Thyroid

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Fabiana Pani[1] MD, Francesco Atzori[2] MD, Germana Baghino[1] MD, Francesco Boi[1] MD, Luciana Tanca[3] MD, Maria Teresa Ionta[2] MD, Stefano Mariotti[1] MD.

(1), Endocrinology Unit and (2) Medical Oncology Unit, Department of Medical Sciences, University of Cagliari; (3) Businco Hospital, Medical Oncology, Cagliari, Italy

**Author's contact information:**

Fabiana Pani; panifabiana@libero.it tel: +3907051096518; 3206259528

Francesco Atzori; francescoatzori74@yahoo.it tel: +3907051096332; 3287565700

Germana Baghino; germanabaghino@yahoo.it tel: +3907051096517; 3490537191

Francesco Boi; boi@medicina.unica.it tel: +3907051096518; 3687643688

Luciana Tanca; l.tanca@virgilio.it tel: +3907051095356; 3334649257

Maria Teresa Ionta; mtionta@yahoo.it tel: +3907051096332; 3388815800

Stefano Mariotti; mariotti@medicina.unica.it tel: +3907051096430; +3907051096429

**Running Title:** Sunitinib and thyroid autoimmunity

**Key terms**: Thyroid Autoimmunity, Hypothyroidism, Radiology-Imaging

## ABSTRACT

**Background:** Sunitinib is a tyrosine kinase inhibitor (TKI) inducing thyroid dysfunction, but the precise mechanism(s) involved remains to be explained, including a role of thyroid autoimmunity.

The objective was to evaluate thyroid function, parameters of autoimmunity, and thyroid ultrasound findings in patients with metastatic cancer and normal thyroid function/autoimmunity before the initiation of Sunitinib therapy.

This is a prospective, observational cohort study.

**Methods***:* Twenty-seven patients with metastatic carcinomas at comparable tumor stages were evaluated over 12-18 months after initiating therapy with Sunitinib given at a daily oral dose of 50 mg for 4 weeks (ON), followed by 1-2 weeks off therapy (OFF).

Serum TSH, free_T4, free_T3, anti-thyroglobulin (TgAb) and anti-thyroid peroxidase (TPOAb) autoantibodies were measured in all cases. Thyroid morphology and volume were evaluated by echo-color Doppler ultrasound.

**Results***:* 16/27 patients (60%) became hypothyroid (TSH range 7-114 mIU/L) within 30-120 days of therapy. The thyroid volume decreased in 24/27 (89%) patients (from 14.6±6.4 [mean±SD] ml to 3.8±2.6 [mean±SD] ml after 12 months, p <0.001), together with the appearance of mild to severe hypoechogenicity. TPOAb (40-3000 IU/ml) became detectable in 7/27 (25%) patients and TPOAb-positive patients displayed a higher degree of hypothyroidism and volume reduction. The progression-free survival (PFS) was significantly longer in patients developing TPOAb (10.8 months) than in the other group of patients (5.8 months).

**Conclusions***:* These data confirm the thyroid-inhibitory effect of Sunitinib, in keeping with the key role of kinases in controlling thyroid function and growth. However, the novel appearance of

TPOAb in a subgroup of patients with more severe hypothyroidism and longer survival indicates that Sunitinib may also trigger/exacerbate thyroid autoimmunity contributing to thyroid failure. The development of TPOAb was associated with a longer PFS.

## INTRODUCTION

Sunitinib is a tyrosine kinase inhibitor (TKI), a group of new multi-targeted drugs used to treat hematological and solid tumors (1-3). TKI represent a valid anti-neoplastic option, both as individual molecules and in combination with conventional cytotoxic chemotherapy and radiotherapy (1-3). Currently, Sunitinib is widely employed in metastatic renal carcinoma (mrCC) (4-6). Compared to conventional cytotoxic chemotherapy agents, TKI have a more favorable toxicity profile and are easier to administer. However, the use of these agents is associated with several side effects including thyroid dysfunction. The latter is particularly frequent with Sunitinib and results in hypothyroidism in more than 50% of patients and, less frequently, in thyrotoxicosis due to a destructive thyroiditis (3). The underlying mechanism(s) underlying Sunitinib-associated thyroid dysfunction is/are not clear, and the course of the disorder is not completely characterized (7-9).

