Clinical Cancer Research

Angiotensin System Inhibitors and Survival Outcomes in Patients with Metastatic Renal Cell Carcinoma

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Abstract

Purpose: The renin-angiotensin system may play a role in carcinogenesis. The purpose of this study was to evaluate the impact of angiotensin system inhibitors (ASI) on outcomes in metastatic renal cell carcinoma (mRCC) patients treated in the targeted therapy era.

Experimental Design: We conducted a pooled analysis of mRCC patients treated on phase II and III clinical trials. Statistical analyses were performed using Cox regression adjusted for several risk factors and the Kaplan–Meier method.

Results: A total of 4,736 patients were included, of whom 1,487 received ASIs and 783 received other antihypertensive agents. Overall, ASI users demonstrated improved overall survival (OS) compared with users of other antihypertensive agents (adjusted HR, 0.838, $P = 0.0105$, 26.68 vs. 18.07 months) and individuals receiving no antihypertensive therapy (adjusted HR, 0.810, $P = 0.0026$, 26.68 vs. 16.72 months). When stratified by therapy type, a benefit in OS was demonstrated in ASI users compared with nonusers in individuals receiving VEGF therapy (adjusted HR, 0.737, $P < 0.0001$, 31.12 vs. 21.94 months) but not temsirolimus or IFNα. An *in vitro* cell viability assay demonstrated that sunitinib in combination with an ASI significantly decreased RCC cell viability compared with control at physiologically relevant doses. This effect was not observed with either agent alone or with other non-ASI antihypertensives or temsirolimus.

Conclusions: In the largest analysis to date, we demonstrate that ASI use improved survival in mRCC patients treated in the targeted therapy era. Further studies are warranted to investigate the mechanism underlying this interaction and verify our observations to inform clinical practice. *Clin Cancer Res; 21(11); 2471–9. ©2015 AACR.*

Introduction

Tumor angiogenesis is an established mechanism of metastatic renal cell carcinoma (mRCC) growth and progression. Critical to this pathway is VEGF, as demonstrated by RCC susceptibility to VEGF blockade with several approved targeted agents. Hypertension is a common condition which affects one of every three American adults (1). It is also commonly seen in patients with mRCC treated with VEGF-targeted therapy. Angiotensin system inhibitors (ASI) are broadly utilized by millions of Americans to treat hypertension, congestive heart failure, and other common medical conditions. ASIs include two major classes of agents: angiotensin-converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB). ACEIs decrease the production of angiotensin II generated from the conversion of antiogensin I to angiotensin II by ACE (2). ARBs block the action of one of two well-described subtypes of angiotensin II receptors (2). Given that angiotensin II can activate both types of receptors, ACEIs diminish activity at both receptors, whereas ARBs diminish only type I receptor-mediated effects.

Increasing evidence suggests that angiotensin II, an important regulator of blood pressure and cardiovascular homeostasis, plays a role in various pathologic processes including VEGF-dependent angiogenesis (3, 4). Preclinical studies have shown that angiotensin II, which mediates its biologic effects via binding to angiotensin II type I and type II receptors, regulates the expression of VEGF and the VEGF receptor (3). Physiologically, both angiotensin II receptors are widely expressed in the kidney (5). They localize to the renal cortex and are expressed by proximal tubular cells, which comprise the cell of origin of both clear cell and papillary RCC (6). The most direct evidence that angiotensin II signaling regulates tumor angiogenesis comes from xenograft studies which demonstrate that angiotensin II receptor knockout mice have reduced angiogenesis and tumor growth rates compared with wild-type mice (7). In addition, studies of human clear cell RCC have demonstrated that angiotensin II receptor expression strongly correlates with tumor aggressiveness and decreased survival (8). Lever and colleagues reported the first clinical evidence that long-term angiotensin II blockade may be protective against cancer (9). Since that time, several retrospective studies have investigated the association between ASIs and cancer progression and survival (10).

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Translational Relevance

Advances in the understanding of the molecular biology of renal cell carcinoma (RCC) have identified angiogenesis as a key factor in the pathogenesis of the disease. A major component of the angiogenic process in RCC is VEGF. Growing evidence suggests that angiotensin II, via activation of the angiotensin II receptor, plays a critical role in VEGF-dependent angiogenesis and key steps in carcinogenesis including cell proliferation and migration. Angiotensin II blockade by broadly utilized angiotensin system inhibitors (ASI) may be a potential therapeutic strategy for patients with metastatic RCC. Elucidation of the impact of ASIs on survival outcomes in patients with RCC is therefore highly relevant to optimizing the current treatment paradigm for patients with metastatic RCC.

Despite increasing evidence to suggest that the renin-angiotensin system may play a role in carcinogenesis and ASIs may be associated with improved outcomes in patients with cancer, there are limited studies investigating the role of ASIs in patients with mRCC treated with targeted therapy. Furthermore, the large number of individuals suffering from hypertension and mRCC presents an opportunity to explore combinatorial treatment regimens. In this analysis, we utilized a large clinical trials database to evaluate the role of ASIs on survival in patients with mRCC treated with a broad range of therapies in the modern era. In addition, we explored the effects of a broad spectrum of antihypertensive agents with or without sunitinib or temsirolimus on RCC cell viability *in vitro*.

