

Predictive Biomarkers and Personalized Medicine

See commentary by Cho and Atkins, p. 2999

Increase in Cholesterol Predicts Survival Advantage in Renal Cell Carcinoma Patients Treated with TemezirolimusChee Khoo Lee¹, Ian C. Marschner^{1,2}, R. John Simes¹, Merryn Voysey^{1,4}, Brian Egleston⁵, Gary Hudes⁵, and Paul de Souza³**Abstract**

Purpose: Temezirolimus is an effective treatment for renal cell carcinoma. It is associated with increases in serum cholesterol, triglyceride, and glucose. We investigated whether changes of these biomarkers could predict its efficacy.

Experimental Design: We examined serial measurements of cholesterol, triglycerides, and glucose from patients randomized to IFN or temezirolimus in the Global Advanced Renal Cell Carcinoma Trial. Using time-dependent proportional hazards models, we quantified the association between changes in these biomarkers from baseline with overall survival (OS) and progression-free survival (PFS). We also assess the extent to which changes of these biomarkers predict the effects of temezirolimus on survival.

Results: Temezirolimus was associated with larger mean increases in cholesterol (1.02 mmol/L; $P < 0.0001$), triglycerides (0.32 mmol/L; $P = 0.0008$), and glucose (1.28 mmol/L; $P < 0.0001$) compared with IFN and improved survival rate (OS: HR = 0.76, $P = 0.02$; PFS: HR = 0.70, $P = 0.001$). Cholesterol increase during study was associated with longer survival (OS: HR = 0.77 per mmol/L, $P < 0.0001$; PFS: HR = 0.81 per mmol/L; $P < 0.0001$). Temezirolimus effect on cholesterol predicted its effect on survival with no additional survival advantage observed after adjusting for cholesterol change during study (OS: HR = 1.14, $P = 0.37$; PFS: HR = 0.88, $P = 0.35$). Temezirolimus effect on triglycerides or glucose did not predict its effect on survival, with survival advantage in favor of temezirolimus still observed after adjusting for these factors ($P = 0.003$ and $P = 0.002$).

Conclusion: Cholesterol increase is a potential predictor for temezirolimus efficacy. Longer survival in patients treated with temezirolimus was observed in those with larger increases in cholesterol. Prospectively designed biomarker studies of temezirolimus or other mTOR inhibitors are recommended. *Clin Cancer Res*; 18(11); 3188–96. ©2012 AACR.

Introduction

The mTOR performs an important role in the regulation of cellular function. In renal cell carcinoma, inactivation of von Hippel–Lindau tumor suppressor gene (*VHL*), a common molecular abnormality in renal cancer, results in abnormal accumulation of hypoxia-inducible factor (HIF), mediated by mTOR, that drives cellular growth and angiogenesis (1–5). Emerging evidence suggests that mTOR also plays a central role in sensing nutrient availability in the cell, particularly in regard to lipid and glucose metabolism (6–8). Under nutrient-poor conditions in a normal cellular

environment, downstream mTOR activation is attenuated. However, in cancer cells, aberrantly high mTOR activity leads to growth and proliferation, even in nutrient-poor conditions (9–11).

Temezirolimus, an mTOR inhibitor, is an effective treatment of renal cancer. In a randomized phase III trial of patients with metastatic renal cell carcinoma and multiple risk factors for short survival, treatment with temezirolimus was associated with improved overall survival (OS) and progression-free survival (PFS), compared with IFN α -2a (12). Better PFS was also reported in a placebo-controlled randomized trial of patients with advanced renal cell carcinoma treated with everolimus, another mTOR inhibitor, after failure of prior VEGF-targeted therapy (13).

Increases in serum cholesterol, triglyceride, and glucose with mTOR inhibitors have been commonly observed in clinical trials (12–15) and represent adverse events that could reflect the mechanism of action of this class of drugs. Therefore, in this study, we investigated whether changes in cholesterol, triglyceride, and glucose levels, compared with baseline, could serve as predictors of clinical efficacy of treatment with temezirolimus.

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Translational Relevance

Temsirolimus and other mTOR inhibitors are associated with increases in serum cholesterol, triglyceride, and glucose. This study used data from patients randomized to temsirolimus or IFN in the Global Advanced Renal Cell Carcinoma Trial to show that cholesterol increase is a predictor for temsirolimus efficacy. This study is hypothesis-generating and will motivate further research to better understand the mechanisms of action of temsirolimus and other mTOR inhibitors. If the result of this study is confirmed in other trials, cholesterol increase may provide an additional phase II trial endpoint to rapidly screen for clinically effective new mTOR inhibitors.

