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Sunitinib in patients with chemotherapy-refractory thymoma and thymic carcinoma: an open-label phase 2 trial

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Summary

Background—No standard treatments are available for advanced thymic epithelial tumours after failure of platinum-based chemotherapy. We investigated the activity of sunitinib, an orally administered tyrosine kinase inhibitor.

Methods—Between May 15, 2012, and Oct 2, 2013, we did an open-label phase 2 trial in patients with histologically confirmed chemotherapy-refractory thymic epithelial tumours. Patients were eligible if they had disease progression after at least one previous regimen of platinum-containing chemotherapy, an Eastern Cooperative Oncology Group performance status of two or lower, measurable disease, and adequate organ function. Patients received 50 mg of sunitinib orally once a day, in 6-week cycles (ie, 4 weeks of treatment followed by 2 weeks without treatment), until tumour progression or unacceptable toxic effects arose. The primary endpoint was investigator-assessed best tumour response at any point, which we analysed separately in thymoma and thymic carcinoma cohorts. Patients who had received at least one cycle of treatment

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Contributors

AT, AR, YT, JBT, ES, and GG contributed to study design, data acquisition, data interpretation, and writing and approval of the final version of the report. PJL contributed to study design, data acquisition, and approval of the final version of the report. AB, CB, AJS, CAC, UG, M-JL, SL, AL, YW, and PM contributed to data acquisition, data interpretation, and approval of the final version of the report. SMS contributed to study design, statistical analyses, and writing and approval of the final version of the report.

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Declaration of interests

We declare no competing interests.

and had their disease reassessed were included in the analyses of response. The trial was registered with ClinicalTrials.gov, number NCT01621568.

Findings—41 patients were enrolled, 25 with thymic carcinoma and 16 with thymoma. One patient with thymic carcinoma was deemed ineligible after enrolment and did not receive protocol treatment. Of patients who received treatment, one individual with thymic carcinoma was not assessable because she died. Median follow-up on trial was 17 months (IQR 14·0–18·4). Of 23 assessable patients with thymic carcinoma, six (26%, 90% CI 12·1–45·3, 95% CI 10·2–48·4) had partial responses, 15 (65%, 95% CI 42·7–83·6) achieved stable disease, and two (9%, 1·1–28·0) had progressive disease. Of 16 patients with thymoma, one (6%, 95% CI 0·2–30·2) had a partial response, 12 (75%, 47·6–92·7) had stable disease, and three (19%, 4·1–45·7) had progressive disease. The most common grade 3 and 4 treatment-related adverse events were lymphocytopenia (eight [20%] of 40 patients), fatigue (eight [20%]), and oral mucositis (eight [20%]). Five (13%) patients had decreases in left-ventricular ejection fraction, of which three (8%) were grade 3 events. Three (8%) patients died during treatment, including one individual who died of cardiac arrest that was possibly treatment-related.

Interpretation—Sunitinib is active in previously treated patients with thymic carcinoma. Further studies are needed to identify potential biomarkers of activity.

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Introduction

Thymic epithelial tumours are rare cancers, yet they are the most common tumours of the anterior mediastinum, accounting for 20% of all mediastinal cancers.[1] On the basis of morphological features and atypia of thymic epithelial cells and their relative proportion to lymphocytes, thymic epithelial tumours are classified as either thymomas (further subclassified into types A, AB, B1, B2, and B3) or thymic carcinomas.[2] Although thymomas and thymic carcinomas share the same neoplastic cell of origin, thymic carcinomas are more aggressive, are less responsive to chemotherapy, and have an increased likelihood of producing distant metastases. 10-year survival for B1 thymomas is 95%, whereas 5-year survival for thymic carcinomas is only 30–50%.[3]

Surgery is the mainstay of treatment for thymic epithelial tumours and is the only potentially curative option. Patients with unresectable disease and recurrence after radical surgery usually receive palliative chemotherapy. Studies of platinum-based regimens for thymic carcinoma have shown objective responses in 55–90% of patients and 5-year survival of 30–55%, although these studies had small numbers of patients with thymic carcinoma.[4,5] No standard treatments are available after failure of platinum-based chemotherapy. With its poor prognosis, the paucity of systemic treatments is particularly evident in thymic carcinoma, for which several targeted agents have yielded disappointing results.[6]

Sunitinib is an oral tyrosine kinase inhibitor, including VEGFR, KIT, and PDGFR.[7] Although limited, available evidence suggests that angiogenesis has an important role in thymic epithelial tumours; VEGF is overexpressed in these cancers, and VEGF expression and microvessel density are associated with invasiveness and stage.[8,9] Higher serum concentrations of VEGF and b-FGF have been noted in patients with thymic carcinoma.[10]

Overexpression of KIT has been reported in about 80% of thymic carcinomas, and mutations in the gene encoding this receptor are noted in about 10% of these cancers.[11,12] PDGF and PDGFRα are also overexpressed in thymic epithelial cells.[13] Anecdotal reports have suggested that drugs targeting VEGF, KIT, or PDGF might be efficacious in thymic epithelial tumours.[14] Strobel and colleagues[15] reported activation of multiple receptor tyrosine kinases and responses to sunitinib in three of four patients with thymic carcinoma. Immune dysfunction at several levels has been recorded in patients with thymic epithelial tumours, and abnormal immune surveillance might underlie tumorigenesis and progression of these cancers.[16] Sunitinib has been reported to modulate immune cells to improve T-cell function and reverse immunosuppression.[17,18]

We designed an open-label phase 2 study to investigate response to sunitinib in patients with thymic epithelial tumours who had progressive disease after at least one previous regimen of platinum-based chemotherapy. Taking into account the different biology and historically discrepant responses and survival of thymoma and thymic carcinoma, we enrolled patients with these tumour types in two separate cohorts.

