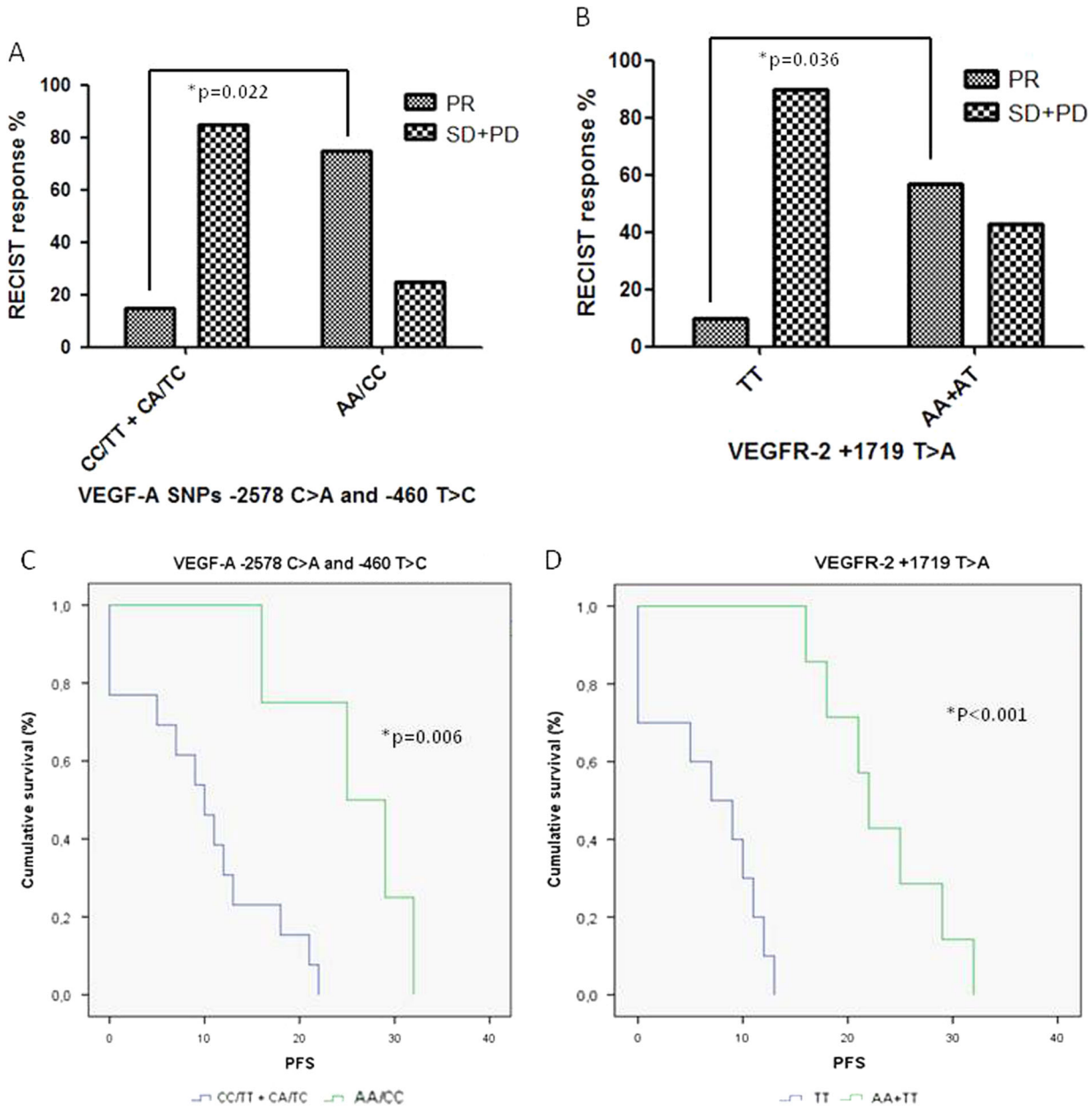

Fig. 1 Comparison of best RECIST response to sorafenib between different genotypes for the VEGF-A SNPs −2578 C>A and −460 T>C **a** and the VEGFR-2 SNP +1719 T>A **b** Kaplan-Meier analysis of PFS according to the VEGF-A SNPs −2578 C>A and −460 T>C **c** and the VEGFR-2 SNP +1719 T>A **d** *PR* partial response, *SD* stable disease, *PD* progressive disease, *PFS* progression-free survival.*Statistically significant difference

Discussion

The role of the VEGF-pathway in DTC tumorigenesis has been widely demonstrated by studies of VEGF-A expression in tumor tissue and its correlation with clinical outcome [28]. To date, VEGF-related markers have demonstrated some value as prognostic factors in DTC [29], but evidence about a possible application as predictive tools of response to anti-angiogenic therapies is almost missing. Indeed, only one study has associated baseline levels of VEGF-A and changes of soluble VEGFR-2 to response to the TKI motesanib in a cohort of patients with advanced thyroid cancer including not only DTC, but also the medullary histotype [30]. SNPs of the VEGF-pathway having demonstrated/putative functional impact may be fully considered as VEGF-related markers. Recently, we showed that the germline VEGF-A SNPs −2578 C>A and −460 T>C predict disease recurrence in a large cohort of low-intermediate risk DTC patients [31]. The present study was the first to report some insights about possible use of germline SNPs of the VEGF-pathway as predictors of response to sorafenib, the firstly approved and mostly used TKI in iodine-refractory DTC. In our analysis, the AA/CC genotype of the VEGF-A SNPs −2578 C>A and −460 T>C, which were in complete LD, and the AA+AT genotype of the VEGFR-2 SNP +1719 T>A proved statistically significant association with both the achievement of PR and improved PFS. The −2578 C>A and −460 T>C are neighbor SNPs located in the promoter of the VEGF-A gene [17]. In vitro experiments demonstrated that the AA genotype of −2578 C>A was associated to decreased VEGF-A production [12], likely due to reduced gene expression, whereas no functional studies exist about the −460 T>C SNP. The VEGFR-2 SNP +1719 T>A is located in the coding region corresponding to the extracellular domain of the receptor [32]. Functional research discovered that the A-allele was associated with decreased binding efficiency to the VEGF-A [13]. Given that genotypes showing predictive impact are associated to weakened angiogenic signal, it is conceivable that sorafenib administration is more effective in those patients with a less efficient angiogenic machinery, as determined by genetic background. Our report is strongly limited by the small sample size, as SNPs association studies requires larger series [33]. Therefore, results have to be considered as preliminary and are aimed to stimulate multicenter studies, which represent the only way of achieving large cohorts of iodine-refractory DTC.

Compliance with Ethical Standards

Conflict of interest All the authors declares that they have no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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