Patients and Methods

The Global Advanced Renal Cell Carcinoma Trial (ARCC, ClinicalTrials.gov number, NCT00065468) was a 3-arm phase III trial in which patients with advanced renal cell carcinoma with an intermediate- or poor-risk classification were randomly assigned to first-line therapy of IFN α -2a (starting dose of 3 million units given s.c. 3 times/wk), temsirolimus (25 mg i.v. weekly), or combination IFN α -2a and temsirolimus (starting dose of IFN 3 million units given s.c. 3 times/wk and temsirolimus 15 mg i.v. weekly; ref. 12). The disease was assessed every 8 weeks by the Response Evaluation Criteria in Solid Tumors (RECIST; ref. 16) and patients continued with treatment until disease progression or symptomatic deterioration or intolerable adverse events, as defined by National Cancer Institute Common Terminology Criteria of Adverse Events (NCI CTCAE v.3.0). Eligible patients had stage IV or recurrent renal cell carcinoma, a Karnofsky performance score of 60 or higher, no previous systemic therapy, and adequate bone marrow, hepatic, and renal function. Patients in this study were also required to have fasting total cholesterol level ≤ 9.1 mmol/L (350 mg/dL) and triglyceride ≤ 4.5 mmol/L (400 mg/dL), but no restriction on fasting blood glucose level. At least 3 of the following 6 predictors of poor prognosis were required: serum lactate dehydrogenase level of more than 1.5 times the upper limit of the normal range, hemoglobin level below the lower limit of the normal range, corrected serum calcium level of more than 2.5 mmol/L (10 mg/dL), less than 1 year from initial diagnosis of renal cell carcinoma to randomization, Karnofsky performance score of 60 or 70, and metastases in multiple organs. All patients gave informed consent. The primary endpoint was OS and secondary endpoints included PFS, tumor response, and clinical benefit. The results of this trial have been published (12).

Cholesterol, triglyceride, and glucose measurements during trial

Cholesterol, triglycerides, and glucose were each measured at baseline before treatment. Measurements of these

biomarkers were repeated every 2 weeks when patients were undergoing treatment in the trial. Change in the biomarker is defined as the difference between biomarker measurement during treatment and its baseline value.

The value of change in serum cholesterol, triglyceride, and glucose concentrations as predictors of temsirolimus efficacy

The objective of this study was to explore the potential value of change in serum cholesterol, triglyceride, and glucose concentrations as predictors of treatment advantage of temsirolimus compared with IFN on OS and PFS. Serum cholesterol, triglyceride, and glucose concentrations were modeled as time-varying covariates over the entire course of treatment. Changes in these biomarkers from pretreatment baseline readings were examined. The primary comparison made was between patients treated with temsirolimus only and those treated with IFN only after adjusting for baseline and on-study biomarker values. Variation in the effect of treatment across different randomization strata was also examined: we compared regional differences between patients treated in the United States and patients treated in the non-U.S. countries and between patients with and without prior nephrectomy. Patients treated with temsirolimus and those treated with a combination of IFN and temsirolimus were compared in sensitivity analyses.

Statistical methods

Baseline patient and disease characteristics were compared by *t* tests for continuous variables and χ^2 tests for categorical variables. The differences between treatment groups in the changes in each biomarker relative to baseline were assessed by using generalized estimating equations (GEE), with an autoregressive correlation structure to account for the multiple measurements on each patient (17). The estimated marginal mean using the regression coefficients and the 95% confidence intervals (CI) from the GEE model are presented for each treatment group. OS was defined as the time from randomization to death from any cause. PFS was defined as the time from randomization to first documented progression as determined by the site investigators' assessment or death. The ARCC trial prespecified the following factors as potentially important baseline characteristics, which might impact on PFS and OS: age; sex; geographic region; nephrectomy status; tumor histologic type; time from metastasis to randomization; Karnofsky performance score; and levels of hemoglobin, serum lactate dehydrogenase, and corrected serum calcium. These factors are adjusted for in multivariable Cox proportional hazards models to estimate HRs and 95% CIs for the baseline and time-varying covariates (18). Extended Kaplan–Meier curves were used to illustrate the effect of time-varying covariates from proportional hazards models (19). The curves describe the survival experience of patients according to their repeated measurements of these biomarkers during the entire study (the values used for the time-varying covariate in the proportional hazards model collapsed into categories according to the median). The

number of patients in each stratum is updated at each event time so that patients can be counted in different strata as their biomarkers change over time.

We also conducted landmark analyses to reduce possible confounding by time on treatment by assessing the impact of change in each individual biomarker at various landmark times on survival outcomes. Patients with early disease progression/deaths or patients lost to follow-up before the landmark times were excluded. Change in the biomarker was defined as the difference in the last reading for the biomarker obtained at landmark time from baseline. For these analyses, PFS and OS times were measured from the landmark times to these survival outcomes. Multivariable Cox proportional hazards models were used to estimate HRs and 95% CIs for treatment effect adjusted for change in biomarker. The landmark times at 1 and 2 months after randomizations were explored.

All *P* values were 2-sided, CIs were at the 95% level, and no adjustments were made for multiple comparisons.

Results

The primary analysis population was 416 patients (207 in the IFN α -2a group and 209 in the temsirolimus group) with a median follow-up of 17.9 months (range, 0.3–

27.5). Table 1 displays the baseline patient and disease characteristics by treatment group, which were well-balanced. There was no significant difference in the baseline serum cholesterol (*P* = 0.63), triglyceride (*P* = 0.11), or glucose (*P* = 0.60) measurements between the 2 treatment groups.

At baseline, serum cholesterol, triglyceride, and glucose measurements were available in 81%, 98%, and 98% of the total patients, respectively. At 2 and 4 months after randomization, 70% and 42% of the total patients had serum cholesterol, triglyceride, and glucose measurements conducted, respectively.