Patients and Methods

Study design

We conducted a pooled retrospective analysis of patients with mRCC treated on phase II and phase III clinical trials sponsored by Pfizer (Table 1; refs. 11–22). We identified 4,736 patients treated for mRCC between January 2003 and June 2013. Patients who received at least one dose of study treatment were included in the analysis. Patients with missing concomitant medication information were excluded from the analysis. In total, 720 patients were excluded from the multivariate analysis.

Table 1. Phase II and phase III studies included in analysis

Clinical trial identifier	Phase	Number of patients enrolled	Number of patients excluded from multivariate analysis
NCT00267748	II	289	79
NCT00077974	II	106	27
NCT00137423	II	107	31
NCT00054886	II	63	13
NCT00338884	II	119	40
NCT00835978	II	213	43
NCT00065468	III	616	94
NCT00678392	III	714	73
NCT00083889	III	735	68
NCT00474786	III	501	83
NCT00631371	III	784	108
NCT00920816	III	409	61

Baseline demographic, clinical and laboratory data were collected. Data regarding ASI use were collected. ASIs included ACEIs and ARB. Users of ASIs or other antihypertensive agents were defined as patients treated with an ASI or other antihypertensive agent at baseline or within 30 days of study treatment initiation. The decision to start an antihypertensive agent before the initiation of study treatment and choice of agent was at the discretion of the treating physician. Each clinical trial had predefined parameters for initiating antihypertensive therapy should treatment-associated hypertension develop; however, the choice of agent was at the discretion of the treating physician. Patient follow-up consisted of imaging assessment every 4 to 12 weeks until disease progression or withdrawal. Treatment-associated toxicities were defined and evaluated according to the Common Terminology Criteria for Adverse Events, Version 3.0.

Treatment outcomes

Survival data were collected for all patients. Overall survival (OS) was defined as the time from initiation of therapy to death from any cause. Progression-free survival (PFS) was defined as the time from initiation of therapy to date of progression or death from any cause, whichever came first. For the evaluation of response, the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 was applied (23). Measurements were performed prospectively in all trials by clinical investigators.

Statistical analysis

The primary outcome was to assess OS in patients treated with ASIs compared with patients receiving other antihypertensive agents or those receiving no antihypertensive therapy. PFS, objective response rates (ORR), and toxicity were secondary endpoints. OS and PFS were calculated using the Kaplan–Meier method. Median OS and PFS along with 95% confidence intervals (CI) were reported. OS and PFS were assessed using multivariate Cox regression analysis, adjusted for age, sex, race, previously described Memorial Sloan-Kettering Cancer Center (MSKCC) risk factors (low performance status, elevated lactic dehydrogenase, elevated corrected calcium, low hemoglobin, absence of prior nephrectomy), presence of baseline hypertension, and the development of treatment-associated hypertension (24). Given that treatment-associated hypertension is a time-dependent covariable, we conducted a 6-month landmark analysis in addition to the primary analysis. All P values are two sided. All HRs presented are adjusted for the above variables and used ASI users as the test group; HR < 1 indicates ASI users had a favorable outcome versus the comparator group. Subgroup efficacy analyses were performed in the following groups: (i) users of antihypertensive therapy, (ii) users of antihypertensive therapy receiving VEGF-targeted therapy, (iii) patients who developed treatment-associated hypertension receiving VEGF-targeted therapy, and (iv) stratified by mRCC therapy type (VEGF-targeted, mTOR-targeted, and IFNα therapy groups).

Cell viability assay

Human Von Hippel–Lindau (VHL)–negative RCC cell lines (A-498 and 769-P) were cultured in DMEM (supplemented with 10% FBS). An equal number of cells (4×10^3) were seeded into 96-well flat-bottomed plates and incubated with various doses (0.001–1,000 μmol/L) of antihypertensive agents (captopril, lisinopril, losartan, amlodipine, and propranolol) with or

without sunitinib at various doses (0.001–1,000 μmol/L), sunitinib alone, or DMSO for 72 hours. Similar experiments were conducted with temsirolimus at various doses (0.001–1,000 μmol/L). Drug concentrations were based on physiologically relevant maximum concentrations (C_{max}) in humans (captopril 1153.38 μmol/L, lisinopril 277.20 μmol/L, losartan 445.88 μmol/L, amlodipine 15.16 μmol/L, propranolol 87.90 μmol/L, sunitinib 52.58 μmol/L, temsirolimus 0.56 μmol/L; refs. 25–28). Cell viability was quantified using the CellTiter 96 AQueous One Solution Cell Proliferation assay (Promega) according to the manufacturer's recommendations. Percent cell viability was calculated by normalizing the absorbance to DMSO. Difference between groups was calculated using a one-way ANOVA followed by Newman-Keuls *post hoc* test to correct for multiple testing. P values < 0.05 were considered statistically significant.

Results

Patient and disease characteristics

Of the 4,736 patients included in the analysis, the majority were less than 65 years of age, male, with good performance status, and clear cell histology (Table 2). ASI users were similar in age to users of other antihypertensive agents; however, both groups were older than patients not on antihypertensive therapy. Most patients underwent prior nephrectomy (70.2%) and a minority (33.2%) received prior systemic therapy. The presence of baseline lung, bone, and liver metastases in the overall cohort was 76.6%, 27.5%, and 26.1%, respectively, and rates were similar between groups. Baseline hypertension was present in nearly half the cohort (47.9%) of whom 83.1% were on antihypertensive therapy at baseline.

