Increase in cholesterol predicts survival advantage in renal cell carcinoma patients treated with temsirolimus

Running title: Cholesterol as a biomarker for temsirolimus efficacy in renal cancer

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Key words: surrogate, mTOR, kidney cancer, cholesterol, metabolism
Abstract

Purpose

Temsirolimus 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 interferon or temsirolimus 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 (PFS). We also assess the extent to which changes of these biomarkers predict the effects of temsirolimus on survival.

Results

Temsirolimus was associated with larger mean increases in cholesterol (1.02 mmol/L; \( P < .0001 \)), triglycerides (0.32 mmol/L; \( P = .0008 \)) and glucose (1.28 mmol/L; \( P < .0001 \)) compared with interferon, and improved survival (OS: HR 0.76, \( P = .02 \); PFS: HR 0.70, \( P = .001 \)). Cholesterol increase during study was associated with longer survival (OS: hazard ratio (HR) 0.77 per mmol/L, \( P < .0001 \); PFS HR 0.81 per mmol/L; \( P < .0001 \)). Temsirolimus 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, \( P = .37 \); PFS HR 0.88, \( P = .35 \)). Temsirolimus effect on triglycerides or glucose, did
not predict its effect on survival, with survival advantage in favor of temsirolimus still observed after adjusting for these factors (p=0.003 and p=0.002).

**Conclusion**

Cholesterol increase is a potential predictor for temsirolimus efficacy. Longer survival in patients treated with temsirolimus was observed in those with larger increases in cholesterol. Prospectively designed biomarker studies of temsirolimus or other mTOR inhibitors are recommended.

**Translational relevance**

Temsirolimus and other mTOR inhibitors are associated with increases in serum cholesterol, triglyceride and glucose. This study utilised data from patients randomized to
temsirilimus or interferon in the Global Advanced Renal Cell Carcinoma Trial to demonstrate 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 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.
Introduction
The mammalian target of rapamycin (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).

Temsirolimus, 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 temsirolimus was associated with improved overall survival (OS) and progression-free survival (PFS), compared with interferon alfa-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 vascular endothelial growth factor-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 temsirolimus.

**Patients and Method**

The Global Advanced Renal Cell Carcinoma Trial (ClinicalTrials.gov number, NCT00065468) was a 3-arm phase III trial in which patients with advanced renal-cell carcinoma with an intermediate-risk or poor-risk classification were randomly assigned to first-line therapy of interferon alfa-2a (starting dose of 3 million U given subcutaneously 3 times per week), temsirolimus (25 mg intravenously weekly), or combination interferon alfa-2a and temsirolimus (starting dose of interferon 3 million U given subcutaneously 3 times per week and temsirolimus 15 mg intravenously weekly) (12). The disease was assessed every 8 weeks by the Response Evaluation Criteria in Solid Tumors (RECIST)(16), and patients continued on 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, adequate bone marrow, hepatic, and renal function. Patients in this study were also required to have fasting total cholesterol level $\leq 9.1$ mmol/L (350 mg/dL) and triglyceride $\leq 4.5$ mmol/L (400 mg/dL), but no restriction on fasting blood glucose level. At least three of the following six 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), <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 prior treatment. Measurements of these biomarkers were repeated every two 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 to interferon on OS and PFS. Serum cholesterol, triglyceride, and glucose concentrations were modelled 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 interferon 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 of America (USA) and patients treated in the non-
USA countries, and between patients with and without prior nephrectomy. Patients treated with temsirolimus and those treated with a combination of interferon and temsirolimus were compared in sensitivity analyses.

**Statistical methods**

Baseline patient and disease characteristics were compared by $t$ tests for continuous variables and $\chi^2$ 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 from the GEE model are presented for each treatment group. OS was defined as the time from randomisation to death from any cause. PFS was defined as the time from randomisation to first documented progression as determined by the site investigators’ assessment or death. The Global Advanced Renal Cell Carcinoma 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 hazard ratios (HRs) and 95% confidence intervals (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 patients can be counted in different strata as their biomarkers change over time.

We also performed 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 HR and 95% CI for treatment effect adjusted for change in biomarker. The landmark times at 1 month and 2 months post-randomisations were explored.