Effect of treatment on the biomarkers

During the study, serum cholesterol increased significantly from baseline in the temsirolimus group, with a mean increase of 0.95 mmol/L (37 mg/dL, *P* < 0.0001; Fig. 1A). In the IFN group, serum cholesterol did not change significantly from baseline during the study; mean change in cholesterol from baseline was –0.07 mmol/L (3 mg/dL; *P* = 0.19). Serum triglyceride and glucose were both significantly increased from baseline for the temsirolimus and the IFN groups; the changes in these biomarkers are summarized in Fig. 1B and C, respectively.

Table 1. Baseline characteristics of patients

Characteristic	Treatment	
	IFN N = 207	Temsirolimus N = 209
Median age (range), y	60 (23–86)	58 (32–81)
Age \geq 65 y	65 (31)	64 (31)
Sex male	148 (72)	139 (67)
Karnofsky performance score \leq 70	171 (83)	168 (80)
Prior nephrectomy	139 (67)	139 (66)
Histologic type clear cell	170 (82)	169 (81)
Lactate dehydrogenase level > 1.5 times upper limit of normal	48 (23)	36 (17)
Hemoglobin level < lower limit of normal	168 (81)	172 (82)
Corrected serum calcium level > 2.5mmol/L (10 mg/dL)	72 (35)	54 (26)
Time from initial diagnosis to randomization < 1 y	164 (79)	174 (83)
\geq 2 sites of organ metastasis	165 (80)	166 (79)
Median baseline cholesterol concentration (range), mmol/L ^a	4.2 (1.5–10.4)	4.4 (1.0–7.5)
Median baseline triglyceride concentration (range), mmol/L ^a	1.4 (0.4–4.5)	1.3 (0.4–4.3)
Median baseline glucose concentration (range), mmol/L ^a	5.6 (3.1–19.8)	5.4 (3.0–14.6)
Treatment with statins at baseline	17 (8)	17 (8)
Treatment with fibrates at baseline	1 (0.5)	0 (0)
Treatment with hypoglycemic agents at baseline	20 (10)	19 (9)
Treatment with statins during study	2 (1)	23 (11)
Treatment with fibrates during study	4 (2)	12 (6)
Treatment with hypoglycemic agents during study	1 (0.5)	26 (12)

NOTE: Values are number (%) unless otherwise labeled.

^aCholesterol values must be multiplied by 39 to convert from mmol/L to mg/dL, triglyceride values must be multiplied by 89 to convert from mmol/L to mg/dL, and glucose values must be multiplied by 18 to convert from mmol/L to mg/dL.

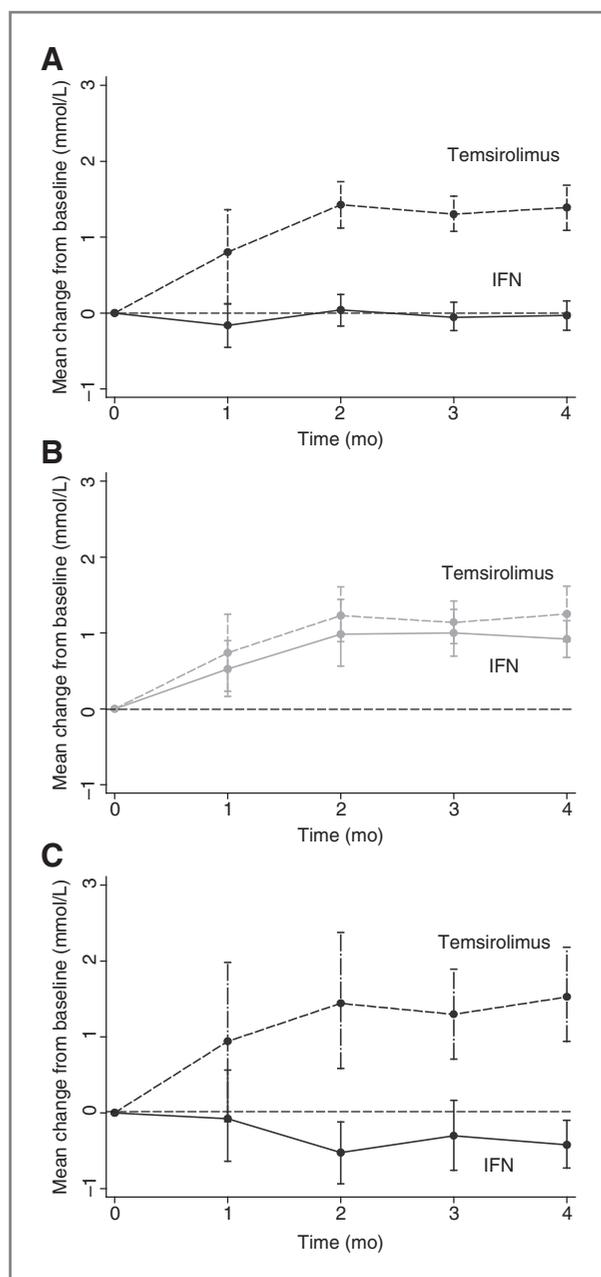


Figure 1. Mean changes in biomarkers from baseline in the IFN and temsirolimus arms. Vertical lines show ranges. A, cholesterol: mean change, IFN arm = -0.07 mmol/L ($P = 0.19$); mean change, temsirolimus arm = 0.95 mmol/L ($P < 0.0001$); mean difference = 1.02 ($P < 0.0001$). B, triglycerides: mean change, IFN arm = 0.53 mmol/L ($P < 0.0001$); mean change, temsirolimus arm = 0.85 mmol/L ($P < 0.0001$); mean difference = 0.32 ($P = 0.0008$). C, glucose: mean change, IFN arm = -0.25 mmol/L ($P = 0.007$); mean change, temsirolimus arm = 1.03 mmol/L ($P < 0.0001$); mean difference = 1.28 mmol/L ($P < 0.0001$).

