

# Phase II study of everolimus in patients with locally advanced or metastatic transitional cell carcinoma of the urothelial tract: clinical activity, molecular response, and biomarkers

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**Background:** This phase II study assessed the safety and efficacy of everolimus, an oral mammalian target of rapamycin inhibitor in advanced transitional carcinoma cell (TCC) after failure of platinum-based therapy.

**Patients and methods:** Thirty-seven patients with advanced TCC received everolimus 10 mg/day until progressive disease (PD) or unacceptable toxicity. The primary end point was the disease control rate (DCR), defined as either stable disease (SD), partial response (PR), or complete response at 8 weeks. Angiogenesis-related proteins were detected in plasma and changes during everolimus treatment were analyzed. PTEN expression and PIK3CA mutations were correlated to disease control.

**Results:** Two confirmed PR and eight SD were observed, resulting in a DCR of 27% at 8 weeks. Everolimus was well tolerated. Compared with patients with noncontrolled disease, we observed in patients with controlled disease a significant higher baseline level of angiopoietin-1 and a significant early plasma decrease in angiopoietin-1, endoglin, and platelet-derived growth factor-AB. PTEN loss was observed only in patients with PD.

**Conclusions:** Everolimus showed clinical activity in advanced TCC. The profile of the plasma angiogenesis-related proteins suggested a role of the everolimus antiangiogenic properties in disease control. PTEN loss might be associated with everolimus resistance.

**Key words:** angiogenesis, angiopoietin-1, everolimus, mammalian target of rapamycin, transitional carcinoma cell

## Introduction

Bladder cancer is the fourth most common malignancy in men. It is estimated that 69 250 new cases of bladder cancer will be diagnosed in the United States in 2011 with 14,990 cancer-related deaths [1]. Transitional cell carcinoma (TCC) represents the most frequent histological type of bladder cancer, accounting for ~90% of cases. Locally advanced or

metastatic TCC has a poor prognosis with a 5-year overall survival (OS) rate <20% [2]. Platinum-based chemotherapy increases OS in the first-line treatment of metastatic TCC, with a median OS reaching 13–15 months compared with 3–6 months if these patients remain untreated [3, 4]. After platinum failure, only one drug, vinflunine, has been shown to date to improve the median progression free survival (PFS) over best supportive care (BSC) (3.0 versus 1.5 months,  $P = 0.001$ ) although no statistically significant OS increase in the intent-to-treat population was observed (6.9 versus 4.6 months,  $P = 0.287$ ) [5]. Due to the lack of therapeutic options, new agents are urgently needed.

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Activation of the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway is detected in a wide range of cancers, including TCC, and results in uncontrolled cell proliferation [6, 7]. The principal causes of PI3K/Akt/mTOR cascade activation are decreased expression of the tumor suppressor phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and deregulation of PI3K/AKT signaling through overexpression or activation of growth factor receptors and activating mutations in *PI3K*. Inactivation of PTEN is detected in up to 30% of TCC and is associated with tumor aggressiveness and worsened patient outcomes [8, 9]. Activating mutations of *PI3K* are identified in up to 27% of TCC and occur principally in three ‘hot spots’ of the *PIK3CA* gene, E545K (52%) and E542K (24%) in the exon 9 and H1047R (13%) in the exon 20 [10–12].

Angiogenesis is implicated in TCC development and depends mainly on the PI3K/Akt/mTOR pathway, via hypoxia-inducible factor and vascular endothelial growth factor (VEGF) production. Elevated VEGF messenger RNA expression in TCC is associated with high stage and grade, vascular invasion, metastases, and poor PFS [13, 14].

Everolimus is a rapamycin derivative, inhibiting mTOR activity, and approved for the treatment of metastatic renal cell carcinoma (RCC), advanced pancreatic neuroendocrine tumors, and inoperable subependymal giant cell astrocytomas associated with tuberous sclerosis [15–17]. Preclinical studies demonstrated that everolimus controls the bladder cancer cell growth *in vitro* and *in vivo* through inhibition of the mTOR pathway and antiangiogenic effects [18–20].

We conducted a phase II study of everolimus in patients with locally advanced or metastatic TCC whose disease progressed after platinum-based therapy. The primary end point was the disease control rate (DCR) at 8 weeks after treatment initiation.