In particular, the role of thyroid autoimmunity in the pathogenesis of TKI is controversial. While thyroid autoimmunity was found to be absent in some prospective series (2,10) of Sunitinib-treated patients, the presence of lymphocytic thyroiditis has been documented even at the histological level in an isolated case report of Sunitinib-induced hypothyroidism (11).

The aim of the present investigation was therefore to longitudinally evaluate thyroid function and thyroid autoimmunity before and during Sunitinib administration, in a homogenous series of patients with metastatic carcinomas, with normal thyroid function and negative thyroid antibodies before starting Sunitinib therapy.

## PATIENTS AND METHODS

### *Patients*

A total of 27 consecutive patients, 24 men and 3 women aged 44-77 (59.9±7.6, mean±SD), with metastatic carcinomas (26 with mRCC and 1 with gastrointestinal stromal tumor [GIST]), currently treated at the Medical Oncology Unit of Cagliari University and Businco Hospitals were enrolled in the study. All patients were at an advanced tumor stage with the following distribution of distant metastases: 20/27 (74%) lung, 4/27 (14%) bone and 3/27 (11%) liver, adrenal and pancreas metastases. Thyroid evaluation was performed at the outpatient clinic of the Endocrinology Unit of Cagliari University Hospital.

All patients were euthyroid with no serological and/or ultrasound evidence of associated thyroid autoimmunity and a negative familial history of thyroid disease. All patients were initially treated with Sunitinib (Sutent®) for 2-18 (mean±SD 8.3±3.9) months. Sunitinib was given at a daily oral dose of 50 mg for 4 weeks (ON), followed by 1-2 weeks (OFF) on the basis of the clinical condition. In three patients Sunitinib was withdrawn after 3-5 months due to progression of the disease and replaced by Axitinib (Inlyta®) given at a daily oral dose of 10 mg.

Tumor measurements were evaluated by the treating oncologist according to the Response Evaluation Criteria in Solid Tumors (RECIST) (12); CT scans were performed every 16 weeks during treatment with Sunitinib, and every 12 weeks during treatment with Axitinib.

All patients signed an informed consent and the study was approved by the Institutional Ethics Committee.

*Biochemical and Instrumental Investigations*

Blood samples were collected before and at the end of the "ON" and "OFF" periods during the Sunitinib administration.

TSH [reference range 0.3-3.0 mIU/L], free T3 (1.8-4.2 pg/ml), free T4 (FT4) (0.9-1.9 ng/dl), anti-thyroglobulin (TgAb) (0-20 IU/mL) and anti-thyroid peroxidase (TPOAb) (0-35 IU/mL) autoantibodies were measured by an ultrasensitive chemiluminescent assay (Immulite 2000-Siemens Germany). All patients underwent a careful clinical examination, followed by thyroid ultrasound using echo-color Doppler technique, performed always by the same operator (FP) and with the same instrument (Sonoline G60S, Siemens Medical Solutions, Issaquah, WA, USA). In all cases the estimated thyroid volume (ETV) was evaluated using the formula of the ellipsoid of rotation (depth x width x length x 0.52) (13). The parenchymal echogenicity and the presence of thyroid nodules was always assessed.

*Statistical Analysis*

Parametric (t-student test) and non-parametric tests (Mann-Whitney test) were used to compare different groups. The hazard ratio (HR) and 95% CI were estimated by stratified Cox proportional-hazards regression. Statistical significance was considered at $p < 0.05$. All calculations were performed using a commercial software GraphPad Prism® (La Jolla, Ca, USA).