Effect of treatment on OS and PFS

The proportions of patients who died were similar in the treatment groups, with 149 deaths (72%) in the temsirolimus group and 143 (68%) in the IFN group; the median survival, however, was longer in the temsirolimus group

(10.9 vs. 7.3 months), favoring treatment with temsirolimus (HR, 0.76; 95% CI, 0.60–0.95; $P = 0.02$).

Similar results were seen for PFS: 187 patients in the temsirolimus arm and 190 in the IFN arm experienced disease progression. The median PFS was 3.8 months in the temsirolimus group and 1.9 months in the IFN group, favoring temsirolimus (HR, 0.70; 95% CI, 0.58–0.86; $P = 0.001$).

The HRs for PFS and OS reported above, based on simple Cox proportional hazards regression, differ slightly from the previously published trial result (12). The trial derived its results, based on Cox proportional hazards regression stratified according to the predefined strata.

Association between biomarkers with OS and PFS

At baseline, serum cholesterol level was associated with survival for the entire study cohort. When analyzed as a continuous measure in univariate analyses, 1 mmol/L (39 mg/dL) increase in cholesterol was associated with 18% reduction in the risk of death (HR, 0.82; 95% CI, 0.74–0.91; $P < 0.001$). One millimole per liter increase in cholesterol was also associated with a 10% reduction in the risk of disease progression (HR, 0.90; 95% CI, 0.83–0.99; $P = 0.03$). Cholesterol remains a significant predictor for OS in multivariate analysis, adjusting for other baseline factors (Table 2).

During the study period, increase in serum cholesterol from baseline was associated with a reduced risk of disease progression (HR, 0.85 per mmol/L increase; 95% CI, 0.78–0.92; or HR, 0.996 per mg/dL increase; 95% CI, 0.993–0.998; $P < 0.0001$), regardless of the treatment arm. The effect on OS was similar (HR, 0.82 per mmol/L increase; 95% CI, 0.74–0.91; or HR, 0.995 per mg/dL increase; 95% CI, 0.992–0.998; $P < 0.0001$). Increase in serum cholesterol from baseline remained significantly associated with a reduced risk of disease progression and death in multivariate analyses adjusted for other baseline factors (Table 3).

For patients with a large increase in serum cholesterol [above the median distribution, ≥ 0.67 mmol/L (26 mg/dL)] from baseline, the median OS in the temsirolimus and IFN groups was 12.9 and 10.3 months, respectively (Fig. 2A). For patients with a smaller change in cholesterol [< 0.67 mmol/L (26 mg/dL) increase in serum cholesterol from baseline], the median OS was 4.2 and 6.9 months in the temsirolimus and IFN groups, respectively. The log-rank test of equality of these 4 curves was significant ($P < 0.0001$).

The median PFS in the temsirolimus and IFN groups, when larger increases in cholesterol were seen, was 5.3 and 3.4 months, respectively, compared with 1.9 months in both treatment arms, when patients experienced smaller increases in cholesterol (Fig. 2B). The log-rank test of equality of these 4 curves was also significant ($P < 0.0001$).

Baseline serum triglyceride and glucose did not predict PFS or OS in univariate or multivariate analyses (Table 2). During the study period, increase in serum triglyceride from baseline was associated with a reduced risk of disease progression and death in univariate or multivariate analyses (Table 3). However, increase in serum glucose from baseline during the study period was not associated with a reduced

Table 2. Baseline serum cholesterol, triglyceride, and glucose levels and the risk of disease progression and death

Marker	PFS				OS			
	Unadjusted analysis		Adjusted analysis ^a		Unadjusted analysis		Adjusted analysis ^a	
	HR ^b (95% CI)	P	HR ^b (95% CI)	P	HR ^b (95% CI)	P	HR ^b (95% CI)	P
Cholesterol (mmol/L ^c)	0.90 (0.83–0.99)	0.03	0.92 (0.83–1.02)	0.11	0.82 (0.74–0.91)	<0.0001	0.87 (0.77–0.99)	0.03
Triglyceride (mmol/L ^c)	1.05 (0.92–1.19)	0.48	1.08 (0.94–1.24)	0.27	0.99 (0.85–1.15)	0.87	1.04 (0.89–1.22)	0.61
Glucose (mmol/L ^c)	1.02 (0.97–1.08)	0.47	1.01 (0.94–1.07)	0.88	1.03 (0.97–1.10)	0.28	1.03 (0.96–1.11)	0.43

^aAnalysis unadjusted (univariate) or adjusted (multivariate) for trial prespecified factors: age; sex; geographic region; nephrectomy status; tumor histologic type; time from metastasis to randomization; Karnofsky performance score; and levels of hemoglobin, serum lactate dehydrogenase, and corrected serum calcium.

