

CLINICAL STUDY

Beneficial effects of sorafenib on tumor progression, but not on radioiodine uptake, in patients with differentiated thyroid carcinoma

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Abstract

Objective: Treatment options for patients with radioactive iodine (RaI) refractory metastases of differentiated thyroid carcinoma (DTC) are limited. We studied the effects of the multitarget tyrosine kinase inhibitor sorafenib on the reinduction of RaI uptake and tumor progression.

Design: Open, single center, single arm 26-week prospective phase II study with open-ended extension.

Methods: We treated 31 patients with progressive metastatic or locally advanced RaI refractory DTC with sorafenib 400 mg b.i.d. The primary endpoint was reinduction of RaI uptake at 26 weeks. Additional endpoints were the radiological response and the influence of bone metastases.

Results: At 26 weeks of sorafenib therapy, no reinduction of RaI uptake at metastatic sites was observed, but 19 patients (59%) had a clinical beneficial response, eight of whom had a partial response (25%) and 11 had stable disease (34%). Seven patients had progressive disease (22%). Sorafenib was significantly less effective in patients with bone metastases. The estimated median progression free survival was 58 weeks (95% confidence interval, CI, 47–68). In general, thyroglobulin (Tg) response (both unstimulated and TSH stimulated) reflected radiological responses. The median time of the nadir of Tg levels was 3 months. Responses were not influenced by histological subtype, mutational status or other variables. No unusual side effects were observed.

Conclusions: Sorafenib has a beneficial effect on tumor progression in patients with metastatic DTC, but was less effective in patients with bone metastases. Diagnostic whole body scintigraphy did not reveal an effect of sorafenib on the reinduction of RaI uptake.

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Introduction

The prognosis of differentiated thyroid carcinoma (DTC) in general is favorable due to the efficacy of the combined treatment of surgery and radioactive iodine (RaI) and the biological behavior of the tumor (1, 2). However, ~50% of patients with distant metastases of DTC die within 10 years of diagnosis (3). Although the role of RaI in recurrent or metastatic thyroid cancer is beyond dispute (4–6), the efficacy of this therapy is hampered by the decreased expression of the sodium iodide symporter (NIS) in DTC during the process of dedifferentiation (7–9). Predictive factors for responsiveness to RaI treatment include a younger age, small metastases, well-differentiated thyroid tumor histology and the absence of uptake of 18-fluoro deoxyglucose (FDG) at FDG-positron emission tomography (PET) scanning. Especially in patients with bone metastases, the efficacy

of RaI is limited to 7–20% of the patients (4, 6, 10, 11). At present, there are no effective therapies available for RaI non-avid DTC. Conventional chemotherapy is hardly effective in DTC, and no longer recommended in international guidelines (12, 13).

In DTC, many genetic alterations have been identified, involving tyrosine kinase signaling pathways (14–16). In nearly all cases of papillary thyroid carcinoma (PTC), genetic defects involving the RET, RAS, and RAF protein kinase signaling cascade are identified. The BRAF^{V600E} mutation has been found in 29–69% of PTC and has been associated with aggressive features including extrathyroidal extension and advanced stage (17). Translocations of RET observed in PTC result in a chimeric protein consisting of an activated RET tyrosine kinase domain (15, 18–32). Follicular thyroid carcinomas (FTC) frequently harbor mutations in one of the three RAS genes.

The RET–RAS–RAF pathway is interconnected with the epithelial growth factor receptor (EGFR) activated cascade that among others leads to vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) synthesis. Therefore, compounds targeting the activated RET–RAS–RAF pathway and beyond may be effective in non-RaI avid DTC. The anti-EGFR compound gefitinib was not successful in 27 patients with DTC, medullary or anaplastic thyroid carcinoma (33). In a phase II study in 60 thyroid carcinoma patients with various histologies, the VEGFR inhibitor axitinib showed a partial response of 30% (median progression free survival (PFS) 18 months) (34). Recently, 3 phase II studies have been published, using multi-kinase inhibitors (35–37). Motesanib diphosphate induced a partial response in 14% of 93 DTC patients (median PFS 40 weeks) (36). Two studies have been published using sorafenib. Sorafenib (BAY 43-9006) is an inhibitor of RET, CRAF, wild-type and mutant (V600E) BRAF, VEGFR1, -2, -3, Flt3, and c-KIT.

