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Integrating cytokines and angiogenic factors and tumour bulk with selected clinical criteria improves determination of prognosis in advanced renal cell carcinoma

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Background: In two clinical trials of the vascular endothelial growth factor (VEGF) receptor inhibitor pazopanib in advanced renal cell carcinoma (mRCC), we found interleukin-6 as predictive of pazopanib benefit. We evaluated the prognostic significance of candidate cytokines and angiogenic factors (CAFs) identified in that work relative to accepted clinical parameters.

Methods: Seven preselected plasma CAFs (interleukin-6, interleukin-8, osteopontin, VEGF, hepatocyte growth factor, tissue inhibitor of metalloproteinases (TIMP-1), and E-selectin) were measured using multiplex ELISA in plasma collected pretreatment from 343 mRCC patients participating in the phase 3 registration trial of pazopanib vs placebo (NCT00334282). Tumour burden (per sum of longest diameters (SLD)) and 10 other clinical factors were also analysed for association with overall survival (OS; based on initial treatment assignment).

Results: Osteopontin, interleukin-6, and TIMP-1 were independently associated with OS in multivariable analysis. A model combining the three CAFs and five clinical variables (including SLD) had higher prognostic accuracy than the International Metastatic Renal Cell Carcinoma Database Consortium criteria (concordance-index 0.75 vs 0.67, respectively), and distinguished two groups of patients within the original intermediate risk category.

Conclusions: A prognostic model incorporating osteopontin, interleukin-6, TIMP-1, tumour burden, and selected clinical criteria increased prognostic accuracy for OS determination in mRCC patients.

Although therapies targeting the vascular endothelial growth factor (VEGF) and the mechanistic target of rapamycin were developed based on understanding the biology of advanced renal cell carcinoma (mRCC), no biomarkers have yet been validated that relate these biological aspects with the individual patient's tumour and need for specific therapy. Although promising tumour-tissue

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and blood-derived markers exist (Jonasch *et al*, 2012; Maroto and Rini, 2014), prognosis for mRCC patients is still largely based upon algorithms utilising clinical features.

In mRCC, many clinical variables have been proposed as prognostic, and some have become validated. The most current prognostic model was presented by the International Metastatic RCC Database Consortium (IMDC) in 2009 (Heng *et al*, 2009). Six variables (two clinical (Karnofsky performance status (KPS) and diagnosis-to-treatment time), four laboratory-based (haemoglobin, corrected calcium, neutrophils, and platelets)) were used to identify three prognostic groups in which different overall survival (OS) outcomes depend on the number of individual risk factors. This model has also been externally validated and found to compare favourably to four other earlier models, including the widely used Memorial Sloan-Kettering Cancer Center (MSKCC) criteria (Motzer *et al*, 2002; Choueiri *et al*, 2007; Escudier *et al*, 2008; Manola *et al*, 2011; Heng *et al*, 2013). Those models, however useful, have reached a ceiling in ability to prognosticate. One possible explanation is that most of the current risk factors represent collateral consequences of biological processes rather than driving forces. Hence, there is a need to incorporate genetic and molecular biomarkers relevant to the natural history and treatment of mRCC into prognostic modelling.

Cytokines and angiogenic factors (CAFs) are broadly profiled to screen for and identify candidate soluble prognostic and predictive markers in RCC (Tran *et al*, 2012; Zurita *et al*, 2012). In mRCC patients with Eastern Cooperative Oncology Group (ECOG) PS 0 to 1 who were treated in clinical trials of pazopanib, we found interleukin-6 as predictive of improved relative progression-free survival (PFS) benefit from pazopanib compared with placebo (Tran *et al*, 2012). Moreover, high osteopontin and interleukin-6 concentrations conferred poor PFS risk independent of clinical factors (Zurita-Saavedra *et al*, 2012), leading us to hypothesise that some CAFs may be independently prognostic for OS and likely to substantially enhance prognostic ability over established criteria. In this analysis, we evaluated the prognostic significance of CAFs for OS together with established clinical parameters (IMDC, MSKCC) and tumour burden.