^bHR is for each mmol/L unit difference in marker level.

^cCholesterol values must be multiplied by 39 to convert from mmol/L to mg/dL, triglyceride values must be multiplied by 89 to convert from mmol/L to mg/dL, and glucose values must be multiplied by 18 to convert from mmol/L to mg/dL.

risk of disease progression or death in multivariate analyses (Table 3).

Change in biomarkers as a predictor of the effect of treatment on survival

In multivariable analyses of treatment with adjustment for change in cholesterol and baseline cholesterol level, temsirolimus (compared with IFN) no longer improved survival outcomes; for OS, adjusted HR (temsirolimus vs. IFN) = 1.14 (95% CI, 0.85–1.53; $P = 0.37$), and for PFS, adjusted HR (temsirolimus vs. IFN) = 0.88 (95% CI, 0.67–1.15; $P = 0.35$). Therefore, changes in cholesterol appear to account for most of the advantageous effect of temsirolimus on OS and PFS (Fig. 3 and Table 4).

When the association between treatment and clinical outcome was adjusted individually for serum triglyceride or for glucose, the relative treatment advantage of temsi-

rolimus over IFN remained statistically significant for PFS and did not change substantially from the unadjusted estimate for OS (Fig. 3 and Table 4). Therefore, the treatment effect of temsirolimus was largely independent of changes in serum triglyceride or glucose.

Regional variations in the change in serum cholesterol and the effect of treatment

There were 122 (29.3%) patients in the U.S. stratum and 294 (70.7%) patients in the non-U.S. stratum. The differences in mean change in serum cholesterol between temsirolimus and IFN for patients from United States and non-U.S. countries were 0.54 mmol/L [95% CI, 0.27–0.81; 21 mg/dL (95% CI, 11–32)] and 1.16 mmol/L [95% CI, 0.96–1.37; 45 mg/dL (95% CI, 37–53)], respectively. The smaller increase in serum cholesterol in the patients from United States was also matched with smaller treatment effect as

Table 3. On-study serum cholesterol, triglyceride and glucose levels, and the risk of disease progression and death

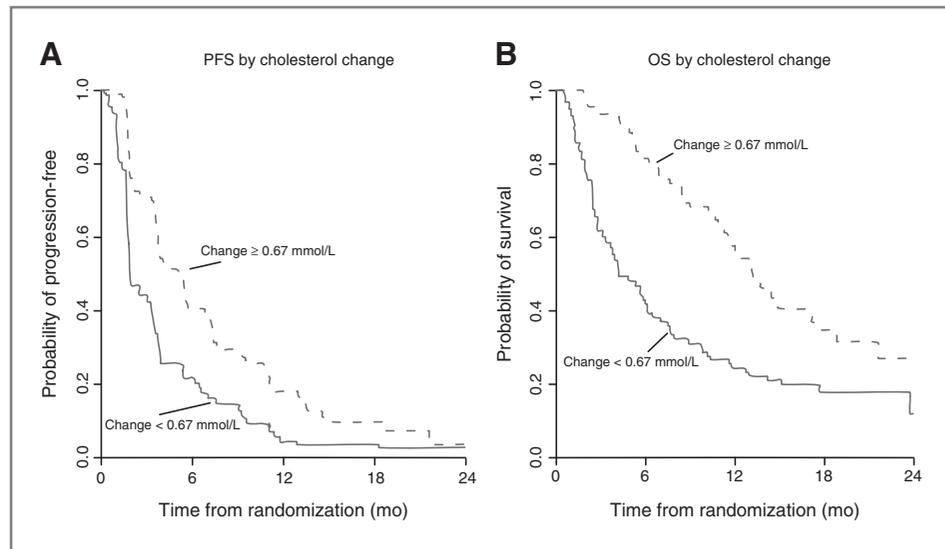
Marker	PFS				OS			
	Unadjusted analysis		Adjusted analysis ^a		Unadjusted analysis		Adjusted analysis ^a	
	HR ^b (95% CI)	P	HR ^b (95% CI)	P	HR ^b (95% CI)	P	HR ^b (95% CI)	P
Cholesterol (mmol/L ^c)	0.85 (0.78–0.92)	<0.0001	0.81 (0.74–0.89)	<0.0001	0.82 (0.74–0.91)	<0.0001	0.76 (0.68–0.85)	<0.0001
Triglyceride (mmol/L ^c)	0.91 (0.83–0.99)	0.03	0.88 (0.80–0.97)	0.01	0.82 (0.74–0.91)	<0.0001	0.83 (0.74–0.93)	0.001
Glucose (mmol/L ^c)	0.99 (0.95–1.03)	0.56	1.00 (0.96–1.05)	0.90	0.94 (0.89–0.99)	0.02	0.95 (0.90–1.00)	0.07

^aAnalysis unadjusted (univariate) or adjusted (multivariate) for trial prespecified factors: age; sex; geographic region; nephrectomy status; tumor histologic type; time from metastasis to randomization; Karnofsky performance score; and levels of hemoglobin, serum lactate dehydrogenase, and corrected serum calcium.

^bHR is for each mmol/L unit change in marker level.

^cCholesterol values must be multiplied by 39 to convert from mmol/L to mg/dL, triglyceride values must be multiplied by 89 to convert from mmol/L to mg/dL, and glucose values must be multiplied by 18 to convert from mmol/L to mg/dL.