Methods

Study design and patients

We did an open-label, single-arm, phase 2 study at two centres in the USA—the Center for Cancer Research, National Cancer Institute (NCI; Bethesda, MD), and the Indiana University Medical Center (Indianapolis, IN). The study population consisted of patients aged 18 years or older with: advanced thymoma or thymic carcinoma not amenable to potentially curative treatments; disease progression after failure of at least one previous line of platinum-based chemotherapy; life expectancy of longer than 3 months; measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1; Eastern Cooperative Oncology Group (ECOG) performance status of 2 or less; blood pressure of 140/90 mm Hg or lower; and adequate organ and bone marrow function. Pathologists at the individual institutions confirmed histological diagnosis. Detailed eligibility criteria are provided in the appendix (p 1).

All patients provided written informed consent. The study was approved by the institutional review boards of the participating centres.

Procedures

We administered 50 mg of sunitinib orally once a day in 6-week cycles. Each 6-week cycle consisted of 4 weeks of treatment followed by 2 weeks without treatment. We allowed two dose reductions (to 37·5 mg and 25 mg) and treatment interruption of up to 3 weeks (for manage ment of adverse events). We continued treatment until disease progression or unacceptable adverse events.

Baseline assessments included patient history; physical examination; CT of the chest, abdomen and pelvis; laboratory tests (haematology, urinalysis, coagulation, blood chemistry, and pregnancy test); and electrocardiogram (ECG). We initially assessed tumour responses (according to RECIST version 1.1) at the end of two treatment cycles. After three patients

had been enrolled, we amended this plan to allow response assessment after every 6-week cycle for 12 months, and every two cycles thereafter. We confirmed responses at least 4 weeks after initial documentation. We graded adverse events according to NCI Common Toxicity Criteria version 4.0.

We obtained blood samples for pharmacodynamic analyses (only from patients enrolled at NCI) before sunitinib was administered on day 1 of cycle one (pretreatment analysis) and before treatment on day 1 of cycles two and three. Correlative assays were only done in patients with available samples. We analysed immune cells including regulatory T cells (Tregs; $CD4^{+}CD25^{hi}Foxp3^{+}$) and CD8+ T cells, which we assessed for expression of immune checkpoint receptors (CTLA4 and PD-1; antibodies for individual T-cell subsets are described in the appendix pp 1–2). We also analysed circulating endothelial cells, circulating endothelial progenitor cells, and circulating tumour cells. We did exon-capture sequencing of 197 cancer-related genes[19,20] on DNA derived from formalin-fixed paraffin-embedded tissue. Assessment and methodological details are provided in the appendix (pp 1–2).

Outcomes

The primary endpoint was objective response, defined as the proportion of patients in each cohort (thymoma or thymic carcinoma) with a complete or partial response at any point. We confirmed responses at least 4 weeks after initial documentation. Secondary endpoints were progression-free survival, overall survival, and duration of response. We measured progression-free survival from the on-study date until date of progression, with censoring at the date of last follow-up if the patient had not progressed. We measured overall survival from the on-study date until date of death, with censoring at the last follow-up date if the patient was alive. We measured duration of response from the time an objective response was first recorded until the date of progressive disease.

Statistical analysis

We assessed the activity of sunitinib based on investigator-assessed tumour response in all assessable patients and overall survival in the modified intention-to-treat population. Patients who received at least one cycle of treatment and had their disease reassessed were judged assessable for response. We assessed safety in patients who received one or more doses of sunitinib. We selected different types of two-stage design to accommodate an appropriate response question because we expected slower accrual of patients with thymomas. We set the type I error at 0·10 and type II error at 0·10 for both cohorts (thymoma and thymic carcinoma). For thymic carcinomas, we used a Simon optimum two-stage design to rule out a 5% objective response (p_0=0·05) and to target a 25% objective response (p_1=0·25).[21] Nine patients with thymic carcinomas were to be enrolled in the first stage and, if one or more responses were seen, a total of 24 patients were to be treated. A total of one or two responses in 24 patients would be judged insufficient for further assessment, whereas three or more responses would be consistent with the 25% target response rate to warrant further study. For thymomas, we used a MiniMax design to rule out a 10% objective response (p_0=0·10) and to target a 30% objective response (p_1=0·30). 16 patients were to be enrolled in the initial stage, to a total of 25 patients if two or more of the first-stage patients had a response. A total of two to four responses in 25 patients would be

insufficient for further assessment, whereas five or more responses in 25 assessable patients would be consistent with the 30% target response rate to warrant further investigation.

We used the Kaplan-Meier method to obtain estimates of overall and progression-free survival; we compared curves with a two-tailed log-rank test, but used an exact test when the number of events was very small. We divided values for biomarkers at their observed median at baseline (before treatment), on day 1 of cycle two, and on day 1 of cycle three, and with respect to changes since baseline. We assessed differences in paired biomarkers, or changes in biomarkers between two timepoints, with a Wilcoxon signed-rank test. We used an exact Wilcoxon rank sum test to compare differences in biomarker values between two groups of patients. No patients died before day 1 of cycle two or day 1 of cycle three in the appropriate curves based on biomarkers; therefore, analyses begin at the on-study date rather than using a landmark approach.

All p values are two-sided and reported without adjustment for multiple comparisons. Because of the large number of exploratory marker analyses, we only judged p values of less than 0·005 statistically significant. SAS version 9.3 and StatXact version 9 were used for statistical analyses. This trial is registered with ClinicalTrials.gov, number NCT01621568.

Role of the funding source

Sunitinib was provided by Pfizer and distributed by the Cancer Therapy Evaluation Program (CTEP). The funder of the study (CTEP) provided input on study design and approved the study protocol and final version of the report, but had no role in data collection, data analysis, or data interpretation. AT, AR, AB, YT, M-JL, SL, JBT, YW, and GG had access to the raw data. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between May 15, 2012, and Oct 2, 2013, 41 patients were enrolled—25 with thymic carcinoma and 16 with thymoma (table 1; appendix p 9). One patient with thymic carcinoma was subsequently deemed ineligible for the trial because he had previously received an investigational multikinase inhibitor. Table 1 summarises baseline demographic characteristics. The median age of all patients was 57·5 years (range 31–81); 18 (45%) of 40 patients were women and 36 (90%) of 40 had an ECOG performance score of 0 or 1. Of 16 patients with thymoma, 13 (81%) had B2 or B3 histological features. 20 (83%) of 24 patients with thymic carcinoma and nine (56%) of 16 with thymoma had extrathoracic metastases. Both cohorts had received a median of two previous treatments: 14 (58%) of 24 patients with thymic carcinoma and 13 (81%) of 16 patients with thymoma had received at least two previous regimens.