## patients and methods

### eligibility

Eligible patients were required to have histologically or cytologically confirmed locally advanced or metastatic TCC, documented progression after first-line platinum-based chemotherapy (in neoadjuvant/adjuvant setting or for distant metastases), disease not amenable to curative treatment, at least one measurable lesion according to RECIST, and an Eastern Cooperative Oncology Group performance status of zero to two. An interval of at least 4 weeks since the last cytotoxic chemotherapy, biological therapy, surgery, or radiotherapy was required. Patients were required to have an absolute neutrophil count  $>1500/\mu\text{L}$ , hemoglobin  $>9\text{ g/dL}$ , platelet count  $>100\,000/\mu\text{L}$ , serum creatinine  $<1.5 \times$  the upper limit of normal (ULN), total bilirubin  $<1.5\text{ ULN}$ , alanine aminotransferase (ALT) and aspartate aminotransferase (AST)  $<2.5\text{ ULN}$ , fasting cholesterol  $<300\text{ mg/dL}$ , and fasting triglycerides  $<2.5\text{ ULN}$ .

Patients were excluded if they received more than two systemic treatments for their metastatic disease and previous treatment with mTOR inhibitors. Patients were also ineligible if they had significant cardiovascular disease, active infection, known central nervous system metastases, autoimmune disease, or other malignancies within 5 years, excluding adequately treated carcinoma *in situ* of the cervix or basal or squamous cell skin cancer.

The translational and clinical parts of the study were approved by an independent ethics committee and the Belgian health authority in accordance with European regulations and conducted in accordance with the Declaration of Helsinki (October 2000). Translational research was prospectively planned, and patients provided informed consent for plasma and tumor storage.

### study objectives and outcome

This was an open-label, multicenter single-arm phase II study. Patients received everolimus monotherapy 10 mg/day continuously until progressive disease (PD) or unacceptable toxicity.

The primary end point was the DCR, defined as complete response (CR), partial response (PR), or stable disease (SD) at 8 weeks after treatment initiation, according to RECIST (version 1.1). The patients with controlled disease were defined as patients with either PR or SD, and patients with noncontrolled disease as patients with PD at week 8. Secondary end points were toxicity, PFS, and OS. PFS was defined as the time interval between the date of inclusion and the date of PD or death. OS was defined as the time interval between the date of inclusion until death or date of last follow-up.

Baseline evaluation was carried out within 2 weeks before inclusion and included medical history, physical examination, electrocardiogram, and chest and abdominal computed tomography. Laboratory tests included hemogram, creatinine, total protein, albumin, bilirubin, AST and ALT, lactate dehydrogenase, alkaline phosphatase, serum-corrected calcium, fasting glucose, triglycerides, and cholesterol. Imaging was repeated every 8 weeks and centrally reviewed. Adverse events were monitored throughout the study and recorded according to the National Cancer Institute—Common Toxicity Criteria (version 3). The relative dose intensity of everolimus was calculated as the actual dose intensity divided by the planned dose intensity, multiplied by 100.

### plasmatic angiogenesis proteins

Hypothesizing that everolimus could have antiangiogenic properties, we carried out angiogenesis-related analyses on plasma samples at baseline (before the treatment) and early during treatment (at day 28), using a Proteome Profiler Array (Angiogenesis Array Kit; R&D Systems, Minneapolis, MN). Each membrane array was incubated with patient plasma and an antibody detection cocktail overnight at 4°C and then with streptavidin-horseradish peroxidase conjugate, followed by a chemiluminescent detection reagent (ECL; Healthcare Life Sciences, Pittsburgh, PA). After exposure to X-ray film, the positive signals were quantified using the ImageQuant TL program (Amersham Bioscience, Piscataway, NJ). For each protein, the results were presented as the percentage of the positive control.

The most significant results were confirmed by ‘sandwich’ enzyme-linked immunosorbent assay (ELISA) following the instructions of the ELISA kits [Angiopoietin-1 Quantikine (DANG10), Endoglin Quantikine (DANG00), and platelet-derived growth factor (PDGF)-AB Quantikine (DHD00B), all from R&D Systems].