Treatment exposure

Patients received treatment with sunitinib (n = 1,059), sorafenib (n = 772), axitinib (n = 896), temsirolimus (n = 457), temsirolimus + IFNα (n = 208), bevacizumab + temsirolimus (n = 393), bevacizumab + IFNα (n = 391), or IFNα (n = 560), of whom 3,044 (64%) received first-line therapy. 992 patients were treated with ACEIs, 703 were treated with ARBs, and 208 were treated with the combination of ACEIs and ARBs. In total, 1,487 patients received ASI therapy and 783 were treated with other antihypertensive agents including β-blockers, calcium-channel blockers, diuretics, and other agents. The most frequently utilized ASIs were enalapril (n = 211), lisinopril (n = 204), valsartan (n = 121), and ramipril (n = 102). A total of 1,191 patients were on an ASI at baseline and 296 patients were started on an ASI within 30 days of starting study treatment.

Impact of ASIs on survival

For the overall cohort, OS was significantly longer in ASI users compared with users of other antihypertensive agents (unadjusted HR, 0.711; 95% CI, 0.625–0.809; adjusted HR, 0.838; 95% CI,

Table 2. Baseline patient and disease characteristics

Characteristic	Antihypertensive agent(s) users		Antihypertensive agent(s) nonuser	Total cohort
	ASI users	Other[a]		
n (%)	*n* = 1,487	*n* = 783	*n* = 2,466	*n* = 4,736
Age at initiation of therapy				
<65 y	871 (58.6%)	447 (57.1%)	1,940 (78.7%)	3,258 (68.8%)
≥65 y	616 (41.4%)	336 (42.9%)	526 (21.3%)	1,478 (31.2%)
Sex				
Male	1,025 (68.9%)	523 (66.8%)	1,815 (73.6%)	3,258 (68.8%)
Female	462 (31.1%)	260 (33.2%)	651 (26.4%)	1,478 (31.2%)
Race				
Caucasian	1,192 (80.2%)	583 (74.5%)	1,889 (76.6%)	3,664 (77.4%)
Other	295 (19.2%)	200 (25.5%)	577 (23.4%)	1,072 (22.6%)
ECOG performance status				
0	891 (59.9%)	384 (49.0%)	1,220 (49.5%)	2,495 (52.7%)
1	574 (38.6%)	386 (49.3%)	1,198 (48.6%)	2,158 (45.6%)
2	16 (1.1%)	8 (1.0%)	36 (1.5%)	60 (1.3%)
Unknown	6 (0.4%)	5 (0.6%)	12 (0.5%)	23 (0.5%)
Pathology				
Clear cell	1,356 (91.2%)	702 (89.7%)	2,177 (88.3%)	4,235 (89.4%)
Nonclear cell	95 (6.4%)	55 (7.0%)	187 (7.6%)	337 (7.1%)
Unknown	36 (2.4%)	26 (3.3%)	102 (4.1%)	164 (3.5%)
Baseline metastatic site				
Lung	1,145 (77.0%)	597 (76.2%)	1,887 (76.5%)	3,629 (76.6%)
Bone	363 (24.4%)	206 (26.3%)	732 (29.7%)	1,301 (27.5%)
Liver	350 (23.5%)	210 (26.8%)	678 (27.5%)	1,238 (26.1%)
Previous nephrectomy				
Yes	1,096 (73.7%)	581 (74.2%)	1,648 (66.8%)	3,325 (70.2%)
No	351 (23.6%)	168 (21.5%)	688 (27.9%)	1,207 (25.5%)
Unknown	40 (2.7%)	34 (4.3%)	130 (5.3%)	204 (4.3%)
Prior type of therapy				
Any prior therapy	529 (35.6%)	274 (35.0%)	770 (31.2%)	1,573 (33.2%)
Cytokine therapy	200 (13.4%)	111 (14.2%)	360 (14.6%)	671 (14.2%)
Targeted therapy	220 (14.8%)	124 (15.8%)	229 (9.3%)	573 (21.1%)
Medical conditions				
Hypertension	1,250 (84.1%)	634 (81.0%)	383 (15.5%)	2,267 (47.9%)
Diabetes mellitus	347 (23.3%)	116 (14.8%)	197 (8.0%)	660 (13.9%)

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

[a]Other antihypertensive agents include B-blockers, calcium-channel blockers, diuretics, and other agents.

Table 3. Impact of ASI use on OS and PFS

		OS			PFS		
	N	**Median (mo)**	***P***	**HR[a] (95% CI)**	**Median (mo)**	***P***	**HR[a] (95% CI)**
Overall cohort ($n = 4,736$)							
ASI users	1,487	26.68		Test	8.34		Test
Other antihypertensive therapy users[b]	783	18.07	**0.0105**	0.838 (0.731–0.960)	6.70	**0.0261**	0.877 (0.782–0.984)
Antihypertensive therapy nonusers	2,466	16.72	**0.0026**	0.810 (0.707–0.929)	6.35	0.4226	0.955 (0.855–1.068)
Overall cohort by type of therapy ($n = 4,736$)							
VEGF therapy ($n = 3511$)							
ASI users	1,192	31.12	<**0.0001**	0.737 (0.640–0.848)	10.15	0.0869	0.907 (0.812–1.014)
ASI nonusers	2,319	20.21			8.09		
mTOR Therapy ($n = 665$)							
ASI users	151	12.66	0.2975	0.867 (0.664–1.134)	5.46	0.5650	0.929 (0.725–1.192)
ASI nonusers	514	10.16			4.05		
IFNα therapy ($n = 560$)							
ASI users	144	15.54	0.2027	1.218 (0.899–1.647)	3.84	0.9084	0.984 (0.747–1.297)
ASI nonusers	416	14.82			3.68		
Patients receiving VEGF-targeted and antihypertensive therapy ($n = 1,769$)							
ASI users	1,192	31.12	**0.0003**	0.725 (0.609–0.861)	10.15	**0.0121**	0.835 (0.726–0.962)
Other antihypertensive therapy users[b]	577	21.94			7.82		
Patients receiving VEGF-targeted therapy who developed treatment-associated hypertension ($n = 2,466$)							
ASI users	982	33.19	**0.0004**	0.742 (0.629–0.875)	10.92	**0.0498**	0.883 (0.779–1.000)
ASI nonusers	1,484	24.64			9.05		