40 of 41 enrolled patients received sunitinib according to the protocol. The patient deemed ineligible because he had previously received an investigational multikinase inhibitor did not receive protocol treatment. The median duration of treatment was 6·1 months (range 0·4–19·0; IQR 3·1–9·1) for patients with thymic carcinoma and 6·9 months (range 1·4–19·8; IQR 4·0–10·8) for those with thymoma. At data cutoff (April 9, 2014), 19 (79%) of 24 patients

with thymic carcinoma had received sunitinib for at least 3 months and three were still on treatment. Of those with thymoma, 14 (88%) of 16 had received treatment for at least 3 months and none remained on treatment.

Of 24 patients with thymic carcinoma, 23 were assessable for tumour response. One patient died 11 days after starting treatment and was not assessable for response. Six patients (26%, 90% CI 12·1–45·3, based on the formal two-stage design; 95% CI 10·2–48·4) of 23 with thymic carcinoma had a partial response (figure 1A, table 2). Nine patients (39%, 95% CI 19·7–61·5) had tumour shrinkage ranging from 10% to 30%. Responses to sunitinib were mostly rapid and durable in patients with thymic carcinoma, with continued administration of the treatment. Median time to response for the six patients with a partial response was 5·6 months (range 2·7–13·8) and the median duration of response was 16·4 months (range 1·4–16·4). Response could not be confirmed in one patient with thymic carcinoma who achieved a partial response at 13·8 months because treatment with sunitinib was discontinued in order to treat brain metastases. At the time of data cutoff, two responses were ongoing (8·3 months each). Treatment was discontinued in one responder because of adverse effects at 3·4 months. All 16 patients with thymoma were assessable for tumour response. Of these, one patient (6%, 95% CI 0·2–30·2) with type B3 histology had a partial response (figure 1B, table 2). The thymoma cohort was closed after enrolment of 16 patients because of insufficient activity.

Disease control (partial response and stable disease) was achieved in 21 (91%, 95% CI 72·0–98·9) of 23 assessable patients with thymic carcinoma and 13 (81%, 54·4–96·0) of 16 assessable patients with thymoma (table 2). Median progression-free survival was 7·2 months (95% CI 3·4–15·2) for patients with thymic carcinoma and 8·5 months (2·8–11·3) for those with thymoma (figure 2A). After median follow-up of 17 months (IQR 14·0–18·4), median overall survival was not reached for patients with thymic carcinoma but was 15·5 months (95% CI 12·6 to undefined) for individuals with thymoma (figure 2B). Overall survival at 1 year was estimated to be 78% (95% CI 58·0–90·4) for thymic carcinoma and 86% (60·9–96·1) for thymoma.

Grade 3 and 4 treatment-related adverse events were recorded in 28 (70%) of 40 patients. Table 3 shows grade 1–2 treatment-related adverse events that arose in at least 10% of patients and all grade 3–5 treatment-related adverse events. The most common treatment-related adverse events of grade 3 or higher were lymphocytopenia (eight [20%] patients), fatigue (eight [20%]), and oral mucositis (eight [20%]). Five (13%) patients had decreases in left-ventricular ejection fraction, three (8%) of which were grade 3 events.

Three (8%) of the 40 patients treated—two with thymic carcinoma and one with thymoma—died while receiving sunitinib. The causes of death in the thymic carcinoma patients were progressive disease and sepsis secondary to urinary-tract infection. The patient who died from progressive disease was a 45-year-old man who had disease progression after two cycles of front-line platinum-based chemotherapy before enrolment to this trial. He continued to have clinical and radiographic evidence of disease progression during treatment with sunitinib and died 5 weeks after starting treatment. Tumour involvement from the superior mediastinum to the inferior border of the heart, with encasement of the great vessels

and severe restriction of the pulmonary artery outflow tract and the left pulmonary artery, was confirmed by autopsy. The patient with thymoma died of cardiac arrest caused by ventricular fibrillation, which was deemed to be treatment-related. He had a previous history of myocardial infarction and atrioventicular block and had undergone coronary-artery bypass grafting and pacemaker placement.

Reasons for study discontinuation in the thymic carcinoma cohort were disease progression in 15 (63%) of 24 patients and adverse events in five (21%) patients. Adverse events that led to treatment discontinuation were grade 3 declines in left-ventricular ejection fraction (two patients), intolerable tumour pain (one grade 3 and one grade 2), and grade 2 mucositis (one patient). Reasons for study discontinuation in the thymoma cohort were disease progression in 11 (69%) of 16 patients, patient's choice in two (13%) patients, and adverse events in two (13%; grade 3 and grade 2 declines in left-ventricular ejection fraction) patients.

15 (63%) of 24 patients with thymic carcinoma needed dose reductions, including seven (29%) patients who had two dose reductions. 11 (69%) of 16 thymoma patients needed dose reductions, including five (31%) patients who had two dose reductions. Adverse events were the primary reason for dose reductions.

In the thymoma cohort, two (13%) patients—one with pure red-cell aplasia and one with hypo gamma-globulinaemia—developed autoimmune disorders while on treatment. The frequency of autoimmune disorders before, during, and after treatment with sunitinib is summarised in the appendix (p 10).