### PTEN status and *PIK3CA* mutation

PTEN immunohistochemistry carried out on archival paraffin-embedded tumor tissues was assessed by a PTEN monoclonal antibody (Cell Signaling, Danvers, MA, #9559). Detection was carried out using a secondary horseradish peroxidase antibody. The tumor cells were assessed for the predominant staining intensity from zero (absent) to three (strong) and for the percentage of stained cells with the predominant staining intensity. For PTEN, positive staining of stromal cells served as an internal control. PTEN-negative tumors showed complete absence of cytoplasmic staining

of the malignant cells while maintaining the stromal cell staining. For the *PIK3CA* mutations detection, DNA was extracted from paraffin-embedded blocks using the DNA extraction kit from Qiagen, Venlo, Netherlands (#51304). Detection of *PIK3CA* mutations, including E545K, E542K, and H1047R, was carried out by real-time PCR using the *PIK3CA* Mutation Test Kit from Qiagen (PK-01).

## statistical methods

This study employed a Simon optimal one-sample two-stage testing procedure to determine the sample size [21]. Based on hypotheses of  $P_0 = 0.10$ ,  $P_1 = 0.25$ ,  $\alpha = 0.15$ , and  $\beta = 0.10$ , 37 patients needed to be recruited into the trial. At least 6 of 37 patients were required to achieve CR, PR, or SD at 6–8 weeks after treatment for the study to meet its primary end point. The trial was to be discontinued if  $\leq 1$  CR, PR, or SD were observed after 17 patients had been treated for 6–8 weeks. Kaplan–Meier analyses were used to calculate PFS and OS. Student's *t*-test was used to compare the mean protein plasma levels of the different time points and subgroups. Association between PTEN status and disease control was assessed using the Fisher's exact test.

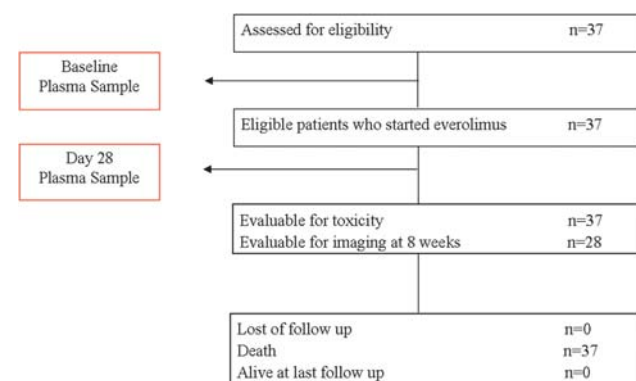
## results

### patient characteristics

Between November 2008 and October 2009, 37 patients from 10 Belgian centers were included (Figure 1). All patients had locally advanced or metastatic TCC and were previously exposed to platinum-based chemotherapy in either the neoadjuvant/adjuvant or palliative setting. Baseline characteristics are described in Table 1.

### dose intensity and acute adverse events

The relative dose intensity of everolimus was 78%. The median duration of everolimus treatment was 60 days (range, 17–491 days). Adverse events were consistent with those previously described [15] and are listed in Table 2. The most frequent grade 1–2 toxic effects were anemia (86%), nausea and vomiting (81%), and fatigue (64%). The main grade 3–4 toxic effects were fatigue (27%) and thrombocytopenia (13%). We observed two grade 5 adverse events: a cerebral bleed and a cardiac infarction not related to everolimus according to the investigators. Although both patients were eligible at enrollment according to the inclusion criteria, the cardiac event occurred in a patient with a past medical history of coronary angioplasty and the cerebral event in a patient with



**Figure 1.** CONSORT diagram.

cardiovascular risks factors (hypertension, hypercholesterolemia, and coronaropathy) in the absence of thrombocytopenia. For these two patients, everolimus did not induce hypertension or a significant increase in lipid levels.

## efficacy

Among the 37 treated patients, confirmed PR was observed in 2 patients, SD in 8, and PD in 27, resulting in a DCR of 27% at 8 weeks. Nine patients with rapid clinical PD were unable to be evaluated at 8 weeks and were considered to have PD as prespecified in the protocol. The maximum percentage reduction in the sum of the largest diameters for assessable patients is displayed in Figure 2. Among the eight patients with SD, three experienced some degree of tumor shrinkage (maximum percentage reduction in the sum of the largest diameters of –1%, –2%, and –9%). Disease control was maintained for 4 months in four patients, for 8 months in two patients, and for 14 months in one patient. Interestingly,