NOTE: Bolded *P* values are statistically significant.

Abbreviation: IMDC, International mRCC Database Consortium.

[a]HR of ASI users to comparator group from multivariate analysis, adjusted for age, sex, race, Memorial Sloan Kettering Cancer Center (MSKCC) risk factors, presence of baseline hypertension, and the development of treatment-associated hypertension.

[b]Other antihypertensive agents include β-blockers, calcium-channel blockers, diuretics, and other agents.

0.731–0.960, $P = 0.0105$, medians of 26.68 vs. 18.07 months) and individuals receiving no antihypertensive therapy (unadjusted HR, 0.630; 95% CI, 0.572–0.694; adjusted HR, 0.810; 95% CI, 0.707–0.929, $P = 0.0026$, medians of 26.68 vs. 16.72 months; Table 3 and Fig. 1). Similarly, PFS was significantly longer in ASI users compared with users of other antihypertensive agents (unadjusted HR, 0.786; 95% CI, 0.704–0.876; adjusted HR, 0.877; 95% CI, 0.782–0.984, $P = 0.0261$, medians of 8.34 vs. 6.70 months) and there was a nonstatistically significant improvement in PFS between ASI users compared with individuals receiving no antihypertensive therapy (unadjusted HR, 0.747; 95% CI, 0.689–0.810; adjusted HR, 0.955; 95% CI, 0.855–1.068, $P = 0.4226$, medians of 8.34 vs. 6.35 months). In multivariate analysis, lack of ASI use (adjusted HR, 1.214; 95% CI, 1.083–1.361), failure to develop treatment-associated hypertension (adjusted HR, 1.976; 95% CI, 1.805–2.165, $P < 0.0001$), and individual MSKCC risk factors were independent predictors of worse OS; however age, sex, race, and baseline hypertension were not predictive of OS. A 6-month landmark analysis demonstrated similar results; lack of ASI use (adjusted HR, 1.305; 95% CI, 1.138–1.496, $P < 0.0001$), failure to develop treatment-associated hypertension (adjusted HR, 1.272; 95% CI, 1.140–1.419, $P < 0.0001$), and individual MSKCC risk factors remained statistically significant.

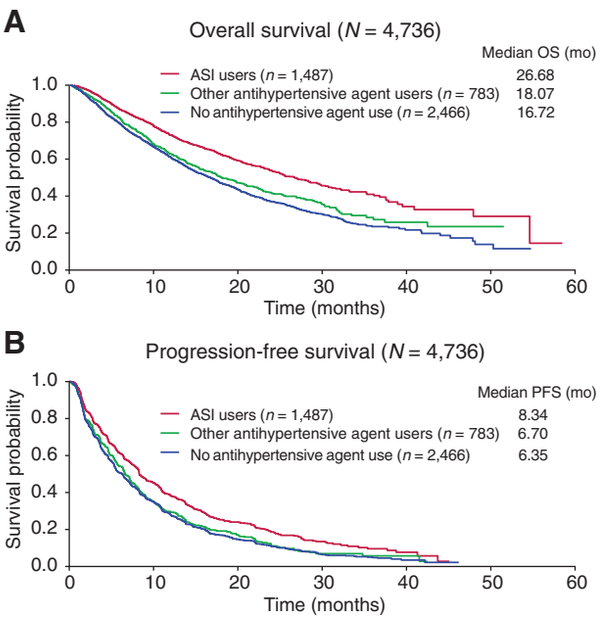

Figure 1.
Kaplan–Meier estimates of (A) OS for the overall cohort, and (B) PFS for the overall cohort stratified by ASI users, other antihypertensive agent(s) users versus no antihypertensive agent use.

When stratified by mRCC therapy type, OS was significantly longer in ASI users compared with nonusers in patients receiving VEGF-targeted therapy only ($n = 3,511$; adjusted HR, 0.737; 95% CI, 0.640–0.848, $P < 0.0001$, medians of 31.12 vs. 20.21 months), and there was no statistically significant difference in OS between ASI users and nonusers in those receiving mTOR-targeted ($n = 665$) or IFNα therapy ($n = 560$; Fig. 2).