Of 33 patients enrolled at NCI (in whom correlative studies were done), 25 patients (in both cohorts) had blood samples available for assessment of circulating endothelial cells. 14 patients had a reduction or no change in the proportion of apoptotic circulating endothelial cells among nucleated cells on day 1 of cycle three; this subgroup showed significantly improved overall survival compared with nine patients in whom the proportion of circulating endothelial cells increased (p=0·002; figure 3A). In the thymic carcinoma cohort, 11 patients had a decrease or no change in the proportion of circulating endothelial progenitor cells among nucleated viable cells on day 1 of cycle three; these individuals had slightly better but not statistically significant overall survival than did two patients in whom the proportion of circulating endothelial progenitor cells increased (p=0·01; figure 3B).

To investigate associations between circulating tumour cells and outcomes, thresholds of one circulating tumour cell per 10 mL peripheral blood up to 11 circulating tumour cells per 10 mL peripheral blood were systematically compared before treatment and on day 1 of cycle two; an optimum threshold of ten cells per 10 mL was identified. Assessment of circulating tumour cells before treatment and on day 1 of cycle two was done in 16 patients and 19 patients, respectively. Patients with ten or more circulating tumour cells per 10 mL peripheral blood before treatment and on day 1 of cycle two had slightly shorter overall survival (p=0·006 and p=0·0095, respectively; figures 3C and 3D). Patients who had a reduction in the circulating tumour cell count of greater than the threshold of 45% had slightly longer (but not significantly so) progression-free survival compared with those in

whom an increase in circulating tumour cells was noted or a reduction of less than 45% from threshold was seen (p=0·02).

Treg PD-1 expression was assessed before treatment and on day 1 of cycles two and three. 28 patients (from both cohorts) had samples available before treatment and on day 1 of cycle two; 22 patients had samples available on day 1 of cycle three. 19 of 28 patients had a non-significant increase in Treg PD-1 expression after treatment (day 1 of cycle two, p=0·0095; day 1 of cycle three, p=0·03; figure 4A). Four patients with a decrease in Treg PD-1 expression on day 1 of cycle three had significantly worse overall survival compared with 18 patients with an increase or no change (p=0·0001; figure 4B). CD8+ T-cell CTLA4 expression also rose significantly in the total study population (day 1 of cycle two, p<0·0001; day 1 of cycle three, p<0·0001; figure 4C). 15 patients with an increase in CD8+ T-cell CTLA4 expression that was greater than the median on day 1 of cycle two of had better overall survival than did 13 patients with smaller increases (p=0·008; figure 4D). Landmark analyses for the above markers are presented in the appendix (pp 3–8).

Immune alterations noted in patients with thymoma differ from those recorded in patients with thymic carcinoma. Furthermore, even within the subtypes of thymoma, immunological findings are likely to vary between the different histological features. Separate analyses of exploratory markers for thymoma and thymic carcinoma are presented in the appendix (p 11). Results differed slightly by histological features and remained roughly consistent with the overall results, despite low numbers of patients. Predictive and pharmacodynamic assessments were based on a subset of patients with available blood samples, and testing of a statistical hypothesis was not prespecified. These preliminary results must, therefore, be interpreted with caution.

22 patients (13 with thymic carcinoma, five with B2 thymoma, three with B3 thymoma, and one with uncategorised thymoma) had adequate formalin-fixed paraffin-embedded tissue for molecular profiling. Four of six patients with thymic carcinoma who had partial responses were included in this analysis. 19 somatic variations affecting 12 genes were identified in eight patients with thymic carcinoma and one with thymoma. In 13 tumours, no somatic variations were detected. The most commonly mutated genes were *TP53* and *DNMT3A* (full data not shown). No association was recorded between a specific mutation and response or survival. None of the patients assessed had *KIT* mutations.

Discussion

Findings of this open-label phase 2 trial of sunitinib in patients with refractory thymic epithelial tumours and good performance status showed that sunitinib was active in patients with thymic carcinoma, whereas activity was limited in those with thymoma. To our knowledge, our trial is the first to show robust and durable clinical activity of a targeted agent in previously treated patients with thymic carcinoma (panel). An objective response was recorded in 26% of patients with thymic carcinoma, with median duration of response of 16·4 months. This finding is particularly meaningful because most patients were heavily pretreated—58% had failed two or more previous lines of treatment.

Thymic carcinomas are less common than thymomas but they are much more aggressive and are more likely to metastasise to the liver, lymph nodes, and bones.[22] At present, no standard treatments are available for patients with thymic carcinoma when platinum-based chemotherapy has failed. Because of the rarity of this disease, no randomised trials have been done to date. Compared with thymomas, thymic carcinomas have proven less responsive to chemotherapy, have shorter progression-free intervals, and are associated with abbreviated overall survival. Prospective trials of several investigational targeted agents for thymic carcinoma have yielded disappointing results in this setting (appendix pp 12–13).

Taking into account the disparate biology and clinical course of thymic carcinoma and thymoma, we enrolled patients in two separate cohorts. With an objective response of 26% in patients with thymic carcinoma, the study met its primary endpoint of a 25% objective response for this cohort. Furthermore, more than 90% of patients with thymic carcinoma—a hitherto difficult-to-manage subgroup of thymic epithelial tumours—achieved disease control. Our study is the first clinical trial to show benefit for patients with thymic carcinoma who have failed platinum-based treatment and, thus, represents an advance for such patients. However, activity of sunitinib was diminished in patients with thymoma, with only one (6%) partial response recorded in an individual with B3 thymoma. Although a distinct entity from thymic carcinoma according to histological classification,[2] this subtype of thymic epithelial tumours does share some similarities with thymic carcinoma at a molecular level.[19,20,23]

Overall, sunitinib was well tolerated in our study, with adverse events reported that did not differ substantially from those described in other cancers. The frequency of declines in left-ventricular ejection fraction was comparable with those reported previously[24] and could be judged acceptable in view of the aggressive nature of the disease and its poor prognosis. Nevertheless, considering concurrent cardiac risk factors in patients with thymic epithelial tumours—eg, previous exposure to anthracyclines, radiation, and high rates of subclinical cardiac tumour involvement[25]—careful monitoring of cardiac function is needed. About two-thirds of patients in both cohorts needed dose reductions. Further studies are needed to address the effect of alternative schedules on adverse events and clinical efficacy.