Figure 2. Extended Kaplan–Meier estimates of PFS (A) and OS (B) and cholesterol change (≥ 0.67 vs. < 0.67 mmol/L) for temsirolimus group.



compared with patients from non-U.S. countries. For PFS, HR was 0.80 (95% CI, 0.54–1.17) and 0.67 (95% CI, 0.53–0.86) for patients from United States and non-U.S. countries, respectively. For OS, HR was 0.79 (95% CI, 0.52–1.21) and 0.74 (95% CI, 0.56–0.97) for patients from United States and non-U.S. countries, respectively.

At baseline, cholesterol was higher in non-U.S. patients than in U.S. patients (4.32 vs. 4.06 mmol/L). The regional differences in treatment effect persists even with adjustment for baseline cholesterol—for PFS, adjusted HR was 0.77 (95% CI, 0.51–1.17) and 0.62 (95% CI, 0.47–0.81) for patients from United States and non-U.S. countries,

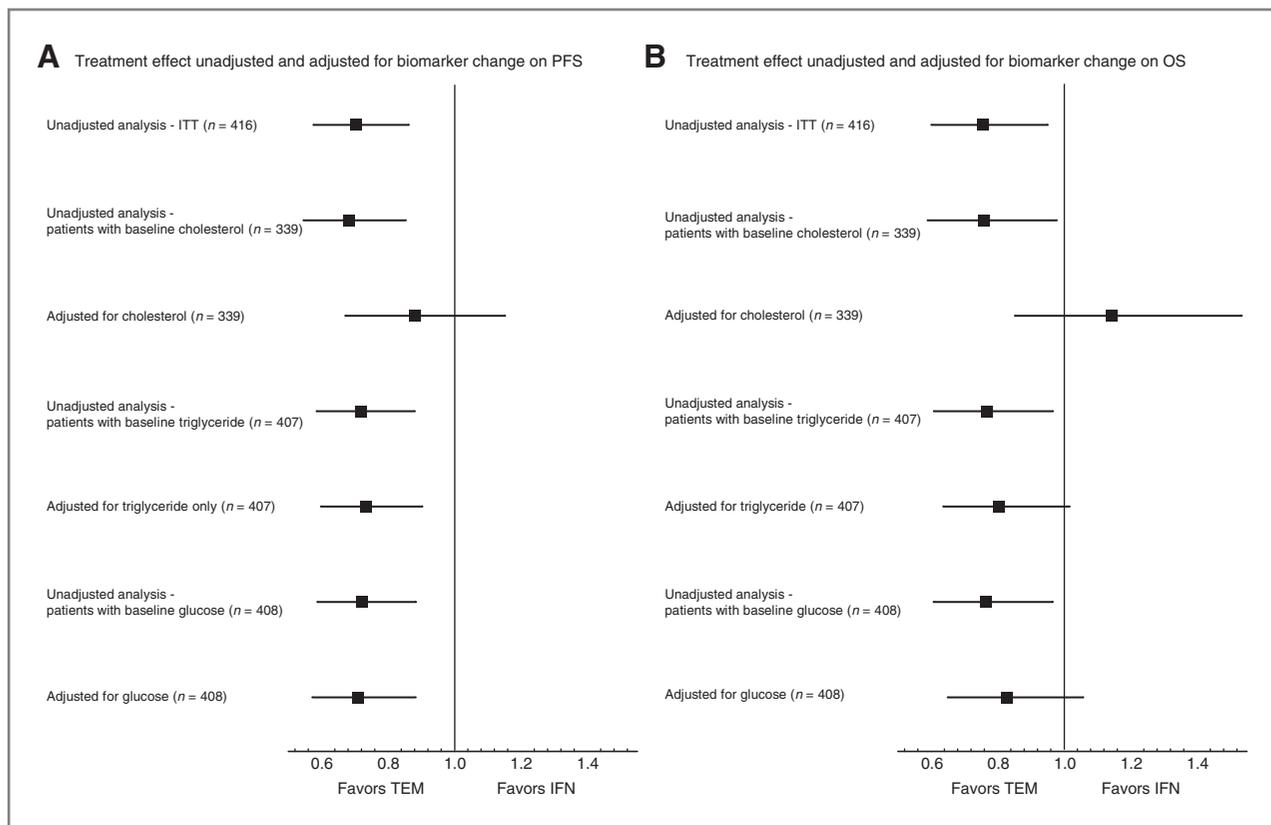


Figure 3. Unadjusted treatment effects and treatment effects adjusted for changes in biomarkers from baseline. A, PFS and B, OS. ITT, intention-to-treat; TEM, temsirolimus.