To investigate the impact of antihypertensive therapy and treatment-associated hypertension in patients specifically receiving VEGF-targeted therapy, we conducted additional subgroup analyses in these patient populations. In patients receiving VEGF-targeted therapy and antihypertensive therapy ($n = 1,769$), ASI use was associated with a statistically significant improvement in OS (adjusted HR, 0.725; 95% CI, 0.609–0.861, $P = 0.0003$, medians of 31.12 vs. 21.94 months) and PFS (adjusted HR, 0.835; 95% CI, 0.726–0.962, $P = 0.0121$, medians of 10.15 vs. 7.82 months). In patients receiving VEGF-targeted therapy who developed treatment-associated hypertension ($n = 2,466$), a similar benefit in OS and PFS was observed (OS adjusted HR,

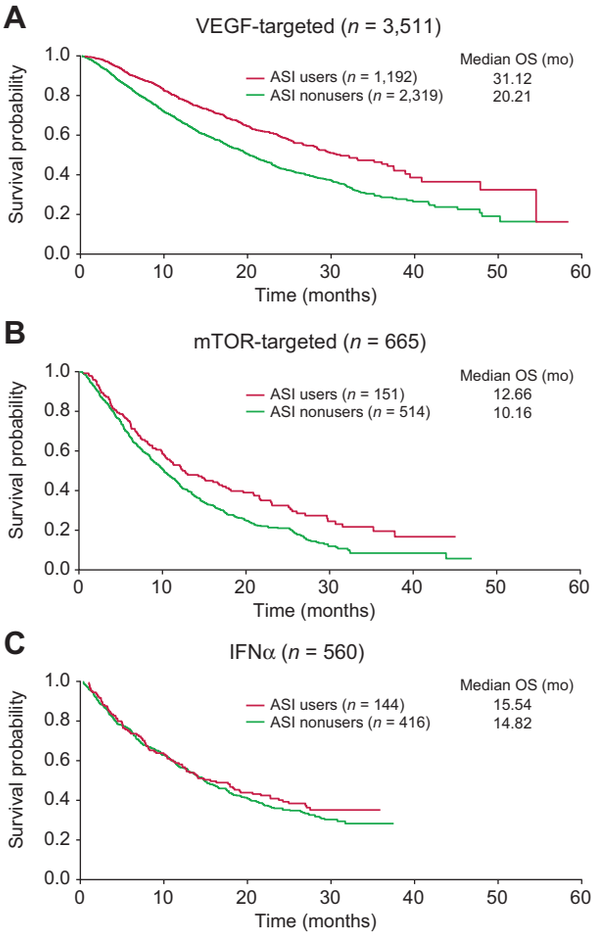

Figure 2.
Kaplan–Meier estimates of (A) OS for patients receiving VEGF-targeted therapy, (B) OS for patients receiving mTOR-targeted therapy, and (C) OS for patients receiving IFNα therapy stratified by ASI users versus nonusers.

0.742; 95% CI, 0.629–0.875, $P = 0.0004$, medians of OS 33.19 vs. 24.64 months,; PFS adjusted HR, 0.883; 95% CI, 0.779–1.000, $P = 0.0498$, medians of PFS 10.92 vs. 9.05 months,). Survival was the longest in patients who received VEGF-targeted therapy, developed treatment-associated hypertension, and were treated with an ASI ($n = 982$; median OS 33.19 months).

Impact of ASIs on response

ORRs were similar in ASI users compared with nonusers in the overall cohort (28.31% vs. 22.74%, $P = 0.7484$). Similar results were seen in the following subgroups: antihypertensive agent users (28.31% vs. 22.35%, $P = 1.000$), antihypertensive agent users receiving VEGF-targeted therapy (32.29% vs. 26.32%, $P = 1{,}000$), and patients receiving VEGF-targeted therapy who developed treatment-associated hypertension (35.64% vs. 33.96%, $P = 0.9002$).

Adverse events

A total of 2,690 patients (62.5%) in the overall cohort developed treatment-associated hypertension. Of the 3,511 patients treated with VEGF-targeted therapy, 2,466 (70.2%) developed treatment-associated hypertension. For the overall cohort, the frequency of grade 3 or higher toxicities was similar between ASI users and nonusers, except hypertension which was more common in the ASI users (17.82% vs. 4.77%; Table 4). For the total cohort, the frequency of cardiac and thromboembolic events was also similar between groups. In patients receiving VEGF-targeted therapy, hypertension was more frequent in ASI users, whereas other adverse events were similar between the groups. In patients receiving mTOR-targeted therapy, hyperglycemia was more frequent in ASI users.

Cell viability assay

Cell viability assays were conducted in 769-P and A-498 RCC cells which mimic clear cell RCC and have previously demonstrated expression of angiotensin II receptor, type I (29, 30). Physiologic concentrations of sunitinib are near 10 μmol/L. The combination of sunitinib with captopril decreased cell viability to 59.3%, 48.1%, and 36.8% at 10, 100, and 1,000 μmol/L concentrations in A-498 RCC cells (Fig. 3A). The combination of sunitinib with lisinopril decreased cell viability to 78.2%, 52.7%, and 37.2% at 10, 100, and 1,000 μmol/L concentrations. In addition, the combination of sunitinib with losartan at 100 μmol/L and above concentrations significantly decreased A-498 RCC cell viability (68.2% and 52.8% at 100 and 1,000 μmol/L). A statistically significant decrease in cell viability was not detected with either agent alone at concentrations below 1,000 μmol/L compared with DMSO.