MATERIALS AND METHODS

Patient data. The study design has been described previously (Sternberg *et al*, 2010; Sternberg *et al*, 2013). Pretreatment plasma samples were obtained from patients with mRCC enrolled in the phase 3 registration study of pazopanib *vs* placebo (VEG105192, ClinicalTrials.gov, NCT00334282; details at: <https://clinicaltrials.gov/ct2/show/NCT00334282>) and provided written informed consent (Tran *et al*, 2012; Sternberg *et al*, 2013). This analysis used baseline ECOG PS instead of KPS.

Analysis of CAFs. Plasma separation methodology is included in Supplementary Online Material. The analysis was completed at a Clinical Laboratory Improvement Amendments (CLIA)-certified facility for seven CAFs (interleukin-6, interleukin-8, E-selectin, hepatocyte growth factor (HGF), VEGF-A (henceforth VEGF), osteopontin, and tissue inhibitor of metalloproteinase-1 (TIMP-1)) with the SearchLight Protein Array (Aushon Biosystems, Billerica, MA, USA), as previously described (Tran *et al*, 2012). These seven were identified from 17 CAFs screened in a previous analysis (Tran *et al*, 2012).

Statistical analysis. Clinical variables and candidate CAFs associated with poor survival were identified using univariable, multivariable, and stepwise Cox proportional hazard models. When testing for proportional hazards, a time-dependent covariate representing interaction between the original covariate and time was used, and the interaction term was tested for statistical

significance. OS results were stratified by treatment assignment at randomisation. Pre-treatment SLD per Response Evaluation Criteria in Solid Tumors was assessed as a \log_2 -transformed continuous variable. Median plasma levels for each CAF were used as the cutoff for high *vs* low analysis, based on the results of sensitivity analyses (Tran *et al*, 2012) and to facilitate clinical application. Concordance (C)-index for each model (median SLD levels were used as the cutoff for high *vs* low analysis in C-index models) was determined as described by Harrell *et al* (1996). Kaplan-Meier analysis was utilised to assess risk groups. Association between CAFs, SLD, and IMDC parameters was assessed using Spearman's correlation coefficient (*R*).

All analyses were *post hoc* and conducted in SAS (v9.2 or later) or R (v3.1.0). *P*-values < 0.05 were considered statistically significant in all models. All tests of statistical significance were two-sided.

RESULTS

Patient disease characteristics and outcomes. Of 435 patients with mRCC enrolled in VEG105192 (Sternberg *et al*, 2010), 343 (79%) with complete information on CAFs at baseline were available for this analysis (pazopanib, *n* = 225; placebo, *n* = 118; Figure 1). At the cutoff date, 322 patients (94%) had discontinued treatment and 114 (33%) were alive. Of the IMDC factors, corrected serum calcium data were missing for 19 patients (5.5%). Because only 5% presented with hypercalcemia and we found no effect for this variable in sensitivity analyses conducted *ad hoc*, we considered those 19 patients as having normal calcium concentrations. A total of 310 patients had data available on diagnosis-to-treatment time: 86 (27.7%) had favourable, 176 (56.8%) had intermediate, and 48 (15.5%) had poor risk according to IMDC prognostic criteria. Baseline characteristics (Table 1) and PFS were similar between the patients included in this analysis and the complete clinical trial set.

Clinical factors and OS. Final OS in the treatment arms was comparable in this study: pazopanib-treated patients, 22.9 months (95% confidence interval (CI), 20.2–25.4), and placebo-treated patients, 20.9 months (95% CI, 15.6–28.8), hazard ratio (HR) 0.91 (95% CI, 0.71–1.16; *P* = 0.224). The similar survival results are likely due to the high rate (54%) of patients' crossover from the placebo arm to pazopanib at the time of progression (Sternberg *et al*, 2013). Of the 11 clinical variables tested (including treatment; Table 2), we found all but corrected serum calcium to be determinants of OS in univariable analysis (Table 3). However, only SLD, serum LDH > 1.5 × upper limit of normal (ULN), high neutrophil count, low serum haemoglobin, and ECOG PS 1 (*vs* 0) were confirmed as predictors of shorter OS in a multivariable stepwise analysis (Table 3). Diagnosis-to-treatment time < 1 year was also a significant factor in the subset with 310 patients (*P* < 0.0001). No effect on gender or ethnic groups was observed.