Circulating endothelial cells and circulating endothelial progenitor cells are potential biomarkers of response to anti-angiogenic treatments.[26] Circulating endothelial cells are apoptotic or necrotic cells, released into the bloodstream as a by-product of tumour vascular turnover, and cell numbers increase after vascular damage.[26] Circulating endothelial progenitor cells might contribute to neovascularisation by releasing pro-angiogenic cytokines and by being incorporated into nascent blood vessels.[27] In this study, reduced or stable levels of circulating endothelial cells levels after administration of sunitinib was associated with improved overall survival in the total study population and in patients with thymic carcinoma. Further investigations are needed to clarify the anti-angiogenic effects of sunitinib in thymic epithelial tumours and the potential correlation of these biomarkers with efficacy.

Circulating tumour cells, which are shed from primary or metastatic tumour deposits, have been exploited for non-invasive quantification of tumour response.[28] In this study, the

baseline level of circulating tumour cells and the change in this amount after administration of sunitinib were identified as potential predictors of overall survival. Prospective characterisation of circulating tumour cells in larger populations of patients is needed to establish the clinical relevance of circulating tumour cells as a prognostic factor in patients with thymic epithelial tumours treated with sunitinib.

After administration of sunitinib, we noted increases in Treg PD-1 expression and CD8+ T-cell CTLA4 expression in most patients, which were associated with improved overall survival. Because T-cell immune activation results in upregulation of immune checkpoint receptors as a homoeostatic mechanism,[29] increased expression of immune checkpoint receptors after administration of sunitinib might be a marker of recent immune activation of T cells in response to the drug, potentially in response to sunitinib-induced tumour shrinkage. At the same time, findings of clinical trials have validated that blockade of PD-L1/PD-1 signalling[30] and CTLA4[31] is a meaningful therapeutic regimen in selected cancers. Thus, T-cell antitumour immunity in thymic epithelial tumours treated with sunitinib might be limited by escalated expression of immune checkpoint receptors. A combi nation of sunitinib with an immune checkpoint inhibitor could, therefore, have a synergistic effect and potentially enhance antitumour responses. A trial addressing this hypothesis would entail administration of immune checkpoint inhibitors sequentially after sunitinib and could, in theory, abrogate the immuno suppressive effects of sunitinib-induced immune check point receptor upregulation.

Tumour molecular profiling was done in patients with available samples to assess variants that might predict response. The panel of genes selected for exon-capture sequencing included those encoding receptor tyrosine kinases that are known targets of sunitinib, including *PDGFRA*, *FLT3*, and *KIT*. Although somatic variations were identified in 62% of patients with thymic carcinoma, they were not predictive of response to sunitinib.

The limitations of our trial include the small sample size, its single-arm design, and the exploratory nature of the correlative studies. Our exploratory findings should be interpreted with caution in view of the limited samples and heterogeneity in immune alterations among subtypes of thymoma and thymic carcinoma; the results need to be confirmed in larger cohorts representing the individual tumour histological features. We used the standard dose of sunitinib, which has been approved for use in several other cancers. However, dose reductions were needed for most patients, which raises the possibility that the standard dose of sunitinib might be too high for patients with thymic epithelial tumours. An ongoing trial is investigating an alternative schedule of sunitinib (50 mg daily for 2 weeks with 1 week off, to constitute a 3-week cycle) in patients with thymic carcinoma, to assess clinical activity and toxic effects (NCT01621568). Although most patients with thymic carcinoma had tumour shrinkage on sunitinib, identification of a mechanism-based predictive marker to select patients who would derive the maximum benefit would strengthen clinical management decisions. However, we did not identify a predictive marker for response to sunitinib. Analyses of existing scientific literature suggest that alterations in KIT, although frequent, might not be a determinant response to sunitinib in thymic carcinoma.[32] We did not assess expression of KIT in tumours, and *KIT* mutations were not recorded in the tumours that were tested.

The responses in thymic carcinoma that we recorded in our study are exceptional compared with other second-line treatments. Despite the limitations of our trial, our findings indicate that sunitinib could be a treatment option for patients with thymic carcinoma whose disease has progressed after platinum-based chemotherapy, a population for whom, currently, no standard treatments are available. To our knowledge, these findings are the first prospective data of a targeted drug to show clinical activity in heavily pretreated patients with thymic carcinoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Panel: Research in context

Systematic review

We searched PubMed between January, 2003, and July, 2011, with the terms "thymoma", "thymic carcinoma", and "sunitinib". We identified one case series[15] in which activation of multiple receptor tyrosine kinases was reported, in addition to responses to sunitinib in three of four patients with thymic carcinoma. Further rationale for our current study was derived from anecdotal reports suggesting activity of sorafenib, another inhibitor of multiple receptor tyrosine kinases, in thymic epithelial tumours. Our phase 2 trial was initiated to assess the clinical activity of sunitinib in thymic epithelial tumours.

Interpretation

The results of this phase 2 trial indicate that sunitinib is an active treatment option for patients with thymic carcinoma whose disease has progressed after platinum-based chemotherapy, a population for whom currently no standard treatments are available. To our knowledge, these findings are the first prospective data of a targeted drug to show clinical activity in heavily pretreated patients with thymic carcinoma. However, sunitinib had limited activity in thymoma and tumour molecular profiling did not identify any somatic variations that were predictive of response to sunitinib. Sunitinib should be considered as a treatment option for patients with thymic carcinoma who progress after first-line platinum-based chemotherapy.