**Table 1.** Patients demographic and clinical characteristics

	N = 37 (%)
Male/Female	28/9
Age, years	
Median (range)	63 (32–86)
ECOG (performance status)	
0	3 (8%)
1	31 (84%)
2	3 (8%)
Tumor grade at diagnosis	
Well differentiated	3 (8%)
Moderately differentiated	7 (19%)
Poorly differentiated	20 (54%)
Unknown	7 (19%)
Platinum-based chemotherapy	
Neoadjuvant	3 (8%)
Adjuvant	8 (22%)
Palliative	30 (81%)
Methotrexate, vinblastine, adriamycin, cisplatin (MVAC regimen)	5 (14%)
Platinum/gemcitabine regimen	36 (97%)
One previous chemotherapy line for palliation	33 (89%)
Two previous chemotherapy lines for palliation	4 (11%)
Platinum-free interval inferior to 6 months	17 (46%)
Location of disease at inclusion	
Non visceral (nodes and bones)	9 (24%)
Visceral	28 (76%)
Lung alone	0 (0%)
Liver alone	2 (5.5%)
Lung and/or liver + nodes + bones	13 (35%)
Lung and/or liver + bones	1 (3%)
Lung and/or liver + nodes	12 (32.5%)
Metastatic sites at inclusion	
Lung	19 (51%)
Liver	18 (49%)
Nodes	29 (78%)
Bones	14 (38%)
Other sites (adrenal gland, Retzius space, mesenteric implants, muscle, thyroid)	5 (14%)

disease control was also observed in patients with poor prognosis factors, including liver metastases [22] (*n* = 3), in patients who had not previously responded to chemotherapy (*n* = 3), and in patients who had a platinum-free interval inferior to 6 months (*n* = 2). Median PFS was 61 days [95% confidence interval (CI) 49–63 days; range, 17–491

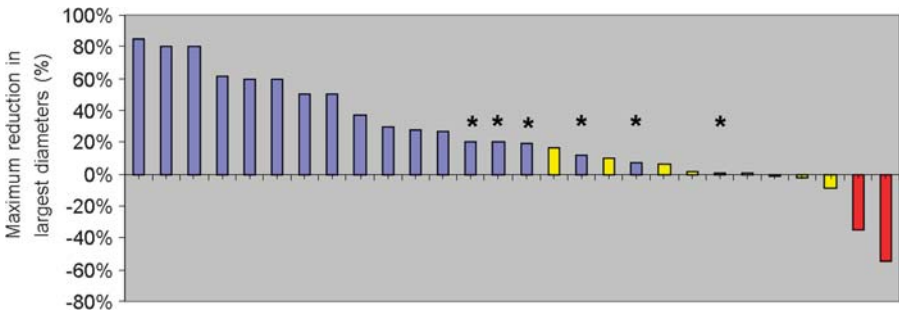
**Table 2.** Everolimus-related adverse events according to National Cancer Institute—Common Toxicity Criteria (version 3)

Toxic effects	Overall	Grade 1–2	Grade 3–4	Grade 5
Fatigue	34 (91%)	24 (64%)	10 (27%)	0 (0%)
Nausea and vomiting	33 (89%)	30 (81%)	3 (8%)	0 (0%)
Anorexia	12 (32%)	10 (27%)	2 (5%)	0 (0%)
Stomatitis	14 (38%)	13 (35%)	1 (3%)	0 (0%)
Diarrhea	15 (40%)	15 (40%)	0 (0%)	0 (0%)
Anemia	35 (95%)	32 (86%)	3 (8%)	0 (0%)
Thrombopenia	18 (49%)	13 (35%)	5 (13%)	0 (0%)
Neutropenia	2 (5%)	2 (5%)	0 (0%)	0 (0%)
Hyperglycemia	20 (54%)	17 (46%)	3 (8%)	0 (0%)
Hypertriglyceridemia	19 (51%)	18 (48%)	1 (3%)	0 (0%)
Liver toxic effects	2 (5%)	1 (3%)	1 (3%)	0 (0%)
Non infectious pneumonia	2 (5%)	1 (3%)	1 (3%)	0 (0%)
Cardiac infarction	0 (0%)	0 (0%)	0 (0%)	1 (3%)
Cerebral bleeding	0 (0%)	0 (0%)	0 (0%)	1 (3%)
Hypertension	3 (8%)	3 (8%)	0 (0%)	0 (0%)
Dermatitis	8 (22%)	8 (22%)	0 (0%)	0 (0%)