In the first study, including DTC, anaplastic and medullary thyroid carcinoma patients, sorafenib induced a partial response in 23% (median PFS 79 weeks). In the second study, including patients with DTC and anaplastic thyroid carcinoma, a partial response was observed in 11% (median PFS 4.5–16 weeks) (37). As bone metastases of DTC are poorly responsive to RaI, the response of bone metastases to the above-mentioned therapies is of interest. However, there were no separate analyses for patients with bone metastases in the above-mentioned studies.

In DTC, a relationship has been identified between genetic alterations in the RET, RAS, RAF cascade and loss of NIS expression (17, 38). Interestingly, in an *in vitro* study, sunitinib was able to reinduce NIS expression in RET/PTC transformed thyroid cells (39). In addition, sunitinib also increased RaI uptake in FRTL-5 cells. We therefore hypothesized that treatment with a multiple kinase inhibitor not only reduces tumor progression, but may also restore RaI uptake in non-RaI avid DTC. Therefore, we performed a single arm phase II trial to determine the efficacy of sorafenib in patients with iodine-refractory metastatic DTC, focusing on the reinduction of RaI uptake and the efficacy in bone metastases.

Patients and methods

Patients

Eligibility criteria for the present study were the presence of progressive metastases or unresectable local recurrence of DTC for which RaI therapy was no longer effective, as indicated by prior negative post-therapeutic whole body scintigraphy (WBS). Progressive disease was defined according to response evaluation criteria in solid tumors (RECIST) criteria in the year before initiation of treatment. Patients who were selected, had to have

undergone total thyroidectomy and RaI ablative therapy. Exclusion criteria were pregnancy, contraindications for the application of recombinant human thyrotropin (rhTSH) and contraindications for the use of sorafenib. In addition, no patients with poorly responsive DTCs were included as it was hypothesized that the likelihood of reinduction of RaI uptake in this category of tumors would be very small. Other eligibility criteria included Eastern Cooperative Oncology Group performance status 0–2 and life expectancy more than 3 months. Patients were ineligible if they had previous exposure to biological therapies. All patients provided written informed consent before enrollment onto the trial. The study protocol was approved by the Institutional Review Board of the Leiden University Medical Center. This study has been registered at ClinicalTrials.gov (# NCT00887107).

Study design

The study was a 26-week open-label, single-arm, phase II study of oral sorafenib in patients with metastatic DTC. The primary objective was to study the effect of sorafenib treatment on reinduction of radioiodine uptake. Secondary objectives were radiological tumor response according to RECIST and the relation between the presence of bone metastases and therapy success. Sorafenib was initially administered at a dose of 400 mg orally twice a day. Eligibility assessments, including a review of medical history and prior treatments, physical examination and laboratory assessments were completed within 2 weeks before initiation of the sorafenib therapy. Baseline radiological evaluation by computed tomography (CT) of target lesions was performed 4 weeks prior to initiation of therapy. From all patients, tumor samples from the primary tumors were obtained for review by the institutional pathologist (H M). After inclusion, the patients underwent a diagnostic scintigraphy 3 days after i.v. administration of 185 MBq I-131 (Mallinckrodt BV, Petten, The Netherlands). Single photon emission computed tomography (SPECT) of the head and neck and chest was performed. Two experienced observers visually analyzed all images. An I-131 standard was used to quantify the uptake in the area of interest at WBS. Patients were prescribed a low iodide diet from 7 days prior to the administration of I-131 (40). The patients received i.m. injections with 0.9 mg rhTSH (Thyrogen, Genzyme, Naarden, The Netherlands) on two consecutive days before the RaI administration. The day after the first WBS, patients started treatment with sorafenib 400 mg bid. Twenty-six weeks after initiation of sorafenib therapy, CT scanning and RaI imaging studies were repeated. All target lesions were imaged using CT. According to RECIST criteria, bone lesions were not evaluated as target lesions. It was carefully planned that CT scans using i.v. contrast were performed after diagnostic RaI scintigraphies. Procedures to exclude