Table 4. Impact of serum cholesterol, triglyceride, and glucose levels on treatment effects for PFS and OS

Treatment (temsirolimus vs. IFN)	PFS		OS	
	HR ^a (95% CI)	P	HR ^a (95% CI)	P
Unadjusted analysis	0.70 (0.58–0.86)	0.001	0.76 (0.60–0.95)	0.02
Adjusted for on-study change and baseline cholesterol only	0.88 (0.67–1.15)	0.35	1.14 (0.85–1.53)	0.37
Adjusted for on-study change and baseline cholesterol and other baseline factors ^b	0.86 (0.65–1.16)	0.33	1.11 (0.81–1.52)	0.51
Adjusted on-study change and baseline triglyceride only	0.73 (0.60–0.90)	0.003	0.80 (0.64–1.02)	0.07
Adjusted on-study change and baseline triglyceride and other baseline factors ^b	0.68 (0.54–0.85)	0.001	0.79 (0.61–1.03)	0.08
Adjusted for on-study change and baseline glucose only	0.71 (0.57–0.88)	0.002	0.83 (0.65–1.06)	0.13
Adjusted for on-study change and baseline glucose and other baseline factors ^b	0.63 (0.50–0.80)	<0.0001	0.78 (0.60–1.02)	0.07

^aHR is for comparison of treatment of temsirolimus versus IFN.

^bAnalysis unadjusted (univariate) or adjusted (multivariate) for baseline and on-study change in marker level. Other baseline factors refer to trial prespecified factors: age; sex; geographic region; nephrectomy status; tumor histologic type; time from metastasis to randomization; Karnofsky performance score; and levels of hemoglobin, serum lactate dehydrogenase, and corrected serum calcium.

respectively. For OS, HR was 0.84 (95% CI, 0.53–1.34) and 0.69 (95% CI, 0.51–0.93) for patients from United States and non-U.S. countries, respectively.

On-study statin treatment was equally common in United States and non-U.S. countries (5.7% vs. 6.1%, $P = 0.88$). When the analyses were repeated to account for on-study statin treatment, similar results were obtained (not shown).

When the analyses were repeated for patients who had nephrectomy compared with those without nephrectomy, similar results were obtained with smaller cholesterol increase in the non-nephrectomy stratum also associated with a smaller treatment effect.

Impact of on-study statin treatment, change in serum cholesterol, and the effect of treatment

Significantly more patients were treated with statin in the temsirolimus group during the study as compared with the IFN group (11.0% vs. 1.0%, $P < 0.0001$). On-study statin treatment does not impact on treatment efficacy; for OS, adjusted HR (temsirolimus vs. IFN) = 1.26 (95% CI, 0.94–1.71; $P = 0.13$), and for PFS, adjusted HR (temsirolimus vs. IFN) = 0.93 (95% CI, 0.70–1.22; $P = 0.60$). On-study statin treatment was a significant predictor in the multivariate model for OS (HR = 0.38; 95% CI, 0.18–0.83; $P = 0.02$) but not for PFS ($P = 0.11$).

Landmark analyses

At 1 month, 276 patients who had not progressed and had baseline cholesterol readings were analyzed. In unadjusted analyses, the HR (temsirolimus vs. IFN) for PFS was 0.81 (95% CI, 0.65–1.00; $P = 0.05$). In multivariable analyses of treatment adjusted for baseline cholesterol reading and change in cholesterol between landmark and baseline time points, the adjusted HR (temsirolimus vs. IFN) for PFS was 0.91 (95% CI, 0.68–1.22; $P = 0.54$). The 289 patients who had not died and had baseline cholesterol readings were analyzed at landmark time of 1 month. The HRs on OS for the unadjusted and adjusted treatment effect

(temsirolimus vs. IFN) were 0.81 (95% CI, 0.64–1.03; $P = 0.09$) and 1.15 (95% CI, 0.83–1.60; $P = 0.40$), respectively.

Significantly fewer patients who had not progressed ($n = 165$) or died ($n = 238$), respectively, with cholesterol readings, were available for landmark analysis at 2 months. The impact of early change in cholesterol on survival outcomes could not be clearly shown because of this limitation (results not shown).

Discussion

The ARCC trial showed that temsirolimus was associated with improved survival compared with IFN α -2a (12). Here, we show in this study that an increase in cholesterol was associated with longer survival and predicted temsirolimus efficacy. Temsirolimus was associated with a larger increase in serum cholesterol than in IFN. Among the patients treated with temsirolimus, those surviving longer were also observed to have larger increase in cholesterol. Moreover, when the effect of treatment on serum cholesterol change was accounted for in multivariable analyses, no additional temsirolimus advantage over IFN was observed suggesting that changes in cholesterol account for the advantageous effect of temsirolimus on survival.

Across the 2 regional strata (United States vs. non-U.S. countries), a greater increase in serum cholesterol concentration was observed in the temsirolimus group relative to the IFN group. However, the size of the increase in serum cholesterol was smaller in patients treated in the United States than in the non-U.S. countries (0.54 vs. 1.16 mmol/L; 21 vs. 45 mg/dL). This was matched by a smaller relative reduction in the rate of disease progression for the United States than the non-U.S. stratum (HR, 0.80 vs. 0.67). While the regional difference in serum cholesterol response is likely a chance finding, it provides a fortuitous opportunity to show that the smaller cholesterol increase in the U.S. stratum is also associated with a smaller treatment effect. We also found differences in the size of the increase in serum cholesterol and matching relative reduction in the rate of

disease progression across the 2 strata of patients with and without nephrectomy. Analyses of these subgroups are intended to illustrate the consistency of the observation across the treatment protocol stratification variables.