## RESULTS

### *Patient Characteristics*

Sex, age, basal and follow-up data on thyroid function and volume and status at the end of the study of all patients are reported in Table 1. Before Sunitinib administration, all patients had normal TSH levels and TgAb and TPOAb were within the reference range (<20 IU/ml and <35 IU/ml, respectively). With the exception of the single patient with GIST who was treated for five months with Imatinib until three months before the inclusion in the present study, none of the patients received any antineoplastic drug before the treatment with Sunitinib. The median duration of treatment with Sunitinib was 64 weeks (range 8-78).

### *Thyroid Function during Sunitinib Administration*

As shown in Table 1, 16/27 patients (60%) developed variable degrees of hypothyroidism (TSH 7-114 mIU/L) after 30-120 days of treatment. Two patients displayed a transient period with suppressed TSH, presumably due to mild thyrotoxicosis secondary to a destructive thyroiditis (TSH <0.01 mIU/L) and one of them later developed thyroid failure (TSH 9.5 mIU/L). Hypothyroid symptoms were mostly mild, with the exception of the patient with a serum TSH of 114 mIU/L. During the first 3 months of sunitinib therapy, increased serum TSH concentration was observed in all patients developing hypothyroidism only during the "ON" period, but subsequently TSH remained abnormally elevated even during the "OFF" period. Serum FT4 displayed a trend to decreasing levels without reaching statistical significance (data not shown). L-T4 therapy (25-75 μg) was started in all patients showing a persistent serum TSH elevation of ≥7 mU/L at the end of the "OFF" period and was followed by a stable normalization of the serum TSH on therapy. The mean serum TSH concentration observed at the last evaluation

before starting L-T4 [mean±SD 14.5±24.6 mU/L (range 7-114 mIU/L)] was significantly higher than basal levels (1.2±0.6 mIU/L, p<0.001 by paired student t-test). This difference remained significant even after excluding the highest TSH concentration of 114 mU/L [mean±SD 17.2±9.7 IU/mL (range 7-30 mIU/L), p<0.01].

*Anti-thyroid Antibodies and Thyroid Function during Sunitinib Administration*

Although both TgAb and TPOAb were within the reference range (<20 IU/ml and <35 IU/ml respectively) at baseline before starting therapy with Sunitinib, 7 (26%) patients developed mildly to significantly elevated titers of TPOAb (40-3000 IU/mL) and they remained elevated after initiating L-T4 replacement therapy (Figure 1A).

In contrast with TPOAb, TgAb did not increase above the reference range during all cycles of treatment (data not shown).

As shown in Figure 1B, the mean of the maximal serum TSH elevation observed during Sunitinib treatment before L-T4 therapy was significantly higher (mean±SD 40.1±18.8 mU/L) in the 7 patients developing TPOAb than in the 20 patients who remained TPOAb-negative (mean±SD 8.7±1.8 UI/mL, p<0.001 by unpaired student t-test). This difference remained significant (mean±SD 17.2±9.7 IU/mL, p<0.01 by unpaired student t-test) after excluding the highest value (114 IU/ml) observed in patient #8.

*Anti-thyroid Antibodies and Tumor Response*

As shown in Figure 2, the median duration of treatment in the 7 patients developing high TPOAb was longer [15 months (range 3-18)] than that observed in the 20 patients in whom TPOAb did not increase [9 months (range 2-14)]. The progression free survival (PFS) of TPOAb-positive patients (10.8 months) was significantly higher than that of TPOAb-negative patients (5.8 months; 95% CI 0.45-0.76, HR for PFS of 0.59 p<0.001by Cox regression analyses).

*Echo-Color Doppler Ultrasonography Evaluation*

At baseline, all patients showed a normal ETV with normal echogenicity, although three of them (patients 1,7,15 in Table 1) displayed one or more thyroid nodules ranging 16-22 mm in maximal diameter. The nodule observed in patient 1 was not suspicious and its benign nature was confirmed by fine needle aspiration cytology (FNAC). In contrast, the nodules observed in patients 7 and 15 displayed some suspicious features, but the patients refused FNAC.