the occurrence of new bone metastases included bone and FDG-PET scintigraphy. Patients visited the hospital every 4 weeks for physical examination and assessment of laboratory safety parameters. Patients were assessed for new symptoms, compliance with study medications, and concomitant medications. Dose adjustments were made as needed for toxicity. The target serum TSH levels were below 0.1 mU/l. After completion of 26-week treatment, patients were allowed to continue sorafenib treatment, when a favorable response (complete or partial remission, or stable disease) had been achieved, until progression according to RECIST criteria. Adverse events were graded with the use of Common Terminology Criteria for Adverse Events (version 3).

To study the relation between efficacy and tumor mutational state, tumor DNA from patients was isolated from the paraffin-embedded material by taking tissue punches (diameter, 0.6 mm) with a tissue microarrayer (Beecher Instruments, Silver Springs, MD, USA) from tumor and normal areas selected on the basis of a hematoxylin eosin (HE)-stained slide. Mutational analysis was performed using PCR amplification followed by sequencing on an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA) ABI 3700. Codons investigated were: B-type Raf kinase (BRAF, V600E and exon 11), HRAS (codon 12/13 and 61), KRAS (codon 12/13 and 61), NRAS (codon 61), and phosphatidylinositol-3-kinase, catalytic, alpha polypeptide (PIK3CA, exons 9 and 20). Primer sequences are available on request.

Laboratory parameters

The following laboratory parameters were assessed: serum TSH, free-thyroxine (T₄), thyroglobulin (Tg), and Tg antibody levels were measured at all visits. TSH and Tg were also measured 1 and 3 days after the last rhTSH injection. Safety parameters were assessed at every visit and included a hematological profile as well as serum levels of sodium, potassium and creatinine in lipids, renal and liver function. Serum TSH was determined with a Modular Analytics E-170 system (Roche Diagnostic Systems), intra-assay variability: 0.9–10.7%, interassay variability: 0.9–12.1%. Serum Tg was determined with IRMA (Tg kit, Brahms, Berlin, Germany) on a Wallac (Wallac, Turku, Finland), intra-assay variability: 0.1–13.9%, interassay variability: 12.3–17.4%. Serum Tg antibodies were determined with IRMA (Sorin Biomedica, Amsterdam, The Netherlands) on a Wallac (Wallac, Turku, Finland, intra-assay variability: 3.6–4.1%, interassay variability: 11.6%).

Statistical methods

Using a Simon two-stage design, the study required 24 subjects to decide whether the proportion responding, P , is $\leq 10\%$ or $\geq 40\%$. If the number of responses was six or more, the hypothesis that $P \leq 10\%$ is rejected with

a target error rate of 5% and an actual error rate of 2.8%. If the number of responses was five or less, the hypothesis that $P \geq 40\%$ is rejected with a target error rate of 5% and an actual error rate of 4%. All enrolled patients were included in an intention-to-treat analysis. Data are reported as mean \pm s.d., median (range) or proportions. Estimates of PFS (time from starting study drug to progression) with associated 95% CIs were obtained using the Kaplan–Meier method. Analyses of variables influencing response to sorafenib were analyzed with binomial logistic regression (26-week data). The calculations were performed using SPSS 16.0 for Windows (SPSS, Chicago, IL, USA).

Results

Between 13 October 2007 and 7 October 2008, a total of 31 patients started sorafenib treatment (Fig. 1). One patient chose not to start sorafenib therapy after giving signed informed consent. Therefore, 32 patients were included in the intention-to-treat analysis. A total of 22 patients completed 26 weeks of treatment. The remaining patients discontinued treatment earlier because of disease progression (four patients, see efficacy section for details), drug-related adverse events