Prognostic value of individual CAFs and model integration. In our original evaluation of CAFs in the biomarker population based on treatment assignment at initial randomisation, six of the seven CAF candidates (all but E-selectin) were found to be prognostic for OS in both the pazopanib and placebo patient sets using univariable analysis (Tran *et al*, 2012). In the stepwise multivariable analysis that included treatment and the clinical criteria above, we assessed the prognostic significance of each CAF individually. Plasma levels of five CAFs (osteopontin, interleukin-6, TIMP-1, HGF, and interleukin-8) remained independently prognostic in the 343-patient set, and four CAFs (osteopontin, interleukin-6, TIMP-1, and interleukin-8) did so when diagnosis-to-treatment time < 1 year was included (Supplementary Table 1).

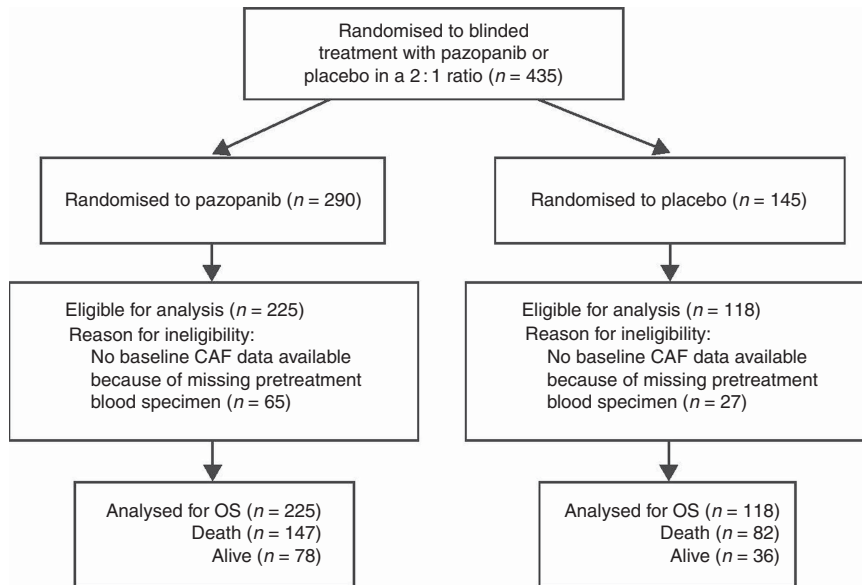


Figure 1. CONSORT diagram. CAF=cytokines and angiogenic factors; OS=overall survival.

Table 1. Patient demographics and disease characteristics

	Overall		CAF subset	
	Pazopanib (n = 290)	Placebo (n = 145)	Pazopanib (n = 225)	Placebo (n = 118)
Median age, years (range)	59 (28–85)	60 (25–81)	59 (31–85)	59.5 (25–81)
Male sex, n (%)	198 (68)	109 (75)	149 (66)	88 (75)
Race, n (%)				
White	252 (87)	122 (84)	196 (87)	97 (82)
Asian	36 (12)	23 (16)	28 (12)	21 (18)
Black	1 (<1)	0	0	0
Other	1 (<1)	0	1 (<1)	0
Median time since initial diagnosis, months (range)	15.7 (0–184)	13.8 (1.0–152)	15.6 (0.7–184)	13.8 (0.8–148)
ECOG performance status, n (%)				
0	123 (42)	60 (41)	93 (41)	44 (37)
1	167 (58)	85 (59)	132 (59)	74 (63)
MSKCC risk category, n (%)				
Favourable	113 (39)	57 (39)	88 (39)	45 (38)
Intermediate	159 (55)	77 (53)	124 (55)	63 (53)
Poor	9 (3)	5 (3)	7 (3)	5 (4)
Unknown	9 (3)	6 (4)	6 (3)	5 (4)
Prior nephrectomy, n (%)	258 (89)	127 (88)	198 (88)	103 (87)
Prior systematic treatment, n (%)				
Treatment naive	155 (53)	78 (54)	119 (53)	59 (50)
Cytokine pretreated	135 (47)	67 (46)	106 (47)	59 (50)
PFS, weeks (95% CI)	40.1 (32.1–56.1)	18.1 (12.1–18.1)	39.6 (32.1–48.1)	13.4 (12.1–19.1)

Abbreviations: CAF = cytokines and angiogenic factors; CI = confidence interval; ECOG = Eastern Cooperative Oncology Group; MSKCC = Memorial Sloan-Kettering Cancer Center; PFS = progression-free survival.