The individual variations of the ETV in all series are reported in Figure 3. The reduction of ETV was clearly higher in TPOAb-positive as compared to TPOAb-negative patients. Although this phenomenon could be partially due to the longer duration of Sunitinib administration in TPOAb-positive patients, the mean ETV observed after 4 months of therapy was already significantly lower (mean±SD 2.1±1.3 ml) than the mean ETV observed in TPOAb-negative patients (mean±SD 6.8±3.4 ml, $p<0.001$ by unpaired student t-test). The decrease in the ETV was much more evident in TPOAb-positive patients both in the "ON" and in the "OFF" phase of Sunitinib therapy (data not shown).

Besides a reduction in the ETV, Sunitinib treatment was associated with a change in parenchymal ultrasound features and vascularization (Figure 4A-B). As shown in Figure 4A-B, we observed a marked hypoechogenicity compared to basal findings, and this decrease in the echogenicity was more evident in TPOAb-positive patients (Figure 4B). As far as the vascularization is concerned, there was a trend to a reduced parenchymal perfusion, which did not reach the level of statistical significance.

Interestingly, in three patients presenting with thyroid nodules, Sunitinib treatment was associated with a marked size reduction of the nodular lesions.

## DISCUSSION

Our study prospectively analyzed thyroid function, thyroid autoantibodies and thyroid ultrasound findings in patients with metastatic cancer during Sunitinib treatment. The high prevalence of mild to severe hypothyroidism (60%) was consistent with previous reports (14,15). Our study provides several additional insights on Sunitinib-associated thyroid dysfunction, which have not been systematically reported before.

Although VEGF inhibition is considered the main mechanism common to all TKIs that cause hypothyroidism (16), it is still unclear whether other effects might be responsible for Sunitinib-induced thyroid dysfunction. Although most of these effects remain still hypothetical, it is worth noting that Sunitinib-induced thyroid dysfunction has been attributed to inhibition of iodine uptake (10), reduced synthesis of thyroid hormone (17), influence on deiodinase activity (18), impairment of MCT8-mediated iodothyronine transport (19), destructive thyroiditis (3), impaired blood flow (20,21) and damage by autoimmune processes (11). Few studies have been carried out so far focusing on a potential role of Sunitinib in modulating thyroid autoimmunity and function. Wolter et al. (8) found positive TPOAb and TgAb in 2/49 patients prospectively evaluated for thyroid dysfunction on Sunitinib therapy, but thyroid antibody tests were performed only at baseline. A higher prevalence of TgAb (but not TPOAb) was found by Rini et al. (22) in 13/44 (30%) patients prospectively evaluated for thyroid dysfunction while on Sunitinib therapy. Of those, 8 patients were TgAb-positive at baseline and 5 patients developed detectable TgAb after Sunitinib therapy, but no correlation was found between the presence of TgAb and either the incidence or the severity of thyroid dysfunction. In a single study (10), where anti-thyroid antibodies were assessed in 24 patients both at baseline and during six Sunitinib cycles, TgAb and TPOAb remained undetectable in all but one patient, who had mild hypothyroidism and positive TPOAb before starting

Sunitinib therapy. Thus, recent reviews on the effect of TKI on thyroid function concluded that, in contrast with IL-2 and interferon-alpha (IFN-α), thyroid autoimmunity cannot be included as an etiological factor in TKI-induced hypothyroidism (10,16), although histological evidence of Sunitinib-associated lymphocytic thyroiditis was obtained in a single case of an euthyroid patient with negative serum anti-thyroid antibodies (11).

In contrast with the above findings, the data obtained in the present study provide strong evidence that thyroid autoimmunity may contribute to Sunitinib-induced thyroid dysfunction in, at least, 25% of patients. This conclusion arises from the following considerations: a) the novel appearance of persistent serum TPOAb in patients without any clinical, serological and ultrasound feature suggestive of thyroid autoimmunity including familial history prior to therapy, b) a higher degree of hypothyroidism, and c) a more pronounced decrease in thyroid volume in TPOAb-positive when compared to TPOAb-negative patients.