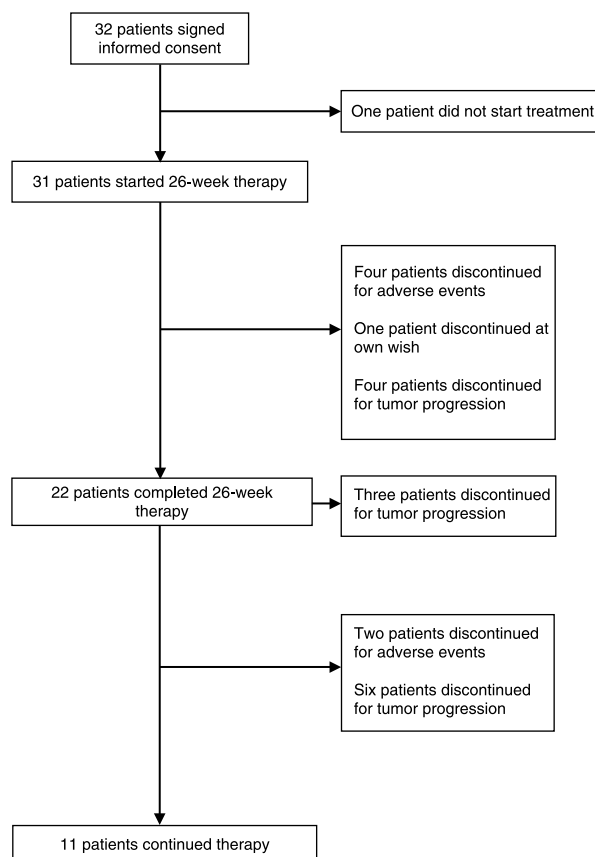


Figure 1 Study flowchart.

(two patients, see adverse events section for details), non-drug related adverse events (two patients) or the patient's request (one patient). Eight of the 22 patients who completed the 26-week trial and continued sorafenib treatment, discontinued treatment later on, two because of adverse events and six because of disease progression. The median duration of sorafenib treatment for all patients was 32 weeks (range 3 days to 79 weeks). Median duration of follow-up was 63 weeks (range 28–80 weeks).

Baseline characteristics are given in Table 1. All patients had undergone prior RAI treatments with a median activity of 15.2 (range 3.7–30.0) GBq. The number of RAI treatments ranged from 1 to 4. None of the patients had RAI uptake before inclusion as indicated by post-therapeutic WBSs and at baseline. Fifteen patients had undergone external beam irradiation. In all patients, external beam irradiation was aimed at one or more bone metastases. As no bone metastases were target lesions this treatment did not interfere with the assessment of efficacy.

Interestingly, the most common histologic carcinoma subtype was Hürthle cell carcinoma. In follicular thyroid with Hürthle cell metaplasia, the majority of cells were Hürthle cells. At study entry, almost all patients had lung metastases ($n=30$), whereas 14 patients had bone metastases. Most patients had metastases at multiple sites. In 10/13 patients PTC's BRAF^{V600E} mutations were identified, one of which (the tall cell variant) also showed a PIK3CA mutation.

Few additional mutations were identified: one KRAS in FVPTC; two NRAS mutations were found (one in a PTC without a BRAF mutation and one in a FTC); and one additional PIK3CA mutation was found in a FTC with Hürthle cell metaplasia.

Efficacy

Data on responses are given in Table 2 and Fig. 2. Disease progression led to treatment discontinuation of patients at 6 weeks (one patient with new bone metastases), 18 weeks (one patient with a new abdominal metastasis, one patient with new pulmonary metastases), 25 weeks (one patient with cerebral metastases), 26 weeks (end of 26-week trial: two patients with new bone metastases, one patient with a new pulmonary metastasis), 32 weeks (two patients with new bone metastases, one patient with progression of a hip lesion, one patient with new liver metastases and one patient with new lung lesions) and 40 weeks (one patient with a new neck lesion).