All $P$ values were two-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 interferon alfa-2a group, 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 two treatment groups.

At baseline, serum cholesterol, triglyceride and glucose measurements were available in 81%, 98% and 98% of the total patients respectively. At two and four months post-randomisation, 70% and 42% of the total patients had serum cholesterol, triglyceride and glucose measurements performed respectively.

Effect of treatment on the biomarkers

During the study, serum cholesterol increased significantly from baseline in the temsirolimus group, with a mean rise of 0.95 mmol/L [37 mg/dL] ($P<0.0001$) (Figure 1A). In the interferon 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 interferon groups; the changes in these biomarkers are summarized in Figure 1B and 1C respectively.
**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 interferon group; the median survival, however, was longer in the temsirolimus group (10.9 months vs 7.3 months), favoring treatment with temsirolimus (HR 0.76; 95% CI, 0.60 to 0.95; \(P=\) .02). Similar results were seen for PFS: 187 patients in the temsirolimus arm and 190 in the interferon arm experienced disease progression. The median PFS was 3.8 months in the temsirolimus group and 1.9 months in the interferon group, favoring temsirolimus (HR 0.70; 95% CI, 0.58 to 0.86; \(P=\) .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 pre-defined strata.

**Association between biomarkers with OS and PFS**

At baseline, serum cholesterol level was associated with survival for the entire study cohort. When analysed as a continuous measure in univariate analyses, one millimole per litre [39 mg/dL] higher in cholesterol was associated with 18% reduction in the risk of death (HR 0.82; 95% CI, 0.74 to 0.91; \(P<\) .001) and 10% reduction in the risk of disease.
progression (HR 0.90; 95% CI, 0.83 to 0.99; \( P = .03 \)). 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 to 0.92; or HR 0.996 per mg/dL increase; 95% CI, 0.993 to 0.998; \( P < .0001 \)) regardless of the treatment arm. The effect on overall survival was similar (HR 0.82 per mmol/L increase; 95% CI, 0.74 to 0.91; or HR 0.995 per mg/dL increase; 95% CI, 0.992 to 0.998; \( P < .0001 \)). 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, \( \geq 0.67 \) mmol/L [26 mg/dL]) from baseline, the median OS in the temsirolimus and interferon groups was 12.9 months and 10.3 months, respectively (Figure 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 months and 6.9 months in the temsirolimus and interferon groups, respectively. The log-rank test of equality of these four curves was significant (\( P < .0001 \)).

The median PFS in the temsirolimus and interferon groups when larger increases in cholesterol were seen was 5.3 months and 3.4 months, respectively, compared with 1.9
months in both treatment arms when patients experienced smaller increases in cholesterol (Figure 2B). The log-rank test of equality of these four curves was also significant ($P<.0001$).

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 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 interferon) no longer improved survival outcomes; for OS, adjusted HR (temsirolimus versus interferon) =1.14 (95% CI, 0.85 to 1.53; $P=.37$), and for PFS, adjusted HR (temsirolimus versus interferon) =0.88 (95% CI, 0.67 to 1.15; $P=.35$). Therefore, changes in cholesterol appear to account for most of the advantageous effect of temsirolimus on OS and PFS (Figure 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 temsirolimus over interferon remained statistically significant for PFS and did not change substantially
from the unadjusted estimate for OS (Figure 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 USA stratum and 294 (70.7%) patients in the non-USA stratum. The differences in mean change in serum cholesterol between temsirolimus and interferon for patients from USA and non-USA countries were 0.54 mmol/L (95% CI, 0.27 to 0.81) [21 mg/dL (95% CI, 11 to 32)] and 1.16 mmol/L (95% CI, 0.96 to 1.37) [45 mg/dL (95% CI, 37 to 53)], respectively. The smaller rise in serum cholesterol in the patients from USA was also matched with smaller treatment effect as compared to patients from non-USA. For PFS, HR=0.80 (95% CI, 0.54 to 1.17) and 0.67 (95% CI, 0.53 to 0.86) for patients from USA and non-USA countries respectively. For OS, HR= 0.79 (95% CI, 0.52 to 1.21) and 0.74 (95% CI, 0.56 to 0.97) for patients from USA and non-USA countries, respectively.