A similar trend was observed in 769-P RCC cells (Fig. 3B). The combination of sunitinib with captopril or lisinopril at 10 μmol/L and above concentrations significantly decreased 769-P RCC cell viability compared with DMSO. The combination of sunitinib with captopril decreased cell viability to 61.4%, 56.8%, and 40.6% at 10, 100, and 1,000 μmol/L concentrations in 769-P cells. The combination of sunitinib with lisinopril decreased cell viability to 66.8%, 59.8%, and 54.5% at 10, 100, and 1,000 μmol/L concentrations in 769-P cells. A statistically significant decrease in cell viability was not detected with either agent alone at 10 and 100 μmol/L concentrations compared with DMSO. No decrease in cell viability was observed when either cell line was incubated with amlodipine or propranolol alone or in combination with sunitinib at concentrations below 1000 μmol/L compared with DMSO.

Similar studies were conducted with temsirolimus in 769-P and A-498 RCC cells (Fig. 3C and D). Physiologic concentrations of temsirolimus are near 0.10 μmol/L. In both cell lines, the combination of temsirolimus with captopril significantly decreased cell viability only at higher dose concentrations compared with DMSO (769-P RCC cells: 70.8% and 56.9% at 100 and 1,000 μmol/L; A-498 RCC cells: 63.5% and 57.8% at 100 and 1,000 μmol/L). Temsirolimus combined with lisinopril or losaratan did not decrease cell viability at concentrations below 1,000 μmol/L compared with DMSO (769-P RCC cells: 62.3% at 1,000 μmol/L lisinopril, 62.6% at 1,000 μmol/L losartan; A-498 RCC cells: 67.8% at 1,000 μmol/L lisinopril, 66.5% at 1,000 μmol/L losartan).

Discussion

This is the largest analysis to date evaluating the impact of ASIs on outcomes, not just in mRCC, but in any type of cancer. To conduct our analysis, we used a large and robust clinical trials

Table 4. Adverse events

	ASI users ($n = 1,487$)	ASI nonusers ($n = 3,249$)	Total ($n = 4,736$)
Total cohort			
Selected adverse events (any grade)			
Angioedema	2 (0.13%)	2 (0.06%)	4 (0.0.84%)
Arterial thrombosis	0 (0%)	0 (0%)	0 (0%)
Cardiac ischemia/infarction	19 (1.28%)	22 (0.68%)	41 (0.87%)
Cerebrovascular event	55 (3.70%)	86 (2.65%)	141 (2.98%)
Cough	349 (23.47%)	681 (20.96%)	1030 (21.74%)
Hyperkalemia	86 (5.78%)	138 (4.25%)	224 (4.73%)
Hypertensive crisis	8 (0.54%)	8 (0.25%)	16 (0.34%)
Renal insufficiency	67 (4.51%)	71 (2.19%)	138 (2.91%)
Most frequent grade 3–5 adverse events (observed in >3% of patients)			
Fatigue	155 (10.42%)	309 (9.51%)	464 (9.80%)
Hypertension	265 (17.82%)	155 (4.77%)	420 (8.87%)
Anemia	84 (5.65%)	279 (8.59%)	363 (7.66%)
Asthenia	85 (5.72%)	186 (5.72%)	271 (5.72%)
Hand–foot syndrome	90 (6.05%)	177 (5.45%)	267 (5.64%)
Diarrhea	109 (7.33%)	147 (4.52%)	256 (5.41%)
Dyspnea	56 (3.77%)	137 (4.22%)	193 (4.08%)
Neutropenia	49 (3.30%)	137 (4.22%)	186 (3.93%)
Disease progression	36 (2.42%)	149 (4.59%)	185 (3.91%)
Proteinuria	65 (4.37%)	94 (2.89%)	159 (3.36%)
Decreased appetite	42 (2.82%)	99 (3.05%)	141 (2.98%)
Patients receiving VEGF-targeted therapy			
Most frequent grade 3–5 adverse events (observed in >3% of patients)			
Hypertension	261 (21.89%)	150 (6.47%)	411 (11.70%)
Fatigue	104 (8.72%)	187 (8.06%)	291 (8.29%)
Hand–foot syndrome	90 (7.55%)	174 (7.50%)	264 (7.52%)
Diarrhea	103 (8.64%)	126 (5.43%)	229 (6.52%)
Asthenia	64 (5.37%)	133 (5.74%)	197 (5.61%)
Anemia	48 (4.03%)	123 (5.30%)	171 (4.87%)
Disease progression	33 (2.77%)	133 (5.74%)	166 (4.73%)
Proteinuria	64 (5.37%)	93 (4.01%)	157 (4.47%)
Neutropenia	41 (3.44%)	90 (3.88%)	131 (3.73%)
Dyspnea	29 (2.43%)	84 (3.62%)	113 (3.22%)
Hyponatremia	43 (3.61%)	65 (2.80%)	108 (3.08%)
Patients receiving mTOR-targeted therapy			
Most frequent grade 3–5 adverse events (observed in >3% of patients)			
Anemia	33 (21.85%)	129 (25.09%)	162 (24.35%)
Fatigue	20 (13.24%)	63 (12.25%)	83 (12.48%)
Hyperglycemia	19 (12.58%)	34 (6.61%)	53 (7.97%)
Dyspnea	15 (9.93%)	37 (7.20%)	52 (7.82%)
Asthenia	5 (3.31%)	29 (5.64%)	34 (5.11%)
Lymphopenia	3 (1.97%)	31 (6.03%)	34 (5.11%)
Hypophosphatemia	7 (4.64%)	26 (5.06%)	33 (4.96%)
Neutropenia	4 (2.65%)	28 (5.45%)	32 (4.81%)
Hypertriglyceridemia	8 (5.30%)	21 (4.09%)	29 (4.36%)
Decreased appetite	6 (3.97%)	20 (3.89%)	26 (3.91%)
Abdominal pain	2 (1.32%)	23 (4.47%)	25 (3.76%)
Pneumonia	3 (1.99%)	21 (4.09%)	24 (3.61%)
Alkaline phosphatase increase	6 (3.97%)	17 (3.31%)	23 (3.46%)
Dehydration	2 (1.32%)	21 (4.09%)	23 (3.46%)
Thrombocytopenia	9 (5.96%)	14 (2.72%)	23 (3.46%)
Stomatitis	5 (3.31%)	16 (3.11%)	21 (3.16%)
Diarrhea	5 (3.31%)	15 (2.92%)	20 (3.01%)