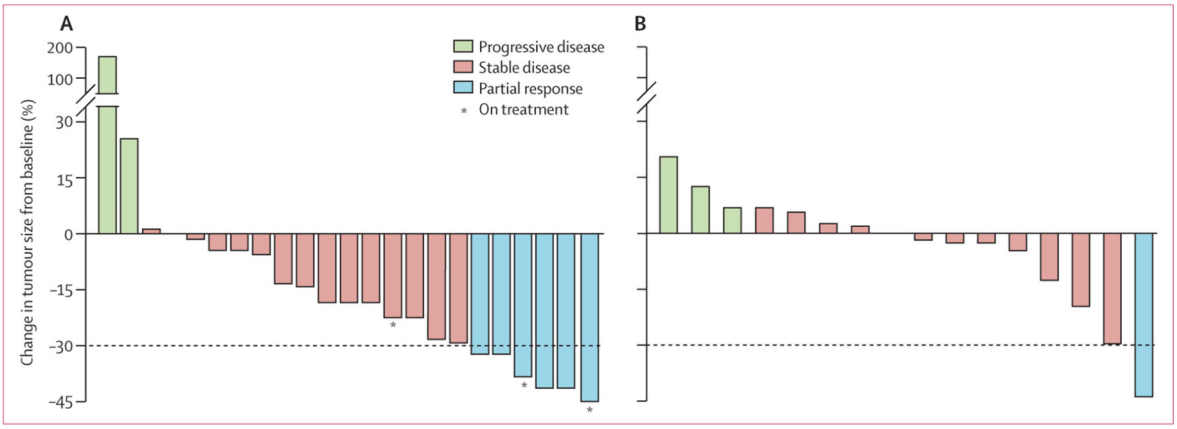

Figure 1. Waterfall plots of tumour responses to sunitinib

Responses in patients with (A) thymic carcinoma and (B) thymoma. Of three patients with thymoma who had progressive disease, two came off treatment because of the appearance of new lesions and one stopped treatment owing to a 20% increase in tumour size. All three had progressive disease at the first restaging timepoint.

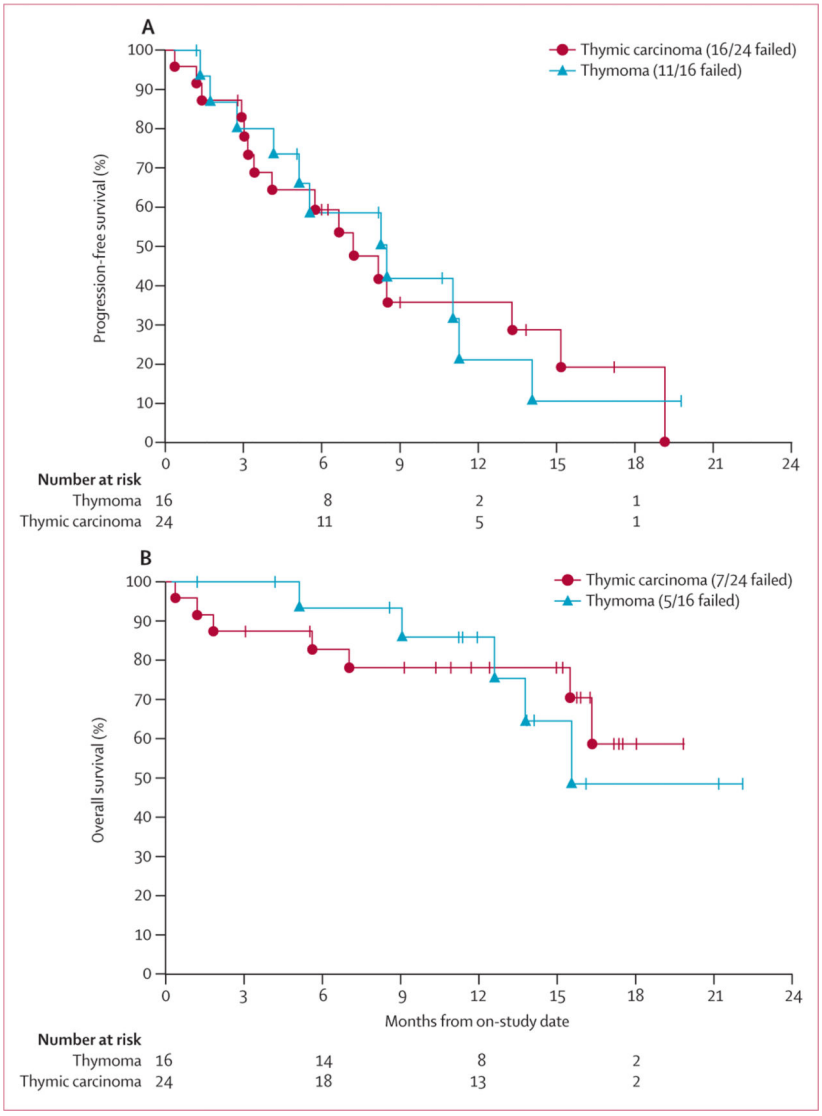

Figure 2. Survival in patients with thymoma and thymic carcinoma

(A) Progression-free survival and (B) overall survival.