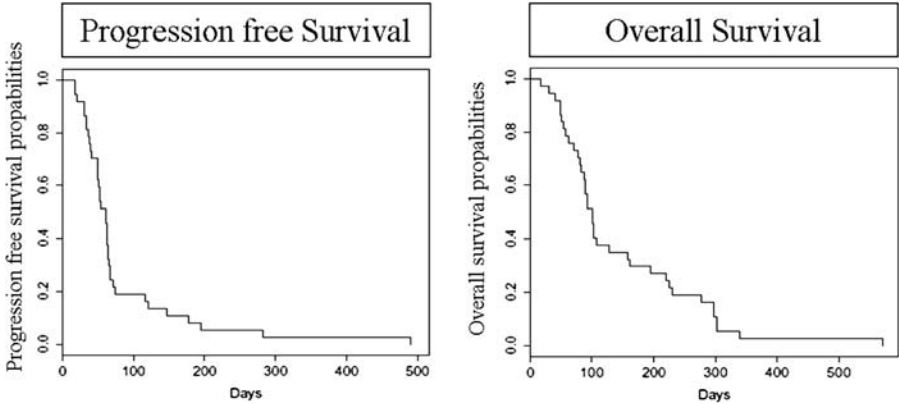
days], and median OS was 101 days (95% CI 82–128 days; range, 17–571 days) (Figure 3).

angiogenesis-related proteins

In 27 patients out of the 28 radiologically assessable at 8 weeks, we analyzed the angiogenesis-related proteins in baseline and day 28 plasma samples, using a Human Angiogenesis Array Kit. Seventeen proteins were detected. For the whole population, at day 28, we observed a decrease in angiogenic proteins such as PDGF-AB (–67.9%; *P* < 0.001) and insulin growth factor-binding protein 2 (IGFBP-2) (–13.1%; *P* = 0.041) and an increase in antiangiogenic proteins such as angiostatin (+113.9%; *P* < 0.001), pentraxine 3 (+27.8%; *P* = 0.003), and platelet factor 4 (+40%; *P* = 0.017). In addition, at day 28, we observed an increase in endoglin level (+32.3%; *P* = 0.027) compared with baseline (Table 3). Compared with patients with noncontrolled disease (*N* = 17), patients with controlled disease (*N* = 10) present a decrease in angiopoietin-1 (–65.2% versus +25.7%; *P* = 0.016) and endoglin (–9.8% versus +62.8%; *P* = 0.008) (Table 3). Baseline angiopoietin-1 expression was higher in patients with controlled disease compared with patients with noncontrolled disease (*P* = 0.040) (Table 4). Of note, both PDGF-AA and PDGF-AB tended to show a higher baseline expression (+34.9% and +56%, respectively; Table 4) and a larger decrease after everolimus (–46.7% versus –9.6% and –80.5% versus –54.8%, respectively;



**Figure 2.** Waterfall plot demonstrating the maximum percentage modification in the sum of the largest diameters of the 28 assessable patients (centrally reviewed). According to RECIST 1.1 criteria, blue plots represent progressive disease, yellow plots represent stable disease, red plots represent partial response, and blue plots \* represent stable disease in target lesions but apparition of new metastases.