database containing over 4,700 patients treated with a broad range of systemic therapies in the modern era. The database contains prospectively collected information, providing a valuable resource for the evaluation of patient characteristics and outcomes.

In our analysis, we demonstrate that ASI users had an improved survival compared with ASI nonusers in patients with mRCC. There have been limited studies investigating the role of ASIs in patients with mRCC. Keizman and colleagues were the first to demonstrate the clinical benefit of ASIs in patients with mRCC treated with VEGF-targeted therapy (31). In a small retrospective study of 127 patients with mRCC treated with sunitinib, ASI users had an improved PFS (HR 0.537, $P = 0.0055$, medians of 13 versus 6 months) and a nonstatistically significant improvement in OS (HR, 0.688, $P = 0.21$, medians of 30 vs. 23 months) compared with ASI nonusers.

We hypothesize that the improved survival may be related to the ability of ASIs to synergize with antiangiogenics to inhibit tumor cell proliferation and angiogenesis (32). Angiotensin II via binding to the angiotensin II receptors in tumor cells can activate

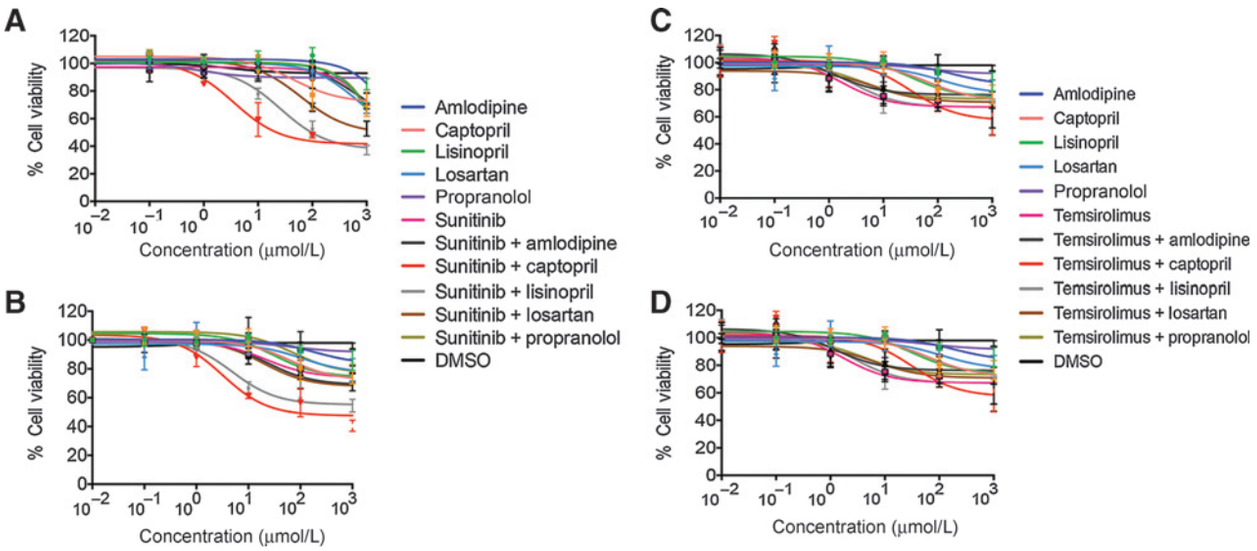

Figure 3.
Cell viability assays of various antihypertensive agents with or without sunitinib at differing concentrations on A-498 (A) and 769-P (B) RCC cells and various antihypertensive agents with or without temsirolimus at differing concentrations on A-498 (C) and 769-P (D) RCC cells. The combination of sunitinib with captopril or lisinopril at 10 μmol/L and above concentrations significantly decreased A-498 and 769-P RCC cell viability when compared with DMSO.

tumor cell proliferation via the PI3K/AKT pathway and EGFR transactivation (33, 34). In addition, angiotensin II can increase expression and production of VEGF, which is hypothesized to be trigged by stabilization of hypoxia-inducible factor, and promoting angiogenesis (35).

To investigate the interaction between ASIs and mRCC therapy, we conducted a subset analysis evaluating the impact of ASIs in patients receiving VEGF-targeted, mTOR-targeted, or IFNα therapy. In this analysis, we demonstrated that ASI use was associated with a significant improvement in OS for patients receiving VEGF-targeted therapy only. We postulate that this is likely related to a synergistic interaction between ASIs and VEGF-targeted therapy given that these agents are thought, at least in part, to augment a similar pathway. Several studies have evaluated the role of ASIs alone in RCC mouse models (36–38). Araujo and colleagues evaluated losartan, captopril, or the combination *in vivo* and demonstrated decreased tumor proliferation with renin-angiotensin system blockade (36). Other studies demonstrated that captopril resulted in significant dose-related reduction in tumor development and candesartan dramatically prevented the development of metastatic lung nodules and inhibited neovascularization and VEGF expression in a RCC mouse xenograft model (37, 38). Preclinical studies involving other cancer types have demonstrated similar effects with ASI treatment (4). Recently, Verhoest and colleagues reported that combination therapy with sunitinib and telmisartan, an ARB, resulted in slower tumor growth, increased tumor necrosis, decreased central microvascular density, and decreased circulating VEGF levels in a murine xenograft model of RCC (30).