At baseline, cholesterol was higher in non-USA patients as compared to USA 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= 0.77 (95% CI, 0.51 to 1.17) and 0.62 (95% CI, 0.47 to 0.81) for patients from USA and non-USA countries respectively. For OS, HR= 0.84 (95% CI, 0.53 to 1.34) and 0.69 (95% CI, 0.51 to 0.93) for patients from USA and non-USA countries, respectively.
On study statin treatment was equally common in USA and non-USA countries (5.7% vs 6.1%, \( P = .88 \)). 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 rise 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 to the interferon group (11.0% vs 1.0%, \( P < .0001 \)). On-study statin treatment does not impact on treatment efficacy; for OS, adjusted HR (temsirolimus versus interferon) = 1.26 (95% CI, 0.94 to 1.71; \( P = .13 \)), and for PFS, adjusted HR (temsirolimus versus interferon) = 0.93 (95% CI, 0.70 to 1.22; \( P = .60 \)). On-study statin treatment was a significant predictor in the multivariate model for OS (HR = 0.38; 95% CI, 0.18 to 0.83; \( P = .02 \)) but not for PFS (\( P = .11 \)).

**Landmark analyses**

At 1 month, 276 patients who had not progressed and had baseline cholesterol readings were analysed. In unadjusted analyses, the HR (temsirolimus versus interferon) for PFS...
was 0.91 (95% CI 0.80 to 1.02; P=.07). In multivariable analyses of treatment adjusted for baseline cholesterol reading and change in cholesterol between landmark and baseline time points, the adjusted HRs (temsirolimus versus interferon) for PFS were 0.91 (95% CI, 0.69 to 1.21; P=.54). The 289 patients who had not died and had baseline cholesterol readings were analysed at landmark time of 1 month. The HRs on OS for the unadjusted and adjusted treatment effect (temsirolimus versus interferon) were 0.91 (95% CI, 0.69 to 1.20; P=.50) and 1.15 (95% CI, 0.83 to 1.60; P=.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 demonstrated because of this limitation (results not shown).

**Discussion**

The Global Advanced Renal Cell Carcinoma Trial demonstrated temsirolimus was associated with improved survival compared with interferon alfa-2a (12). Here, we demonstrate in this study, an increase in cholesterol was associated with longer survival and predicted temsirolimus efficacy. Temsirolimus was associated with a larger increase in serum cholesterol as compared to interferon. Amongst 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
interferon was observed suggesting that changes in cholesterol account for the advantageous effect of temsirolimus on survival.

Across the two regional strata (USA versus non-USA countries), a greater increase in serum cholesterol concentration was observed in the temsirolimus group relative to the interferon group. However, the size of the rise in serum cholesterol was smaller in patients treated in the USA than non-USA 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 USA than the non-USA 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 demonstrate that the smaller cholesterol rise in the US stratum is also associated with a smaller treatment effect. We also found differences in the size of the rise in serum cholesterol and matching relative reduction in the rate of disease progression across the two strata of patients with and without nephrectomy. Analyses of these subgroups are intended to illustrate 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 demonstrated 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 two 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 deferolimus, maximum change in serum cholesterol in the first two cycles was significantly associated with a response to treatment (15). In a phase II study of temsirolimus, elevated serum lipids during the first two cycles of treatment was 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 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 performed to demonstrate that disease-free survival is a valid surrogate endpoint for overall survival 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 three 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 using available trial data and therefore should be regarded 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 rise in cholesterol (Figure 1A). However, the results of landmark analyses at 1 month give similar qualitative conclusions as the time-varying Cox proportional-hazards analyses (Figure 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 demonstrated 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 demonstrates that the treatment benefit of temsirolimus over interferon is associated with increases in serum cholesterol. This analysis, however, is
hypothesis - generating and laboratory studies are required to investigate the exact biological 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 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.

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References


Figure legends

Figure 1
Mean changes in biomarkers from baseline in the interferon and temsirolimus arms. Vertical lines show ranges.