**Figure 3.** Progression-free survival and overall survival probabilities.



**Table 3.** Changes in the plasma angiogenesis-related proteins between baseline and day 28

Angiogenesis-related proteins detected with Human Angiogenesis Array Kit	Changes in protein expression between baseline and day 28 after treatment. The value represents the variation in % of the mean levels between the baseline and the day 28 plasmatic samples.			
	Whole population (N = 27), P value, paired Student's t-test	Noncontrolled disease, (N = 17) %	Controlled disease, (N = 10) %	Difference in inpatient coefficient variation of protein expression between noncontrolled and controlled disease patients. P value, unpaired Student's t-test
Angiogenin	−1.5%, <i>P</i> = 0.610	−2.7	+0.5	3.2%, <i>P</i> = 0.571
Angiopoietin-1	−36.3%, <i>P</i> = 0.122	+25.7	−65.2	90.9%, <b><i>P</i> = 0.016</b>
Angiostatin	+113.9%, <b><i>P</i> &lt; 0.001</b>	+106.9	+128.2	21.3%, <i>P</i> = 0.976
CXCL16	+0.9%, <i>P</i> = 0.840	+5.9	−7.3	13.2%, <i>P</i> = 0.140
Endoglin	+32.3%, <b><i>P</i> = 0.027</b>	+62.8	−9.8	72.6%, <b><i>P</i> = 0.008</b>
Endostatin	+14%, <i>P</i> = 0.075	+21.2	+2.6	18.6%, <i>P</i> = 0.229
IGFBP-1	+1%, <i>P</i> = 0.788	+2.3	−1.1	3.4%, <i>P</i> = 0.635
IGFBP-2	−13.1%, <b><i>P</i> = 0.041</b>	−7	−22.7	15.7%, <i>P</i> = 0.117
IGFBP-3	−0.1%, <i>P</i> = 0.976	−1.8	+2.8	4.6%, <i>P</i> = 0.333
MMP-9	+3.5%, <i>P</i> = 0.469	+4.9	+1.1	3.8%, <i>P</i> = 0.698
Pentraxin-3	+27.8%, <b><i>P</i> = 0.003</b>	+21.7	+39.5	17.8%, <i>P</i> = 0.452
PDGF-AA	−27.3%, <i>P</i> = 0.099	−9.6	−46.7	37.1%, <i>P</i> = 0.157
PDGF-AB	−67.9%, <b><i>P</i> &lt; 0.001</b>	−54.8	−80.5	25.7%, <i>P</i> = 0.110
Platelet factor 4	+40%, <b><i>P</i> = 0.017</b>	+41.1	+37.6	3.5%, <i>P</i> = 0.742
Prolactin	+32.6%, <i>P</i> = 0.075	+42.4	+12.3	30.1%, <i>P</i> = 0.213
Serpin-1	−1.6%, <i>P</i> = 0.570	−0.6	−3.0	2.4%, <i>P</i> = 0.649
Thrombospondin-1	+9.8%, <i>P</i> = 0.652	+13.7	+2.5	11.2%, <i>P</i> = 0.777

The most relevant proteins are shown in bold type.

IGFBP, insulin growth factor-binding protein; MMP-9, matrix metalloproteinase 9; PDGF, platelet-derived growth factor.

Table 3) in patients with controlled (versus noncontrolled) disease.

Angiopoietin-1, endoglin as well as PDGF-AB profiles were thus also analyzed with an ELISA method (Figure 4). The patients with controlled disease showed significant decreases in angiopoietin-1 (−80%, *P* = 0.011), endoglin (−18%, *P* = 0.010), and PDGF-AB (−54%, *P* = 0.006) (Figure 4). Moreover, angiopoietin-1 baseline levels were higher in patients with controlled disease than in patients with noncontrolled disease (*P* = 0.011) (Figure 4A). Furthermore, the differences in inpatient coefficient of protein expression variation between noncontrolled and controlled disease patients were significant for these three biomarkers: angiopoietin-1 (456 pg/ml; *P* = 0.013), endoglin (1.59 ng/ml; *P* = 0.005), and PDGF-AB (890.92 pg/ml; *P* = 0.017) (supplemental Figure S1, available at *Annals of Oncology* online).

### PTEN status and PIK3CA mutations

Archival tumor tissues were available for 20 patients, including 6 with controlled disease and 14 with noncontrolled disease at week 8. Absence of PTEN expression was found in 8 of the 14 patients (57%) with noncontrolled disease and in 0% of the patients with controlled disease (supplemental Table S1, available at *Annals of Oncology* online). PTEN expression was detected in all the 6 patients with controlled disease and in 6 of 14 patients (43%) with noncontrolled disease (supplemental Figure S2, available at *Annals of Oncology* online). This data suggest an association between PTEN loss and everolimus resistance (*P* = 0.041). Only three (15%) tumors with a *PIK3CA*

mutation were detected (supplemental Table S1, available at *Annals of Oncology* online).

### discussion

Everolimus was found to have activity in palliative TCC patients. This study met its primary end point as 10 patients (27%) achieved at least SD at 8 weeks. Although median PFS and OS were low (61 days and 101 days, respectively), some patients achieved long-term SD. Even if a randomized phase II study would have eliminated the potential bias of patient selection, this clinical benefit is noteworthy, particularly in this heavily pretreated population for whom there is no available effective treatment. Furthermore, disease control was observed in some patients with poor prognosis factors according to Bellmunt et al. [22] or in some patients with an aggressive and chemoresistant disease. Until recently, there was no established treatment for metastatic TCC after platinum failure. Vinflunine has been approved by the European Medicines Agency for these patients based on the findings that vinflunine improved OS and PFS over BSC, although the OS improvement was statistically significant only in the eligible population and not in the intention-to-treat analysis [5]. Other chemotherapy agents have been investigated in monotherapy in this setting, including paclitaxel, docetaxel, irinotecan, ixabepilone, oxaliplatin, ifosfamide, topotecan, and pemetrexed, with overall response rate (ORR) between 5% and 27.7%, time to progression or PFS ranging from 1.5 to 3.8 months, and median OS ranging from 5 to 9 months [23–30].