A complete multivariable model incorporating all CAFs, treatment, and clinical variables showed that high (>median) concentrations of three CAFs (osteopontin (>191 627 pg ml⁻¹), interleukin-6 (>13.07 pg ml⁻¹), and TIMP-1 [>676 070 pg ml⁻¹]) and four clinical factors (LDH >1.5 × ULN, SLD, elevated neutrophil counts, and ECOG PS 1) were independent predictors of poor OS (Table 4). The concentrations of the three CAFs were not significantly different in treatment-naïve (n = 178) vs cytokine pretreated (n = 165) patients (median osteopontin 197.9 pg ml⁻¹ vs 180.6 pg ml⁻¹, P = 0.11; interleukin-6 12.8 pg ml⁻¹ vs 13.5 pg ml⁻¹, P = 0.99; and TIMP-1 638.6 pg ml⁻¹ vs 693.8 pg ml⁻¹, P = 0.21). The variable ‘treatment’ did not reach significance (P = 0.06; HR, 0.77); however, diagnosis-to-treatment time <1 year was significant (P < 0.0001) in the subset of 310 patients for whom it was available (Table 4).

In the new prognostic ‘CAF model’ (eight variables, including diagnosis-to-treatment time), the group with very favourable prognosis (no risk factors) comprised 16 patients (5.2%) with median OS not reached (NR; 95% CI, 27.1 months-NR). The following two new groups emerged: favourable (1–2 adverse factors; 107 patients (34.5%)) and intermediate (3–4; 98 patients (31.6%)), with a median OS of 38.9 (95% CI, 30.7-NR) and 19.4 months (95% CI, 15.4–22.9), respectively. The poor prognosis group (≥5 risk factors) comprised 89 patients (28.7%) with a median OS of 7.7 months (95% CI, 5.3–10.9). There were clear distinctions in OS among risk groups (log-rank P < 0.0001). Patient distribution and OS according to the IMDC risk categories and the new CAF model are shown in Figure 2A and B, respectively.

The predictive performance of our final model was internally validated using a bootstrap resampling procedure (n = 300) with

Table 2. Patient distribution as related to components of IMDC and/or MSKCC classifications

Variable, n (%)	Patients (N = 343) ^a
ECOG PS 1	206 (60)
Diagnosis-to-treatment time < 1 year	134 (43)
Haemoglobin <LLN	156 (46)
Platelets >ULN	73 (21)
Neutrophils >ULN	62 (18)
LDH >1.5 × ULN	33 (10)
Calcium >ULN	16 (5)
Bone metastases	99 (29)
Number of metastasis sites ≥ 1	276 (80)

Abbreviations: ECOG PS = Eastern Cooperative Oncology Group performance status; IMDC = International Metastatic RCC Database Consortium; LDH = lactate dehydrogenase; LLN = lower limit of normal; MSKCC = Memorial Sloan-Kettering Cancer Center; ULN = upper limit of normal. The IMDC prognostic variables are KPS, diagnosis-to-treatment time, haemoglobin, corrected calcium, neutrophils, and platelets [3]. The MSKCC prognostic variables are KPS, diagnosis-to-treatment time, haemoglobin, corrected calcium, and lactate dehydrogenase (LDH) [4].
^aN = 343 patients except for calcium (n = 324) and diagnosis-to-treatment time < 1 year (n = 310).