At 26 weeks, 19 patients (59%) had achieved a clinical beneficial response, eight of whom had a partial response (25%) and 11 had stable disease (34%; Table 2). There were no complete responses. At 26 weeks, the cumulative number of patients with progressive disease was seven. Interestingly, four patients with progressive disease based on new lesions had no progression or a considerable decrease in the sum of the longest diameter of target lesions. Radiological response at 26 weeks was not influenced by gender ($P=0.593$), age ($P=0.172$), initial tumour lymphnodes metastases (TNM) stage ($P=0.488$), histology ($P=0.614$), or the presence of the BRAF^{V600E} mutation ($P=0.760$). The prevalence of other mutations was too low to allow statistical analysis. However, when bone metastases were present, the response to sorafenib was significantly worse than in the absence of bone metastases ($P=0.004$, Table 2). Although this study was not formally designed for the establishment of PFS, estimation of median PFS was 58 weeks (95% CI, 47–68). Likewise, PFS was influenced by the presence of bone metastases. PFS was 69 weeks (CI: 58–80) in patients without bone metastases and 47 weeks (CI: 32–62) in patients with bone metastases ($P=0.046$).

In general, Tg response reflected radiological response and there were no differences between the responses of unstimulated versus TSH stimulated Tg measurements (Table 2, Fig. 2). The median time of the nadir of Tg levels was 3 months. The median change in Tg at 26 weeks versus baseline was $-16 \mu\text{g/l}$ (range -2746 to 17836). Remarkably, one patient with progression in a non-target lesion had a decrease in unstimulated Tg, but an increase in TSH stimulated Tg. In one 54-year-old woman with pulmonary metastases of a Hürthle cell FTC, a remarkable rise in serum Tg from 1664 to 99 900 $\mu\text{g/l}$ was observed 8 weeks after

Table 1 Baseline characteristics.

	All patients ($n=32$)
Gender (no.; %)	
Female	13 (39)
Male	19 (61)
Time from diagnosis (year; median, range)	3 (0–18)
Age (year; median, range)	65 (53–82)
Histology (no.; %)	
Papillary	13 (41)
Tall cell	1 (3)
Hürthle cell metaplasia	2 (6)
Follicular variant papillary	3 (9)
Follicular	15 (47)
Hürthle cell metaplasia	11 (34)
Mixed papillary follicular	1 (3)
Initial TNM stage (no.; %) (5 unknown)	
T _{1–3} N0/1 M0	12 (44)
T ₄ N0/1 M0	6 (23)
M1	9 (33)
Tumor extent at study entry (no.; %)	
Thyroid bed only	1 (3)
Lungs only	8 (25)
Lungs and bones only	7 (22)
Locally advanced ^a and distant metastases	9 (33)
Other	7 (22)
Radioiodine dose (GBq, median, range)	15.2 (3.7–30.0)
External beam radiation (no.; %)	15 (47)

^aIncluding both thyroid bed ($n=5$) and neck lymph nodes ($n=4$).

Table 2 Tumor response.

	All patients (n=32)	Bone metastases		Histology ^a			
		Absent	Present	Papillary thyroid carcinoma (PTC)	Follicular variant PTC	Follicular thyroid carcinoma (FTC)	Hürthle cell metaplasia
Reinduction of radioiodine uptake at 26 weeks	0			Total	Hürthle cell metaplasia	Total	Hürthle cell metaplasia
Radiological response (n; % of total patients)							
Complete response	0 (0) ^b	0 (0)	0 (0)	0 (0) ^c	0	0 (0)	0 (0)
Partial response	8 (25)	5 (16)	3 (9)	2 (22)	0	2 (67)	4 (49)
Stable disease	11 (34)	7 (22)	4 (13)	6 (67)	1	0 (0)	5 (36)
Clinical benefit	19 (59)	12 (38)	7 (22)	8 (89)	1	2 (67)	9 (64)
Progressive disease	7 (22)	0 (0)	7 (22)*	1 (11)	0	1 (33)	5 (36)
Serum thyroglobulin levels (µg/l)							
Baseline (median; range)	77 (4.4–8570)						
26-weeks (n=22)	46 (0.9–19 500)						
Delta Tg (versus baseline, median, range)	–16 (–2746–17 836)						
Nadir	29 (0.5–7634)						
Time to nadir (months, median, range)	3 (0–5)						
Progression free survival (weeks, median, CI)	58 (47–68)						

**P* = 0.005 versus no bone metastases.

^aOne patient with mixed PTC and FTC left the study because of an adverse event and could not be evaluated.