Other targeted therapies, including antiangiogenic agents, have also been investigated in this setting with ORR between 0 and 22% [31–34]. Pazopanib, a multitarget tyrosine kinase, appears to be promising based on an interim report, with PR observed in 22%, SD in 61%, and 65% of patients progression-free at 2 months [35]. Another phase II study presented at the American Society of Clinical Oncology (ASCO) Annual Meeting 2011 evaluated everolimus in advanced TCC after failure of

**Table 4.** Differences in baseline plasma angiogenesis-related proteins (mean levels) between patients with controlled disease at week 8 and patients with noncontrolled disease

Angiogenesis-related proteins detected with Human Angiogenesis Array Kit	Baseline mean levels differences in controlled disease (N = 10) compared with noncontrolled disease (N = 17) patients. P value, unpaired Student's <i>t</i> -test
Angiogenin	+2.5%, <i>P</i> = 0.506
Angiopoietin-1	+242.5%, <b><i>P</i> = 0.040</b>
Angiostatin	−15.2%, <i>P</i> = 0.528
CXCL16	+3.1%, <i>P</i> = 0.586
Endoglin	+25.5%, <i>P</i> = 0.257
Endostatin	+4.0%, <i>P</i> = 0.524
IGFBP-1	+0.4%, <i>P</i> = 0.778
IGFBP-2	+8.8%, <i>P</i> = 0.355
IGFBP-3	+1.0%, <i>P</i> = 0.669
MMP-9	+0.3%, <i>P</i> = 0.945
Pentraxin-3	−10.3%, <i>P</i> = 0.541
PDGF-AA	+34.9%, <i>P</i> = 0.348
PDGF-AB	+56.0%, <i>P</i> = 0.196
Platelet Factor 4	−10.1%, <i>P</i> = 0.514
Prolactin	−17.2%, <i>P</i> = 0.269
Serpin-1	+5.9%, <i>P</i> = 0.112
Thrombospondin-1	−0.7%, <i>P</i> = 0.828

+Represents higher mean value in controlled patients; −represents lesser mean value in controlled patients.

The most relevant proteins are shown in bold type.

IGFBP, insulin growth factor-binding protein; MMP-9, matrix metalloproteinase 9; PDGF, platelet-derived growth factor.

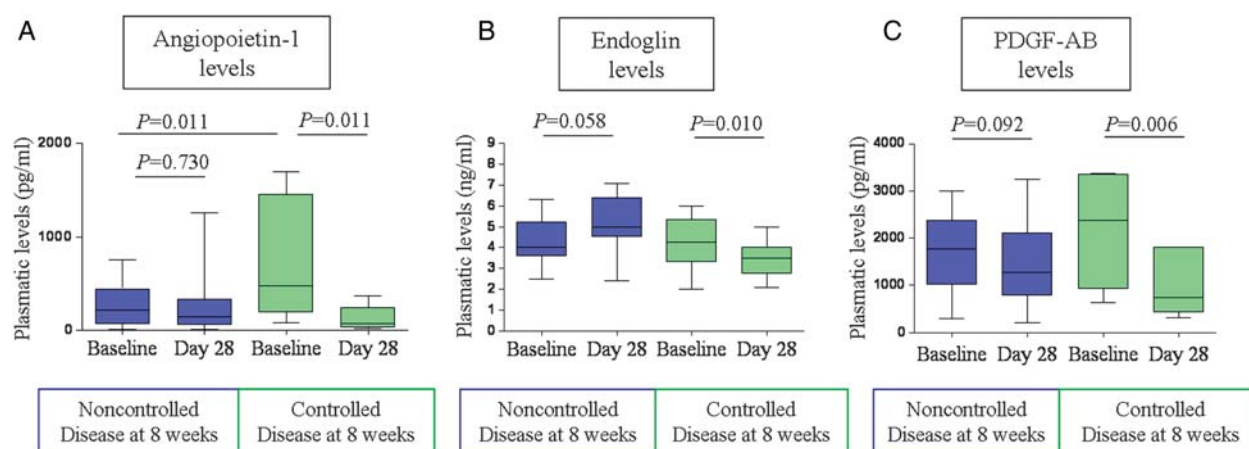
platinum-based therapy [36, 37]. Median PFS and OS were 3.3 and 10.5 months. However, 8 of the 43 patients enrolled in this trial who received one or less treatment cycle secondary to rapid progression or toxicity unrelated to everolimus were deemed inevaluable for PFS and were replaced. This probably accounts for the differences in PFS and OS between the trial reported here and the one presented at the ASCO meeting 2011.