1A
Cholesterol: mean change, interferon arm = -0.07 mmol/L ($P=.19$); mean change, temsirolimus arm = 0.95 mmol/L ($P<.0001$); mean difference = 1.02 ($P<.0001$).

1B
Triglycerides: mean change, interferon arm = 0.53 mmol/L ($P<.0001$); mean change, temsirolimus arm = 0.85 mmol/L ($P<.0001$); mean difference = 0.32 ($P=.0008$).

1C
Glucose: mean change, interferon arm = -0.25 mmol/L ($P=.007$); mean change, temsirolimus arm = 1.03 mmol/L ($P<.0001$); mean difference = 1.28 mmol/L ($P<.0001$).

Figure 2
Extended Kaplan–Meier estimates of progression-free survival (2A) and overall survival (2B), and cholesterol change ($\geq 0.67$ mmol/L vs <0.67 mmol/L) for temsirolimus group.

Figure 3
Unadjusted treatment effects and treatment effects adjusted for changes in biomarkers from baseline. 3A, progression-free survival; 3B, overall survival.

ITT, intention-to-treat; TEM, temsirolimus; IFN, interferon
Figure 2

A  Progression-free Survival by Cholesterol Change

B  Overall Survival by Cholesterol Change
Figure 3

A  Treatment Effect Unadjusted and Adjusted for Biomarker Change on Progression-free Survival

Unadjusted Analysis - ITT (n=416)

Unadjusted Analysis - patients with baseline cholesterol (n=339)

Adjusted for cholesterol (n=339)

Unadjusted Analysis - patients with baseline triglyceride (n=407)

Adjusted for triglyceride only (n=407)

Unadjusted Analysis - patients with baseline glucose (n=408)

Adjusted for glucose (n=408)

B  Treatment Effect Unadjusted and Adjusted for Biomarker Change on Overall Survival

Unadjusted Analysis - ITT (n=416)

Unadjusted Analysis - patients with baseline cholesterol (n=339)

Adjusted for cholesterol (n=339)

Unadjusted Analysis - patients with baseline triglyceride (n=407)

Adjusted for triglyceride (n=407)

Unadjusted Analysis - patients with baseline glucose (n=408)

Adjusted for glucose (n=408)
Table 1 – Baseline characteristics of patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Treatment</th>
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<tr>
<td></td>
<td>Interferon</td>
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<td></td>
<td>n=207</td>
</tr>
<tr>
<td>Median age (range) (years)</td>
<td>60 (23–86)</td>
</tr>
<tr>
<td>Age ≥65 years</td>
<td>65 (31)</td>
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<tr>
<td>Sex male</td>
<td>148 (72)</td>
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<tr>
<td>Karnofsky performance score ≤70</td>
<td>171 (83)</td>
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<td>Prior nephrectomy</td>
<td>139 (67)</td>
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<tr>
<td>Histologic type clear-cell</td>
<td>170 (82)</td>
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<tr>
<td>Lactate dehydrogenase level &gt;1.5 times upper limit of normal</td>
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<tr>
<td>Hemoglobin level &lt; lower limit of normal</td>
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<tr>
<td>Corrected serum calcium level &gt;2.5 mmol/L (10 mg/dL)</td>
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<td>Time from initial diagnosis to randomisation &lt; 1 year</td>
<td>164 (79)</td>
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<td>≥2 sites of organ metastasis</td>
<td>165 (80)</td>
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<tr>
<td>Median baseline cholesterol concentration (range) (mmol/L)#</td>
<td>4.2 (1.5–10.4)</td>
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<tr>
<td>Median baseline triglyceride concentration (range) (mmol/L)#</td>
<td>1.4 (0.4–4.5)</td>
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<tr>
<td>Median baseline glucose concentration (range) (mmol/L)#</td>
<td>5.6 (3.1–19.8)</td>
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<tr>
<td>Treatment with statins at baseline</td>
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<tr>
<td>Treatment with fibrates at baseline</td>
<td>1 (0.5)</td>
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<tr>
<td>Treatment with hypoglycemic agents at baseline</td>
<td>20 (10)</td>
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<td>Treatment with fibrates during study</td>
<td>4 (2)</td>
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<tr>
<td>Treatment with hypoglycemic agents during study</td>
<td>1 (0.5)</td>
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</table>

*Values are number (%) unless otherwise labelled.