Table 3. Clinical factors that predict shorter patient overall survival

	HR	95% CI	P-value
Univariable covariate model^a			
ECOG PS 1 vs 0	1.827	1.385–2.412	<0.0001
Diagnosis-to-treatment time < 1 year	1.900	1.443–2.503	<0.0001
Haemoglobin <LLN	1.941	1.495–2.521	<0.0001
Neutrophils >ULN	2.074	1.523–2.826	<0.0001
LDH >1.5 × ULN	3.265	2.171–4.909	<0.0001
Calcium >ULN	1.679	0.958–2.942	0.0701
Bone metastases	1.357	1.025–1.796	0.0332
Number of metastasis sites > 1 vs ≤ 1	2.192	1.492–3.221	<0.0001
Baseline SLD	1.532	1.351–1.738	<0.0001
Platelets >ULN	1.528	1.131–2.063	0.0057
Multivariable stepwise OS model			
ECOG PS 1 vs 0	1.368	1.024–1.826	0.0338
Diagnosis-to-treatment time < 1 year	1.892	1.422–2.519	<0.0001
Haemoglobin <LLN vs others	1.565	1.191–2.056	0.0013
Neutrophils >ULN	1.85	1.352–2.533	0.0001
LDH >1.5 × ULN vs others	2.044	1.348–3.101	0.0008
Baseline SLD	1.395	1.234–1.577	<0.0001

Abbreviations: CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; HR = hazard ratio; LDH = lactate dehydrogenase; LLN = lower limit of normal; OS = overall survival; SLD = sum of longest diameters; ULN = upper limit of normal.
^aTreatment variable included in the model; N = 343, except for diagnosis-to-treatment time < 1 year (n = 310).

the same selection criteria as the original model (Table 4). We found > 50% frequency for each of the included variables except for TIMP-1 (46%) in the 343-patient set (TIMP-1 came up 68% of the time in n = 310). The C-index for our new CAF model (n = 310) was 0.75 (95% CI, 0.70–0.79).

Comparative assessment of new CAF model with IMDC criteria. The C-index for our CAF model was substantially higher than that for the IMDC (0.67 (95% CI, 0.63–0.72)). To assess whether the prognostic ability of the IMDC criteria could be improved by the independently prognostic CAFs and SLD, we re-evaluated the effect of the inclusion of these variables and re-calculated the C-index. As expected, the C-statistic improved to 0.71 (95% CI, 0.67–0.75) with interleukin-6 and osteopontin, to 0.72 (95% CI, 0.68–0.77) with the three CAFs, and to 0.73 (95% CI, 0.69–0.77) with the three CAFs and SLD. The C-index of IMDC criteria plus SLD was 0.70 (95% CI, 0.66–0.74). Similar to the effect

Table 4. Stepwise model of independent predictors of overall survival with treatment, CAFs, and clinical risk factors, and bootstrap resampling for internal validation

	HR	95% CI	P-value
Variable (N = 343)^a			
IL-6	1.563	1.156–2.114	0.0037
TIMP-1	1.367	1.029–1.817	0.0311
OPN	1.485	1.066–2.068	0.0192
ECOG PS 1 vs 0	1.387	1.036–1.855	0.0278
Neutrophils >ULN	1.681	1.225–2.306	0.0013
LDH >1.5 × ULN	2.221	1.461–3.378	0.0002
Baseline SLD	1.227	1.077–1.397	0.0021
Variable (N = 310)^b			
TIMP-1	1.522	1.122–2.065	0.0070
OPN	1.611	1.161–2.236	0.0043
ECOG PS 1 vs 0	1.485	1.090–2.023	0.0121
Diagnosis-to-treatment time < 1 year	1.798	1.346–2.401	
Neutrophils >ULN	1.634	1.163–2.296	0.0047
LDH >1.5 × ULN	2.320	1.490–3.614	0.0002
Baseline SLD	1.221	1.069–1.395	0.0032

Abbreviations: CAF = cytokines and angiogenic factors; CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; HR = hazard ratio; IL = interleukin; LDH = lactate dehydrogenase; NEU = neutrophils; OPN = osteopontin; SLD = sum of longest diameters; TIMP-1 = tissue inhibitor of metalloproteinase-1; ULN = upper limit of normal.
^aBootstrap resampling: IL-6 = 79%, TIMP-1 = 46%, OPN = 67%, SLD = 78%, NEU = 74%, LDH = 92%, ECOG PS = 53%.
^bBootstrap resampling: TIMP-1 = 68%, OPN = 57%, diagnosis-to-treatment time = 95%, SLD = 69%, NEU = 62%, LDH = 91%, ECOG PS = 68%.

observed with our CAF model, the incorporation of interleukin-6, osteopontin, or SLD resulted in an obvious separation of the prognosis for patients categorised as intermediate risk by the IMDC criteria (Figure 2C illustrates OS by IMDC risk groups plus SLD).