^bPercentage of total (n=32) patients.

^cPercentage of evaluable patients per histology class.

initiation of therapy. A chest CT scan revealed an impressive decrease in the number, size, and density of all pulmonary metastases.

Reinduction of radioiodine uptake could be evaluated in 21 patients who had completed 26-week treatment with sorafenib. In one patient, WBS could not be performed for technical reasons. In three patients with extensive metastases, both at baseline and at 26 weeks, RaI WBS revealed uptake at the thyroid bed, but not at the sites of metastases. In 20 patients, there was no RaI uptake at metastatic sites after 26 weeks of sorafenib therapy. In one patient with an occipital skeletal metastasis that did not accumulate RaI at baseline, very slight RaI uptake was observed at 26 weeks. However, a post-therapeutic WBS performed after subsequent 7600 MBq RaI therapy did not reveal any uptake, and consequently it was concluded that no clinically relevant reinduction of RaI therapy had occurred in this patient.

Adverse events

Adverse events led to discontinuation of the study in six patients at respectively 3 weeks (angioedema), 5 weeks (myocardial infarction), 7 weeks (hematuria), 12 weeks (concomitant small cell lung carcinoma), 38 weeks (dyspnea, not related to tumor progression), and 50 weeks (congestive heart disease). Doses were reduced in 18 (56%) patients to control toxicities. The median dose at 26 weeks was 400 mg/day.

The most prevalent adverse events are mentioned in Table 3. The hand-foot syndrome occurred in the first few weeks of treatment and subsided in most patients after dose reduction and topical treatment. Most patients received nutritional support for weight loss. In three patients with considerable weight loss, a percutaneous gastric feeding catheter had to be inserted. Mineral deficiencies were treated with supplementation. Hypertension was treated with antihypertensive drugs. Hematological abnormalities were mild and did not require treatment. In order to maintain serum-free T₄ levels and TSH levels at the required values, five patients required a thyroxine dose reduction, whereas in six patients, the dose needed to be increased. No relationship was observed between toxicity and performance state and age.

Discussion

We studied the efficacy of the multiple target tyrosine kinase inhibitor sorafenib in patients with iodine-refractory metastatic DTC, focusing on the reinduction of RaI uptake and the efficacy in patients with bone metastases. After 26 weeks of treatment, we found a partial response rate of 25% and a clinical beneficial response in 59% of the patients. Patients with bone metastases responded significantly worse. Reinduction of RaI uptake as studied by diagnostic WBS was not observed in any patient.