Treatment with everolimus was well tolerated in our study population. Thrombocytopenia (13%) was the most frequent grade 3–4 adverse event. This frequency is higher than that reported in the RCC phase III study [15] and may be linked to the bone marrow impairment related to the previous use of chemotherapy, which is not administered in RCC. Two grade 5 adverse events, one cardiac infarction, and one cerebral bleed occurred during treatment. Although atheroembolic and hemorrhagic events are unlikely with mTOR inhibitors [15–17], we cannot exclude that these events could be related to everolimus since our translational research suggested that everolimus has antiangiogenic properties.

Although exploratory and limited by the low number of patients, our translational research suggests new hypotheses on mTOR inhibitor mechanisms.

The plasmatic analyses suggest antiangiogenic properties of everolimus, with decrease in proangiogenic proteins and increase in antiangiogenic proteins (Table 3). Furthermore, the decrease of some angiogenic proteins in patients with controlled disease could reflect the role of angiogenesis inhibition in disease control. Compared with patients with noncontrolled disease, a decrease in angiopoietin-1, PDGF-AB, and endoglin were observed in patients with controlled disease (Figure 4). Angiopoietin-1 and PDGF-AB are two proteins implicated in vessel maturation and stabilization [38–40] and endoglin is a proliferation-associated antigen expressed only by dividing endothelial cells in newly forming tumor vessels [41]. These observations support the hypothesis that everolimus could control cancer growth through an inhibition of vessel proliferation but also of vessel maturation.

Because PTEN acts as a break of Akt activation, PTEN loss is associated with constitutional activation of PI3K/Akt/mTOR



**Figure 4.** Plasma levels (enzyme-linked immunosorbent assay) before and after 28 days of treatment of angiopoietin (A), endoglin (B), and platelet-derived growth factor (PDGF)-AB (C) in noncontrolled disease patients (blue) compared with controlled disease patients (green). The box-whisker plots show the median, interquartile range, 10th, 90th, and 5th and 95th percentiles.

signaling. Although some preclinical models demonstrated that PTEN loss could sensitize tumor cells to mTOR inhibitors [42], clinical studies in renal, endometrial, and breast cancers did not show any correlation [43–45]. Furthermore, a recent *in vitro* study on breast cancer cells showed that cells with PTEN loss were not sensitive to mTOR inhibitors [46]. A clinical trial carried out in PTEN-deficient glioblastoma suggested that rapamycin induced Akt activation, which was associated with shorter PFS [47]. In our study, PTEN loss was found exclusively in patients with noncontrolled disease at week 8. This observation could highlight the importance of the negative feedback mediated by S6 Kinase 1 toward the PI3K/Akt pathway [48]. Indeed, it could be postulated that everolimus induces Akt activation via the removal of this negative feedback. Akt activation could be sustained in case of PTEN loss and therefore might act as a cancer cell survival mechanism inducing everolimus resistance.

There are some limitations in our study interpretation. The number of patients is limited and our trial was not designed and powered to study the role of angiogenesis-related proteins, PTEN status, or *PIK3CA* mutations as biomarkers. Another limitation is that PTEN status and *PIK3CA* mutations were carried out predominantly on archival tissue and not on fresh biopsies.

In conclusion, everolimus has clinical activity in advanced TCC after failure of platinum-based chemotherapy. Angiopoietin-1, endoglin, and PDGF-AB are potential biomarkers that should be investigated further in clinical trials. PTEN loss might be associated with everolimus resistance.

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## Prognosis after high-dose chemotherapy followed by autologous stem-cell transplantation as first-line treatment in primary CNS lymphoma—a long-term follow-up study

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**Background:** High-dose chemotherapy followed by autologous stem-cell transplantation (HCT–ASCT) is a promising approach in eligible patients with primary central nervous system lymphoma (PCNSL). We report long-term data of patients who were treated according to HCT–ASCT containing protocols.

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