Association of CAFs with SLD and nonindependent IMDC parameters. We investigated associations between the seven initial CAFs and SLD, as well as the IMDC parameters that were not found to be independent predictors in our patient set (haemoglobin, platelets, and calcium). Six of the seven CAFs (all but E-selectin) showed statistically significant but generally weak positive correlations with baseline SLD (highest R was 0.41, for osteopontin), supporting a prognostic significance for the selected CAFs that goes well beyond a mere reflection of tumour burden (Supplementary Table 2). Moderate negative correlations were seen between the variables haemoglobin and osteopontin (R = -0.52, P < 0.0001) and interleukin-6 (R = -0.51, P < 0.0001), and a positive but weaker correlation between platelets and interleukin-6 (R 0.41, P < 0.0001) (Supplementary Table 3), suggesting a partial causal relationship for these CAFs with the anaemia and thrombocytosis usually found in patients with aggressive RCC. There was no correlation with calcium for any of the CAFs.

DISCUSSION

This retrospective analysis evaluated seven preselected CAFs relevant to mRCC biology (Tran *et al*, 2012) and identified three (osteopontin, interleukin-6, and TIMP-1) as strongly prognostic for OS and independent of established clinical criteria. Two aspects of this analysis differentiate our study from previous research to identify prognostic, circulating biomarkers (Negrier *et al*, 2004; Montero *et al*, 2009; Pena *et al*, 2010). First, our strategy included CAF screening, selection, and validation, and used specimens from two separate, relatively large clinical trials (Tran *et al*, 2012). Second, the CAF analyses were performed under robust CLIA conditions. Results from three independent platforms were highly correlated (Tran *et al*, 2012), supporting the notion that any CLIA-