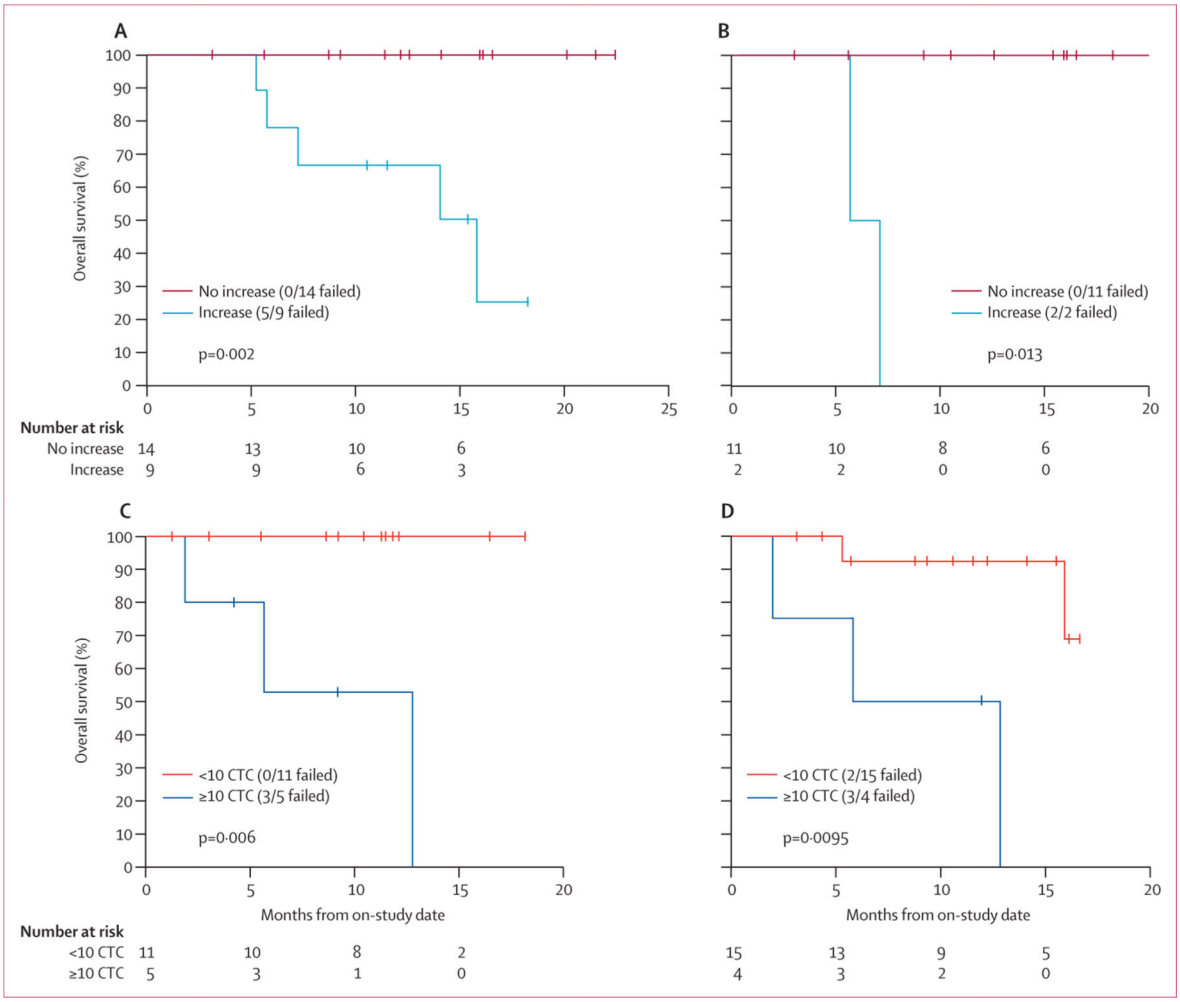

Figure 3. Survival according to predictive biomarkers

(A) Overall survival in the total study population of patients with either no increases (ie, reductions or no changes) or increases in the proportion of apoptotic circulating endothelial cells among nucleated cells on day 1 of cycle three. (B) Overall survival of patients with thymic carcinoma with either no increases (ie, reductions or no changes) or increases in the proportion of circulating endothelial progenitor cells among nucleated viable cells on day 1 of cycle three. (C) Overall survival of patients with ten or more circulating tumour cells (CTCs) per 10 mL of peripheral blood at baseline, and of those with less than ten cells. (D) Overall survival of patients with ten or more CTCs per 10 mL of peripheral blood on day 1 of cycle two, and of those with less than ten cells.

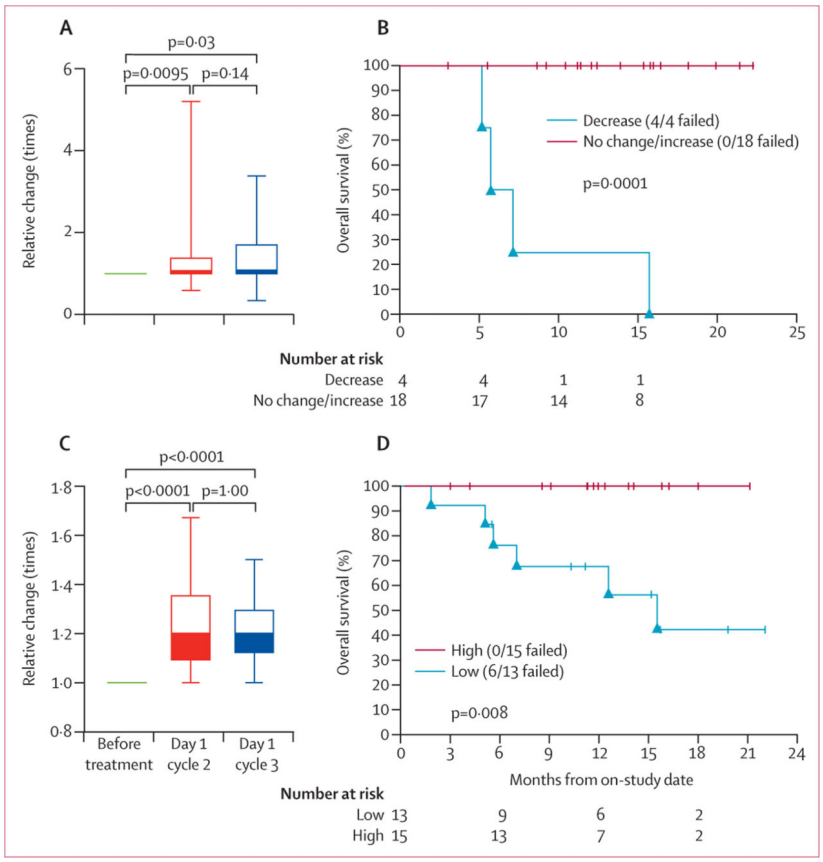

Figure 4. Changes in immune subsets with sunitinib in thymic epithelial tumours

(A) Relative changes in peripheral blood Treg PD-1 expression before treatment, on day 1 of cycle two, and on day 1 of cycle three. (B) Overall survival of patients either with decreases or with no changes or increases in Treg PD-1 expression on day 1 of cycle three. (C) Relative changes in CTLA4 expression on peripheral blood CD8+ T cells before treatment, on day 1 of cycle two, and on day 1 of cycle three. (D) Overall survival of patients with a high (ie, above th e median) increase in CD8+ T-cell CTLA4 expression on day 1 of cycle two, and of those with a low (ie, below the median) increase in CTLA4 expression.