In this study, we demonstrate that ASIs when combined with sunitinib significantly decrease cell viability of RCC cells *in vitro*, an effect which was not seen with temsirolimus or with either agent alone, suggesting synergy between agents. Although prior studies have demonstrated that sunitinib at physiologic concentrations acts primarily on tumor endothelium rather than tumor cells to inhibit RCC growth, we demonstrate that at physiologic concentrations of the combination of sunitinib and captopril or lisinopril, cell viability is decreased (39). Although not statistically significant, ORR was higher in ASI users compared with nonusers, and thus further preclinical investigations are necessary to explore the interactions of both agents on tumor cells and the tumor microenvironment.

To distinguish from prior preclinical work, in this study we utilized two distinct RCC cells lines and evaluated a broad spectrum of antihypertensives including ASIs, β-blockers, and calcium-channel blockers. Further studies are warranted to investigate the effect of combination therapy on angiogenesis and cell signaling. This type of work will help illuminate the potential mechanisms underlying our clinical observations

Hypertension is commonly associated with VEGF blockade, as demonstrated by recent studies in mRCC documenting an incidence of 30% to 40%. It is postulated that treatment-associated hypertension develops secondary to decreased nitric oxide and increased endothelin-1 production resulting in vasoconstriction, decreased endothelial cell viability, and vessel rarefaction (40). Growing evidence suggests that treatment-associated hypertension is a clinical biomarker of efficacy in patients receiving angiogenesis-targeted therapy (41, 42). Rini and colleagues conducted a retrospective analysis of 544 patients with mRCC treated with sunitinib which demonstrated that the development of treatment-associated hypertension was associated with significantly improved PFS ($P < 0.001$, medians of 12.5 vs. 2.5 months,) and OS ($P < 0.001$, medians of 30.9 vs. 7.2 months), while adverse events related to hypertension were similar between the two groups (41). In our analysis, we confirm that the OS for patients who develop treatment-associated hypertension was higher compared with the overall cohort (33.19 vs. 26.64 months for ASI users who developed treatment-associated hypertension on VEGF-targeted therapy compared with ASI users in the overall cohort). To account for treatment-associated hypertension as a potential confounder, we conducted a separate analysis in patients receiving VEGF-targeted therapy who developed

treatment-associated hypertension ($n = 2,466$). Despite the development of treatment-associated hypertension, we demonstrated that there was an OS benefit specifically attributable to ASI use in these patients, which was not attributable to other antihypertensive agents or the sole effect of developing hypertension.

Although this is the largest database using prospectively collected clinical trials information to assess the impact of ASIs in cancer patients in general and in mRCC patients specifically, there are several limitations. All patients in the database were enrolled in clinical trials, which could lead to decreased generalizability of the study findings, given that patients with severe comorbidities, unstable medical conditions, or poor performance status are typically excluded from therapeutic clinical trials. In addition, data collection was not specifically designed to examine ASI use. Furthermore, there was significant variability with regard to choice of ASI and type of mRCC therapy received. Indications for ASI treatment and modifications were at the discretion of the treating physician and were not collected in the database. In addition, data were lacking about dosing, schedule, and duration of ASI use, though we presume on-label usage of these commonly utilized agents. Finally, though patients receiving a broad range of targeted therapies were included in the analysis, not every approved agent to treat mRCC was represented. Given these limitations, specific recommendations about ASI use in patients with mRCC receiving targeted therapy cannot be made based on the results of this analysis.

In conclusion, we demonstrate that ASI use improved survival outcomes in patients with mRCC treated in the era of targeted therapy. Although hypertension is a known on-target effect and a demonstrated clinical biomarker of efficacy to VEGF-targeted therapy, we show that even in cancer patients who develop treatment-associated hypertension secondary to VEGF blockade, ASI users experience an OS and PFS benefit compared with ASI nonusers. We do not believe a randomized prospective clinical trial of a VEGF inhibitor with and without an ASI is easy to conduct in this population. Although our study was based on a pooled analysis of prospectively collected data, further studies are warranted to verify our observations and inform the clinical practice of ASI use in combination with VEGF-targeted agents in patients with mRCC. Furthermore, we showed that ASIs have a synergistic effect with sunitinib on cell viability and additional preclinical studies are required to investigate the mechanisms underlying this interaction.

Disclosure of Potential Conflicts of Interest

R.R. McKay reports receiving a commercial research grant from Pfizer. X. Lin is an employee of Pfizer. T.K. Choueiri reports receiving a commercial research grant from Pfizer and is a consultant/advisory board member for Bayer, GlaxoSmithKline, and Pfizer. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): G.E. Rodriguez, X. Lin, V.S. Sabbisetti, T.K. Choueiri

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R.R. McKay, X. Lin, O.-P.R. Hamnvik, R. Simantov, T.K. Choueiri

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T.K. Choueiri

Study supervision: T.K. Choueiri

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