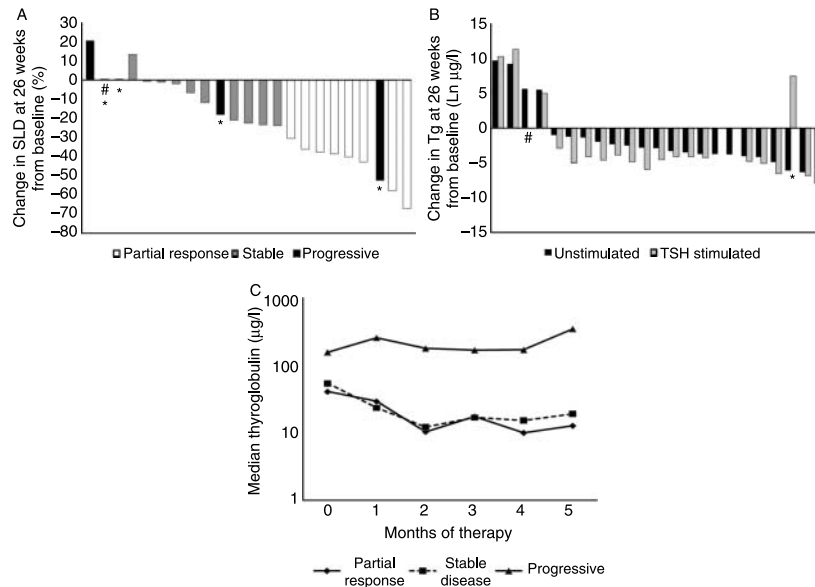


Figure 2 (A) The change from baseline in tumor dimensions (sum of longest diameters (SLD)) is shown for 22 patients who completed 26 weeks of treatment with sorafenib and one patient who discontinued treatment at 18 weeks for disease progression (new soft-tissue lesion (#)). Not shown are data for three patients with progressive disease due to new lesions. Four patients with progressive disease based on new lesions had no progression or a considerable decrease in SLD (*). Target and non-target lesions are defined according to the response evaluation criteria in solid tumors (RECIST). (B) The natural logarithm of the change at 26 weeks from baseline in unstimulated and TSH stimulated serum thyroglobulin (Tg) levels is shown for 22 patients who completed 26 weeks of treatment with sorafenib and one patient who discontinued treatment at 25 weeks for disease progression (new cerebral metastasis (#)). One patient with progression in a non-target lesion had a decrease in unstimulated Tg but an increase in TSH stimulated Tg (*). TSH stimulated Tg measurements were performed 3 days after the last injection of 0.9 mg rhTSH. (C) Medians of unstimulated serum thyroglobulin (Tg) levels of 25 evaluable patients during 26 weeks of treatment with sorafenib stratified according to radiological response. Three of these patients with progressive disease discontinued treatment before 26 weeks. From one other patient, who also discontinued treatment before 26 weeks for disease progression, no Tg levels were obtained.

RaI therapy is the only available conventional therapy for patients with metastases of DTC. Hürthle cell carcinomas respond less favorably to RaI, which is compatible with the fact that the most prevalent histology was Hürthle cell metaplasia. The fact that 10/13 PTC harbored BRAF^{V600E} mutations also illustrates the unfavorable prognostic characteristic of our patient group (41). The extensive characterization in recent years of the molecular pathways involved in the pathogenesis of DTC has revealed potential targets for new therapies. The identification of tyrosine kinase activated pathways in DTC together with the advent of novel classes of tyrosine kinase inhibitors has provided new therapeutic perspectives for patients with non-RaI avid DTC. The results of our study with respect to radiological response are comparable with those observed in another study with sorafenib by Gupta *et al.* (35) and better than observed in the study of Kloos *et al.* (37). The latter study, however, included patients with anaplastic carcinoma. Comparison of the results of phase II studies with different tyrosine kinase inhibitors in DTC is hampered by differences in patient categories (including histologies, tumor stages, sites of metastases, and tumor extent), study design, and analytical methods. Nonetheless, the results obtained with sorafenib in different studies, including our own

suggest that sorafenib is among the most successful and promising compounds for metastatic DTC. We found a significant difference in the response to sorafenib between patients with or without bone metastases, the latter category responding worse. Nevertheless, 23% of patients with bone metastases had a favorable response. Those patients had metastases at multiple sites, mostly pulmonary that regressed under sorafenib, whereas the bone metastases were stable.

The explanation for a less favorable response of bone metastases is not clear. It can be hypothesized that both pharmacokinetic factors (lower tissue levels in bone) and tumor-related factors are involved. As it is hardly possible to obtain tissue from bone metastases, the relationship between the genetic profile of bone metastases and responsiveness to sorafenib is not easy to study. Another explanation may be that it has been observed that the role of VEGFR signal transduction in tumor propagation in conscript vascular systems such as soft-tissue metastases may differ from less well-defined vascular beds as the bone marrow. As a consequence, VEGFR targeted therapies may have less prominent beneficial effects in bone metastases. In addition it is also conceivable that the presence of bone metastases influences the response of soft-tissue metastases to sorafenib. Although the mechanism is not

Table 3 Adverse events.