The reason(s) for the apparent discrepancy between the previous reports and the present study on a possible autoimmune contribution in the etiology of Sunitinib-induced thyroid dysfunction are not immediately clear. However, it should be noted that thyroid autoimmunity was systematically assessed only in one study (10) which had a shorter duration (6 Sunitinib cycles) when compared to our investigation, which was extended up to 18 cycles. Other still unknown factors such as differences in the genetic background of the populations studied may also be involved in this context, e.g. the known increased susceptibility of the Sardinian population to developing autoimmune diseases (23-25).

The appearance of Sunitinib-induced thyroid autoimmunity was apparently related to a better outcome. However, since patients developing TPOAb also developed more severe hypothyroidism, the improved survival of TPOAb-positive patients could be simply related to thyroid failure, as already suggested in previous reports (26,27,28). Thus, whether and

to what extent thyroid autoimmunity might be the expression of a widespread activation of the immune system contributing to the control of tumor growth, remains a matter of future investigation.

Another difference of our study is the availability of longitudinal thyroid ultrasound evaluation. While this confirmed previous studies showing a reduction of thyroid volume and nodules (29,30), it also allowed to better characterize the time course of this effect, showing that a significant reduction of thyroid volume was observed already in the early phase of treatment, when it was partially dependent from the ON or OFF phase of Sunitinib administration, and that it reached its maximal effect after 4-6 months of therapy. The reduction of thyroid volume was associated to a reduction of thyroid echogenicity, which was more evident in TPOAb-positive patients, providing further support to the involvement of thyroid autoimmunity in Sunitinib-induced thyroid damage.

The longitudinal ultrasound follow-up also allowed evaluation of thyroid vascularization during Sunitinib therapy. Although we found a trend towards reduction in vascularity, the difference did not reach statistical significance. This finding contrasts with the marked anti-angiogenetic effect of TKI, which is considered one of the most important factors involved in Sunitinib-induced hypothyroidism. This paradox could be possibly explained by considering the stimulating effect of increased TSH on thyroid vascularization, which may compensate the drug-induced effect (31). Our study also allowed a precise characterization of the effect of Sunitinib on the volume reduction of thyroid nodules. Although the lack of cytological data on 2 of 3 patients with nodules is a limitation because they refused FNA, the complete disappearance of all nodules within 4-9 months of Sunitinib therapy represents a remarkable finding, which deserves further investigation.

In conclusion, the data of the present study confirm and extend our knowledge on the

thyroid-inhibitory effects of Sunitinib. Besides the functional inhibition expected on the basis of the key role of kinases in thyroid function and growth, the novel appearance of anti-thyroid antibodies in one third of patients with more severe hypothyroidism and a higher degree of thyroid volume reduction, indicates that Sunitinib is able to trigger/exacerbate thyroid autoimmunity, which in turn, may contribute to thyroid damage and could play a role as a potential biomarker of response.

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**Corresponding author**:

Fabiana Pani, MD, Department of Medical Sciences "M. Aresu", University of Cagliari, Endocrinology Unit, SS 554 09042 Monserrato, Cagliari, Italy

Phone: +39-07051096430 - +39-0706754035

Fax: +39-07051096429

email panifabiana@libero.it

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**Table 1**. Age, sex, thyroid parameters, follow-up duration and final status of 27 patients with metastatic cancer submitted to sunitinib therapy