Emerging evidence suggests that Akt/mTOR plays a key role in the intersecting pathways involved in lipid metabolism, glucose metabolism, and regulation of cell cycles. Under conditions of excess energy intake over expenditure, insulin engages its receptor, resulting in lipogenesis mediated by the Akt/mTOR pathway and promotion of glucose uptake, glycolysis, and lipid and cholesterol synthesis (20–24). Constitutive activation of Akt/mTOR, as shown in many cancers, including renal cell cancer, also results in the stimulation of this pathway, which ultimately causes expression of lipogenic enzymes, such as acetyl-CoA carboxylase and fatty acid synthase, mediated by sterol regulatory element-binding protein-1 (SREBP-1), a master transcriptional regulator for lipids (22). A recent study further indicates that mTOR complex 1, a functional subunit of the 2 distinct mTOR complex, regulates cholesterol biosynthesis through its substrate, 4E-BP1, on SREBP-2 (23). Notionally, blocking mTOR function reduces these cellular functions, leading to apoptosis and autophagy, thereby reducing metabolic requirements at the cellular level. Cholesterol transport is also impaired with mTOR blockade in endothelial cells, leading to antiangiogenesis (24). The exact biochemical mechanisms subsequently leading to increased serum cholesterol, triglyceride, and glucose, however, remain poorly understood.

Nevertheless, these *in vitro* data are supported by *in vivo* and clinical studies. In guinea pigs, rapamycin alters the insulin signaling pathway, resulting in elevated serum triglyceride and glucose (25). In a phase I study of deforolimus, maximum change in serum cholesterol in the first 2 cycles was significantly associated with a response to treatment (15). In a phase II study of temsirolimus, elevated serum lipids during the first 2 cycles of treatment were also significantly associated with radiographic response (26).

Despite preclinical and some early-phase clinical trial data, our study based on a single large phase III study shows that serum cholesterol, but not triglyceride or glucose, is a potential biomarker for temsirolimus efficacy. It remains unclear that why serum triglyceride and glucose do not show the same attributes. This may be related to the stringent requirement and other limitations of the statistical approach used in the present study (27–29). A recently developed meta-analytic approach, using data from many randomized trials, to directly measure the association between the treatment effects on a surrogate and the true clinical survival endpoint has been proposed as an alternative approach for surrogate endpoint analysis (30–32). Such an approach has been carried out to show that disease-free survival is a valid surrogate endpoint for OS in colorectal cancer (33, 34).

The main strengths of our study include the large sample size, the availability of survival data, regular repeated measurements of cholesterol, triglycerides, and glucose, and treatment superiority in the experimental arm allowing for

analysis of treatment-by-biomarker interaction. Differences in the outcomes allowed for direct comparisons across the 3 different biomarkers; cholesterol, but not glucose or triglycerides, was linked to survival benefit. Furthermore, the observation of increased cholesterol and improved survival was consistent across all study stratification variables.

This study has a number of limitations. First, this analysis was conducted *post hoc* with available trial data and therefore should be considered as hypothesis-generating for future studies, rather than definitive. Second, landmark analyses to assess the impact of early change in cholesterol on survival outcomes were limited because fewer patients were available and fewer outcome events were documented after the landmark time of 2 months which coincides with the maximal increase in cholesterol (Fig. 1A). However, the results of landmark analyses at 1 month give similar qualitative conclusions as the time-varying Cox proportional hazards analyses (Fig. 3) which assess cholesterol change throughout the trial to provide greater power to detect its impact on survival. Finally, serum cholesterol, triglyceride, and glucose were measured on all trial subjects as part of the ARCC trial protocol to monitor for potential toxicities from treatment. The laboratories used current standard protocols and quality control procedures for these routine assays. However, not all samples collected were fasting specimens, which might impact on the reproducibility of results. As the primary objective of the ARCC trial was not to investigate the impact of cholesterol, triglyceride, and glucose changes on clinical outcomes, the trial protocol did not specify quantitative methods and reproducibility assessments, and this information was not collected to allow a complete assessment and reporting of assay methods to address the REMARK criteria (35).

This study is not sufficiently powered to investigate whether treatment with statins interacts with temsirolimus-mediated inhibition of mTOR as only 25 patients received statins during the study. Other studies have shown that statins appear to reduce mTOR activation and improved chemotherapy sensitivity (36, 37). It remains speculative whether statins could potentiate the effects of temsirolimus; this hypothesis should be tested further in future prospective trials.

In summary, this study shows that the treatment benefit of temsirolimus over IFN is associated with increases in serum cholesterol. This analysis, however, is hypothesis-generating and laboratory studies are required to investigate the exact biologic mechanisms of treatment action. Whether increase in serum cholesterol merely reflects successful target (mTOR pathway) inhibition or is mechanistically required for the antitumor response cannot be determined from our analysis. Nevertheless, our analysis indicates that cholesterol increase is potentially an important biomarker of treatment benefit with temsirolimus in terms of survival outcomes. If confirmed in other trials, change in serum cholesterol concentration may provide an additional phase II trial endpoint to screen for clinically effective new mTOR inhibitor agents for future phase III trials.

Disclosure of Potential Conflicts of Interest

B. Egleston has a commercial research grant from Pfizer and G. Hudes and P. de Souza are consultants/advisory board members of 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. Hudes

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.K. Lee, I.C. Marschner, R.J. Simes, M. Voysey, B. Egleston, G. Hudes, P. de Souza

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