# Cholesterol 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; glucose values must be multiplied by 18 to convert from mmol/L to mg/dL
Table 2 – Baseline Serum Cholesterol, Triglyceride and Glucose Levels and the Risk of Disease Progression and Death

<table>
<thead>
<tr>
<th>Marker</th>
<th>Progression-free Survival</th>
<th>Overall Survival</th>
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<tr>
<td></td>
<td>Unadjusted Analysis</td>
<td>Adjusted Analysis†</td>
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<tr>
<td></td>
<td>HR* (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Cholesterol (mmol/L#)</td>
<td>0.90 (0.83 - 0.99)</td>
<td>.03</td>
</tr>
<tr>
<td>Triglyceride (mmol/L#)</td>
<td>1.05 (0.92 - 1.19)</td>
<td>.48</td>
</tr>
<tr>
<td>Glucose (mmol/L#)</td>
<td>1.02 (0.97 - 1.08)</td>
<td>.47</td>
</tr>
</tbody>
</table>

*Hazard ratio (HR) is for each mmol per litre unit difference in marker level.
†Analysis 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.

# Cholesterol 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; glucose values must be multiplied by 18 to convert from mmol/L to mg/dL.
Table 3 – On-study Serum Cholesterol, Triglyceride and Glucose Levels and the Risk of Disease Progression and Death

<table>
<thead>
<tr>
<th>Marker</th>
<th>Progression-free Survival</th>
<th>Overall Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted Analysis</td>
<td>Adjusted Analysis†</td>
</tr>
<tr>
<td></td>
<td>HR* (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Cholesterol (mmol/L#)</td>
<td>0.85 (0.78 to 0.92)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Triglyceride (mmol/L#)</td>
<td>0.91 (0.83 - 0.99)</td>
<td>.03</td>
</tr>
<tr>
<td>Glucose (mmol/L#)</td>
<td>0.99 (0.95 - 1.03)</td>
<td>.56</td>
</tr>
</tbody>
</table>

*Hazard ratio (HR) is for each mmol per litre unit change in marker level

†Analysis 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.

# Cholesterol 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; glucose values must be multiplied by 18 to convert from mmol/L to mg/dL.
Table 4 – Impact of Serum Cholesterol, Triglyceride and Glucose Levels on Treatment Effects for Progression-free and Overall Survival

<table>
<thead>
<tr>
<th>Treatment (Temsirolimus Vs Interferon)</th>
<th>Progression-free Survival</th>
<th>Overall Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR* (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Unadjusted Analysis</td>
<td>0.70 (0.58 - 0.86)</td>
<td>.001</td>
</tr>
<tr>
<td>Adjusted for on-study change and baseline cholesterol only</td>
<td>0.88 (0.67 – 1.15)</td>
<td>.35</td>
</tr>
<tr>
<td>Adjusted for on-study change and baseline cholesterol and other baseline factors†</td>
<td>0.86 (0.65 - 1.16)</td>
<td>.33</td>
</tr>
<tr>
<td>Adjusted on-study change and baseline triglyceride only</td>
<td>0.73 (0.60 – 0.90)</td>
<td>.003</td>
</tr>
<tr>
<td>Adjusted on-study change and baseline triglyceride and other baseline factors†</td>
<td>0.68 (0.54 - 0.85)</td>
<td>.001</td>
</tr>
<tr>
<td>Adjusted for on-study change and baseline glucose only</td>
<td>0.71 (0.57 – 0.88)</td>
<td>.002</td>
</tr>
<tr>
<td>Adjusted for on-study change and baseline glucose and other baseline factors†</td>
<td>0.63 (0.50 - 0.80)</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

*Hazard ratio (HR) is for comparison of treatment of temsirolimus versus Interferon

†Analysis 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.
Increase in cholesterol predicts survival advantage in renal cell carcinoma patients treated with temsirolimus

Chee Khoon Lee, Ian Marschner, John Simes, et al.

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