Event	All Number of patients (% of total (n=32))	Grades (% of category)			
		1	2	3	4
Hand foot syndrome	21 (66)	33	33	33	0
Weight loss	18 (56)	35	50	15	0
Diarhoea	16 (50)	31	69	0	0
Alopecia	15 (47)	87	13	0	0
Rash	15 (47)	60	40	0	0
Mucositis	14 (44)	100	0	0	0
Hypertension	13 (41)	31	31	38	0
Hypocalcemia	13 (41)	100	0	0	0
Thrombopenia	9 (28)	100	0	0	0
Hypophosphatemia	9 (28)	100	0	0	0
Anemia	8 (25)	100	0	0	0
Myocardial infarction	1 (3)	0	0	0	100
Congestive heart disease	1 (3)	0	0	100	0
Hematuria	1 (3)	0	100	0	0

According to Common Terminology Criteria for Adverse Events (version 3).

clear it could be hypothesized that the systemic release of proteins or cytokines, such as interleukin 6 or TGF beta from the bone microenvironment plays a role in this phenomenon.

The fact that there was a mixed response in some subjects (both regression and progression) is explained by the heterogeneous nature of metastases in thyroid carcinoma, which can be caused by tumor-related factors and the microenvironment of the metastatic site. This may lead to differences in tumor dependence on tyrosine kinase activated pathways, which may consequently lead to differences in response to sorafenib. RECIST does not take into account these phenomena, which are very important for the selection of lesions that may or may not respond to tyrosine kinase inhibitors.

Another purpose of our study was to assess the effects of sorafenib on RaI uptake. We did not find reinduction of RaI uptake in any patient. Although a clear relation has been found *in vitro* between genetic alterations in DTC and decreased NIS gene expression (17, 38), multiple mechanisms may be involved in decreased NIS functionality, including impaired NIS membrane trafficking (42, 43), epigenetic changes in NIS and/or NIS promoter genes (44). Although *in vitro* studies have shown that multiple target tyrosine kinase inhibitors may lead to reinduction of RaI uptake (39, 45), it may well be that these additional mechanisms have prevented a beneficial effect of sorafenib on RaI uptake in our study. Another explanation for the lack of an effect of sorafenib on RaI uptake may be the lower sensitivity of diagnostic WBS as compared to post-therapeutic scintigraphy. For this reason, we used SPECT acquisition in our diagnostic scintigraphies, which has a higher sensitivity than conventional WBS. Nevertheless, we cannot exclude that a limited sensitivity may indeed have contributed to our

observation of a failure of sorafenib to reinduce RaI uptake. As baseline CT scans were performed 4 weeks prior to radioiodine scintigraphies, i.v. contrast could have interfered with the diagnostic scintigraphy at baseline. As no urinary iodine measurements were performed, this cannot be entirely ruled out. However, the fact that all patients had negative post-therapeutic WBSs previously ascertains the true radioiodine non-avid nature of the lesions.

Although uptake of RaI is an important determinant of tumor response, another important factor is radio-sensitivity. Indeed, a subgroup of thyroid carcinomas does not respond to RaI therapy, despite RaI uptake. Genetic alterations in radiation-induced apoptotic pathways, including p-53 or cdk-21 may be involved. Therefore, in the design of redifferentiation studies in thyroid carcinoma, this aspect should always be taken into account, ideally involving genetic studies in these pathways.

Serum Tg levels in general paralleled radiological changes. However, tumor lysis during sorafenib or comparable compounds can lead to elevated Tg levels as was observed in one patient. This could be due to tumor lysis, but it could in theory also be caused by increased Tg synthesis as a result of enhanced differentiated behavior of thyroid carcinoma cells.

Although sorafenib therapy had considerable adverse effects, no unexpected or new adverse effects were observed. Most of the adverse effects were temporary and could be managed with dose reduction or therapeutic measures. Weight loss was considerable, especially in patients with low weight at baseline, requiring dietary support or even catheter feeding.

In conclusion, the results of our study confirm the potential role of sorafenib in patients with non-RaI avid metastases of DTC. In addition, we found that patients with bone metastases respond less favorably, and that

diagnostic WBS did not reveal an effect of sorafenib on the reinduction of RaI uptake in these patients. Future phase III studies should confirm the efficacy of sorafenib for DTC.

Declaration of interest

J W Smit received a research grant from Bayer B V Mijdrecht for performing this study. The other authors have nothing to disclose.

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