| Patient # | Age/Sex | Hypo/TPOAb | TSH[a] | ETV[b] | Follow-up (months) | Final status |
|---|---|---|---|---|---|---|
| 1 | 66/M | Yes/No | 9.8→1.8 | 16.5→2.7 | 13 | I |
| 2 | 72/M | No/No | 1.5 | 9.6→5.1 | 10 | N |
| 3 | 60/M | Yes/No | 19.0→1.5 | 12.0→5.1 | 14 | I |
| 4 | 61/M | Yes/Yes | 25.0→1.7 | 10.1→0.6 | 18 | I |
| 5 | 68/M | No/No | 2.5 | 12.3→5.2 | 14 | N |
| 6 | 74/M | Yes/No | 7.5→2.1 | 15.5→5.3 | 13 | I |
| 7 | 77/F | Yes/Yes | 9.5→1.6 | 8.6→1.6 | 16 | I |
| 8 | 62/M | Yes/Yes | 114.0→3.3 | 11.0→1.4 | 15 | D |
| 9 | 58/M | No/No | 5.0 | 22.7→8.4 | 13 | N |
| 10 | 58/M | Yes/No | 9.2→1.6 | 16.0→2.2 | 9 | W |
| 11 | 51/M | Yes/No | 7.0→1.2 | 23.3→6.4 | 9 | W* |
| 12 | 60/M | No/No | 1.6 | 11.7→5.9 | 10 | N |
| 13 | 54/M | No/No | 2.3 | 12.4→5.4 | 11 | W* |
| 14 | 55/M | Yes/No | 30.0→1.2 | 12.0→3.2 | 9 | D |
| 15 | 51/M | Yes/No | 7.1→1.5 | 20.8→7.6 | 8 | W* |
| 16 | 66/F | Yes/Yes | 30.3→1.4 | 12.5→6.1 | 9 | I |
| 17 | 54/M | Yes/No | 12.0→1.2 | 7.9→3.3 | 8 | I |
| 18 | 54/M | No/No | 1.8 | 15→11.4 | 6 | D |
| 19 | 55/M | Yes/Yes | 10.0→1.4 | 13.1→2.9 | 15 | I |
| 20 | 60/M | Yes/Yes | 8.7→1.6 | 20.8→4.7 | 9 | I |

| | | | | | | |
|---|---|---|---|---|---|---|
| 21 | 44/M | Yes/No | 7.1→1.3 | 11.6→8.0 | 3 | I |
| 22 | 64/F | No/No | 1.1 | 10.3→5.1 | 3 | N |
| 23 | 66/M | No/No | 2.2 | 5.2→3.7 | 3 | N |
| 24 | 58/M | No/No | 1.7 | 9.5→4.9 | 3 | D |
| 25 | 57/M | No/No | 1.3 | 21.6→20.5 | 3 | W |
| 26 | 63/M | Yes/Yes | 9.8→3.6 | 20.1→17.7 | 3 | I |
| 27 | 51/M | No/No | 1.6 | 13.0→11.1 | 2 | N |

[a]: In patients developing hypothyroidism the first value represents the highest TSH concentration observed before the beginning of L-T4 therapy and the second value is the last measurement available. In the other patients the value represents the last TSH measurement available.

[b]: The first value represent the basal volume, the second value the last measure available.

* Shifted to Axitinib.

I: Improved; N: No change; W: Worsened; D: Died

## LEGEND OF FIGURES

**Figure 1A**. Serum TPOAb titers observed in 27 patients with metastatic cancer before (basal) and during 1-18 months of sunitinib treatment. Dashed lines indicate the normal range of TPOAb. TPOAb titers >35 UI/ml were considered positive; **1B**. Maximal values of serum TSH concentrations (mean±SD) observed in 27 patients with metastatic cancer during sunitinib treatment at the last control before beginning L-T4 therapy. Serum TSH concentrations were significantly higher ($p<0.001$ by unpaired t test) in the 7 patients developing TPOAb (TPOAb positive) than in the 20 patients in whom TPOAb remained undetectable (TPOAb negative).

**Figure 2**: Kaplan Meier estimate of Survival probability in 7 patients who developed TPOAb (●) and in 20 patients who remained TPOAb-negative (■) during sunitinib therapy.