Table 1

Baseline characteristics of patients

	Thymoma (n=16)	Thymic carcinoma (n=24)
Age (years)	54 (31–74)	58 (41–81)
Sex		
Men	7 (44%)	15 (63%)
Women	9 (56%)	9 (38%)
Ethnic origin		
White	13 (81%)	23 (96%)
Black	3 (19%)	1 (4%)
ECOG performance status*		
0	4 (25%)	6 (25%)
1	11 (69%)	15 (63%)
2	1 (6%)	3 (13%)
Histological type, thymoma		
B1	2 (13%)	· ·
B2	5 (31%)	· ·
B3	8 (50%)	· ·
Uncategorised	1 (6%)	· ·
Histological type, thymic carcinoma		
Poorly differentiated	· ·	8 (33%)
Well differentiated, squamous-cell	· ·	4 (17%)
Non-keratinising, squamous-cell	· ·	4 (17%)
Poorly differentiated, squamous-cell	· ·	3 (13%)
Uncategorised	· ·	3 (13%)
Basaloid	· ·	1 (4%)
Neuroendocrine	· ·	1 (4%)
Presence of extrathoracic metastases		
Any	9 (56%)	20 (83%)
None, disease restricted to thorax	7 (44%)	4 (17%)
Previous systemic treatment		
Number of regimens	2 (1–7)	2 (1–5)
Two or more regimens	13 (81%)	14 (58%)
Chemotherapy received in the first-line setting		
Cisplatin, doxorubicin, cyclophosphamide	12 (75%)	10 (42%)
Cisplatin, doxorubicin, cyclophosphamide, belinostat	0	5 (21%)
Carboplatin, paclitaxel	2 (13%)	4 (17%)
Carboplatin or cisplatin, etoposide	1 (6%)	4 (17%)
Carboplatin, gemcitabine	0	1 (4%)
Carboplatin, pemetrexed	1 (6%)	0

Data are number of patients (%) or median (range).

*Based on an assessment of activities of daily living, on a scale from 0 (fully active) to 5 (dead).

Table 2

Clinical activity of sunitinib in thymic malignant diseases

	Thymic carcinoma (n=23)		Thymoma (n=16)	
	Patients (%)	**95% CI**	**Patients (%)**	**95% CI**
Objective response*	6 (26%)	10·2–48·4†	1 (6%)	0·2–30·2
Stable disease	15 (65%)	42·7–83·6	12 (75%)	47·6–92·7
Progressive disease	2 (9%)	1·1–28·0	3 (19%)	4·1–45·7
Disease control	21 (91%)	72·0–98·9	13 (81%)	54·4–96·0

*Objective response includes only partial responses; no complete responses were recorded. All partial responses except one were confirmed by repeat imaging at least 4 weeks later.

†A 90% two-sided CI was constructed based on the parameters for the two-stage design, 12·1–45·3.

Table 3

Treatment-related adverse events

	Grade 1–2*	Grade 3	Grade 4	Grade 5
Haematological adverse events				
Lymphocyte count decreased	23	3	5	0
Platelet count decreased	24	2	2	0
White blood cells decreased	26	4	0	0
Neutrophil count decreased	25	4	0	0
Anaemia	27	0	1	0
Hepatic and renal adverse events				
Hypophosphataemia	9	3	0	0
Hyponatraemia	4	3	0	0
Aspartate aminotransferase increased	26	1	0	0
Alanine aminotransferase increased	17	1	0	0
Alkaline phosphatase increased	9	0	0	0
Bilirubin increased	9	1	0	0
Cholecystitis	0	1	0	0
Hypoalbuminaemia	25	1	0	0
Hypokalaemia	0	1	0	0
Hyperkalaemia	0	1	0	0
Hypocalcaemia	9	0	0	0
Hypomagnesaemia	5	0	0	0
Cardiovascular and respiratory adverse events				
Hypertension	13	5	0	0
Ejection fraction decreased†	0	3	0	0
Dyspnoea	5	1	0	0
Hypoxia	0	1	0	0
Cardiac arrest	0	0	0	1
Cough	4	0	0	0
Gastrointestinal adverse events				
Mucositis, oral	18	8	0	0
Diarrhoea	20	2	0	0
Pancreatitis	0	1	0	0
Anorexia	19	0	0	0
Nausea	17	0	0	0
Dyspepsia	14	0	0	0
Dysguesia	13	0	0	0
Vomiting	13	0	0	0
Oral pain	9	0	0	0
Abdominal pain	8	0	0	0
Gastro-oesophageal reflux disease	5	0	0	0
Dry mouth	4	0	0	0

	Grade 1–2*	Grade 3	Grade 4	Grade 5
Other adverse events				
Fatigue	31	8	0	0
Tumour pain	0	2	0	0
Febrile neutropenia	0	1	0	0
Lung infection	0	1	0	0
Palmar-plantar erythrodysesthesia	13	1	0	0
Headache	5	1	0	0
Oedema of the limbs	9	0	0	0
Oedema of the face	8	0	0	0
Alopecia	7	0	0	0
Hypothyroidism	7	0	0	0
Rash, maculopapular	7	0	0	0
Non-cardiac chest pain	5	0	0	0
Peripheral sensory neuropathy	5	0	0	0
Dry skin	4	0	0	0
Weight loss	4	0	0	0

The highest grade per event per patient is shown.

*Only grades 1 and 2 treatment-related adverse events that occurred in 10% of patients or more are shown.

†Additionally, two patients had grade 2 declines in ejection fraction.