**Figure 3**. Individual values of estimated thyroid volume (ETV) in 27 patients with metastatic cancer before (basal) and during 1-18 months of sunitinib treatment. Spots (●) indicate TPOAb-negative and squares (■) indicate TPOAb positive patients.

**Figure 4A-B.** Selected thyroid ultrasound images from sunitinib-treated patients with metastatic cancer. A. Before sunitinib therapy patient #4 showing normal thyroid ultrasound (left lobe); B. Patient #4 eight months after sunitinib therapy, showing marked reduction of the left lobe size associated to a hypoechoic thyroiditis-like pattern.

Figure 1

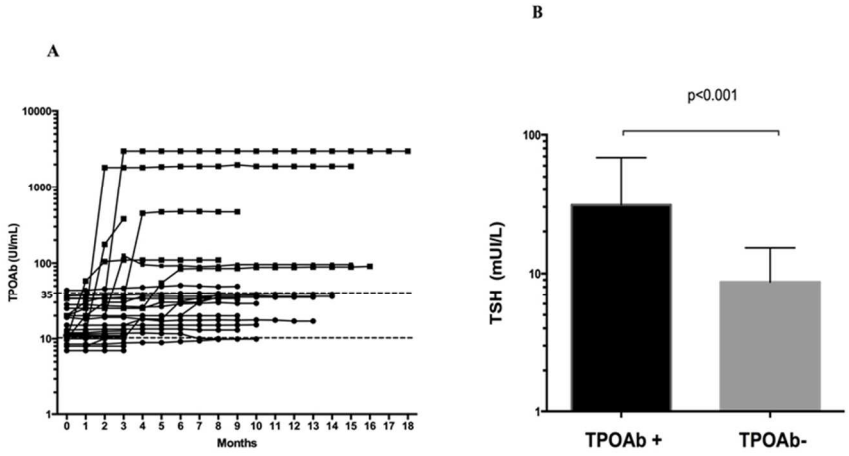


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Figure 2

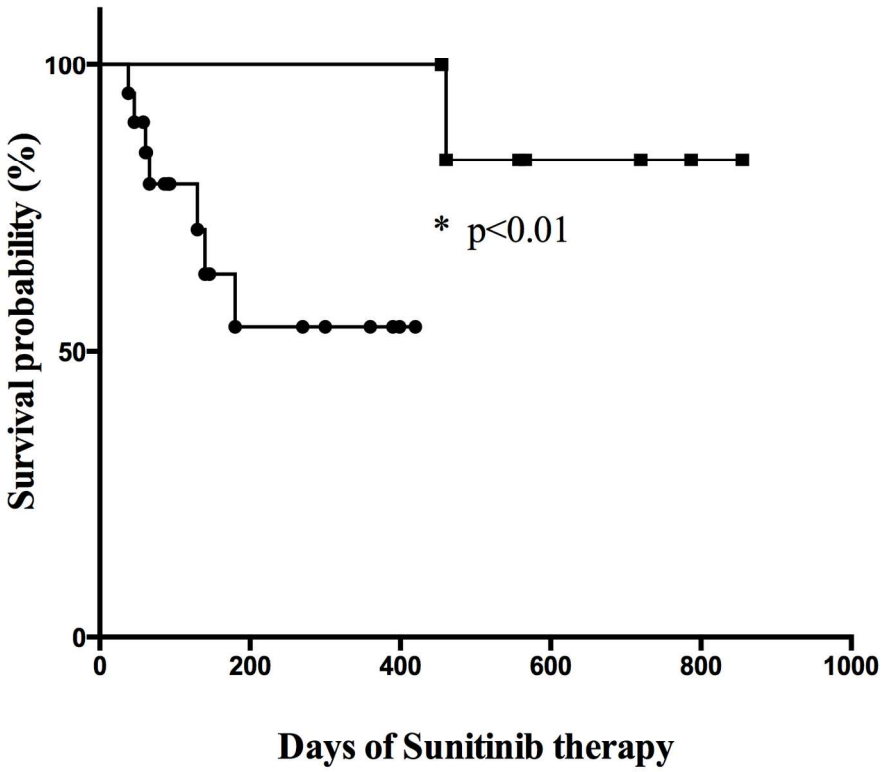


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Figure 3

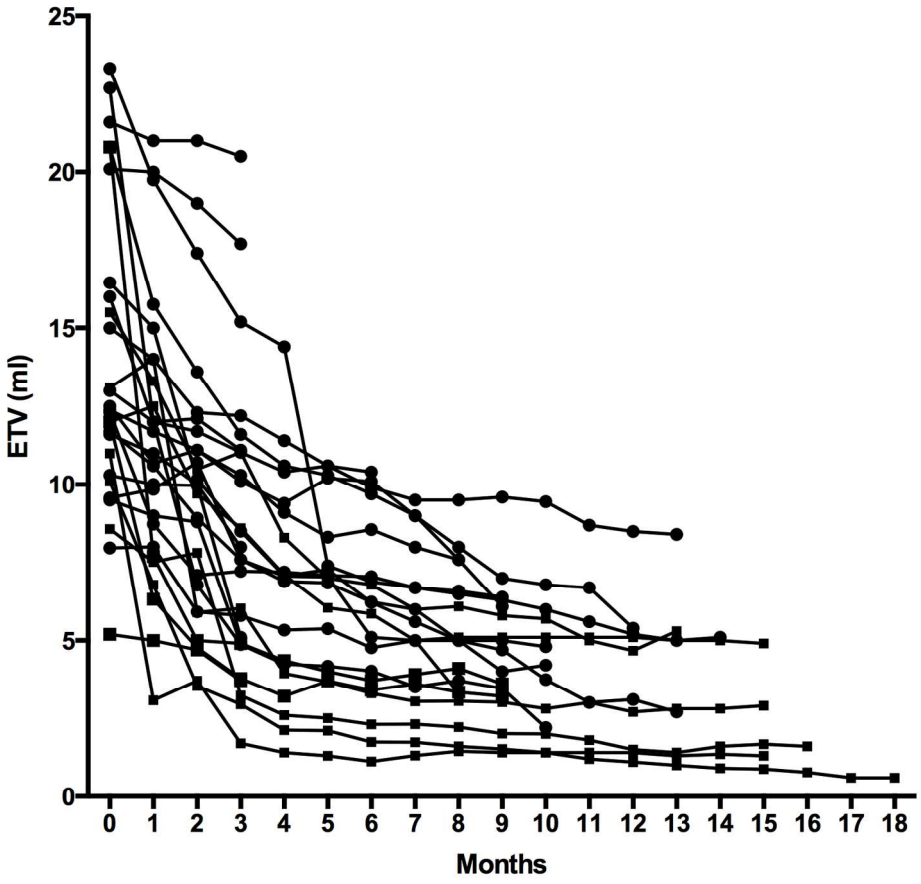


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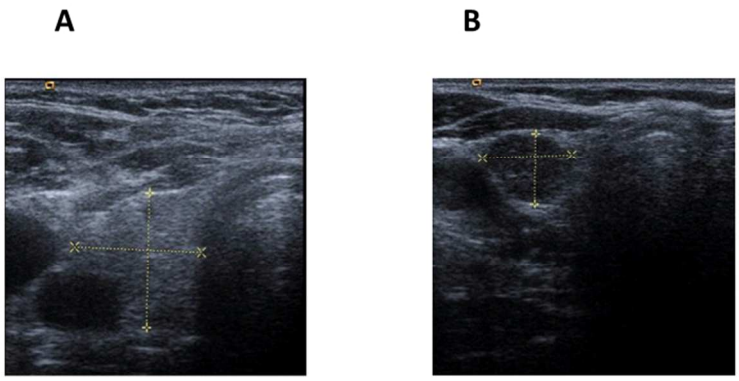
Figure 4

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