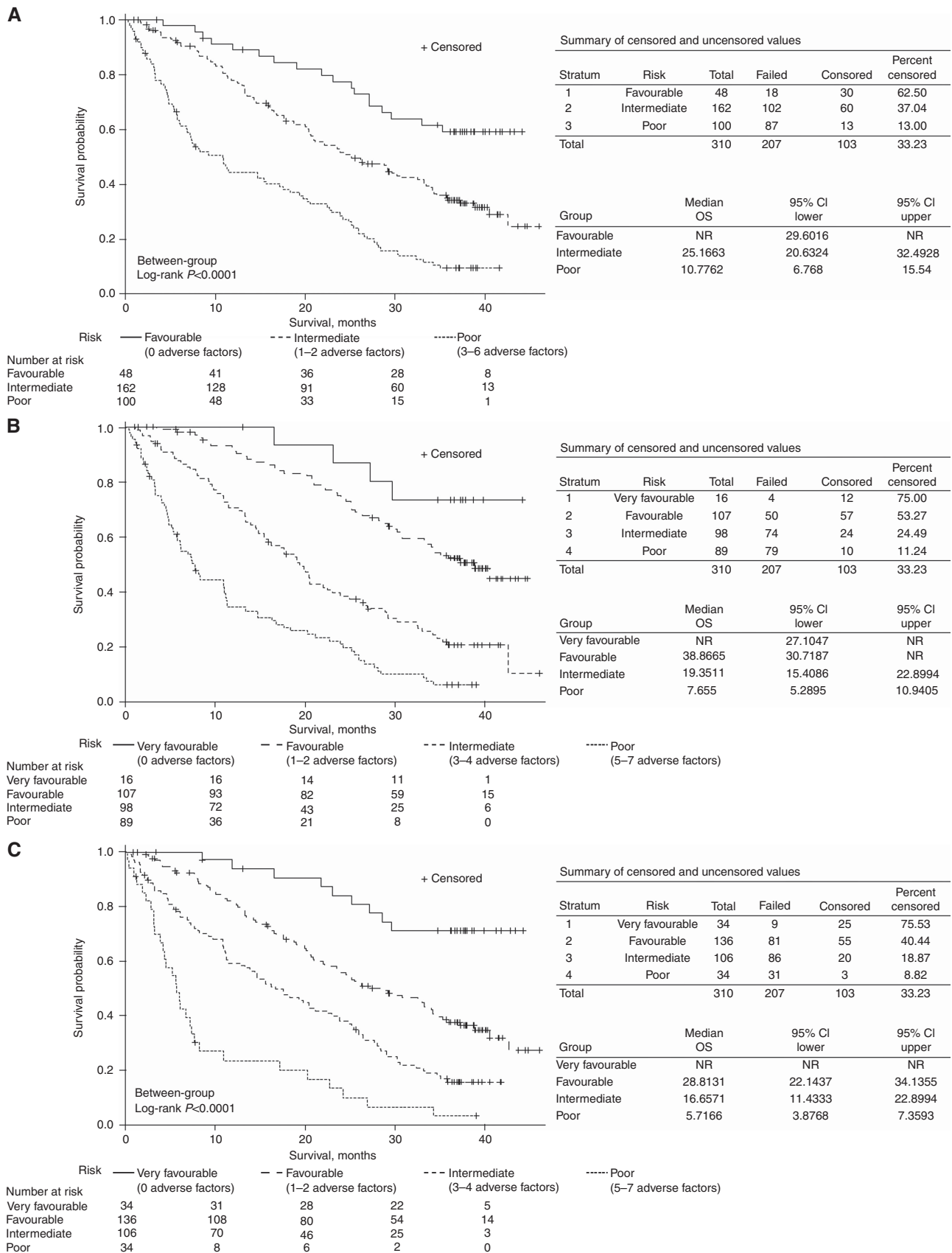


Figure 2. OS by (A) IMDC risk groups, (B) CAF model with IL-6, and (C) IMDC risk groups plus SLD. Adverse factors for (A) PS (0 vs >0), diagnosis-to-treatment time (<1 year), haemoglobin (<LLN), calcium (>ULN), neutrophils (>ULN), platelets (>ULN); (B) PS (0 vs >0), diagnosis-to-treatment time (<1 year), neutrophils (>ULN), SLD (>median), LDH>1.5 × ULN, OPN, IL-6, TIMP-1; (C) as (A) above plus SLD (>median). CAF=cytokines and angiogenic factors; CI=confidence interval; IMDC=International Metastatic RCC Database Consortium; LDH=lactate dehydrogenase; LLN=lower limit of normal; NR, not reached; OS=overall survival; PS=performance status; SLD=sum of longest diameters; ULN=upper limit of normal.

certified assay suitable for routine measurement of osteopontin, interleukin-6, and TIMP-1 could be clinically useful. However, 'high' assay-specific levels would need to be defined for tests other than the SearchLight used here (Tran *et al*, 2012).

Although similar to other phase 3 clinical trials in the frontline mRCC systemic treatment setting the VEG105192 study only included patients with ECOG PS 0–1, 15% of our set had poor prognosis per IMDC criteria. Therefore, we grouped ECOG PS as 0 vs >0 (instead of 0–1 vs 2-higher) in our CAF model (and also for the IMDC), and based the risk groups on this categorisation. When individual CAFs were evaluated in multivariable stepwise models that included the preselected treatment and clinical prognostic factors as variables, high levels of five CAFs (osteopontin, interleukin-6, TIMP-1, interleukin-8, and HGF) showed association with poor OS. However, only osteopontin, interleukin-6, and TIMP-1 were confirmed in the complete model (which included all CAFs, treatment, and clinical variables). Other groups have suggested that interleukin-8 and HGF, and also VEGF, are associated with prognosis and even response and resistance to VEGF signalling pathway inhibitors (Escudier *et al*, 2008; Escudier *et al*, 2009; Huang *et al*, 2010; Nixon *et al*, 2013), but we did not confirm independent prognostic value for any of them in the presence of the other stronger CAFs and clinical variables.

Our results confirm and substantially expand previous studies showing that high levels of interleukin-6 and TIMP-1 are associated with poor OS in patients with mRCC (Negrier *et al*, 2004; Montero *et al*, 2009; Pena *et al*, 2010). Negrier *et al* (2004) reported a negative association with survival for high serum levels of interleukin-6 after analysis of 25 factors that included clinical variables, and circulating VEGF and interleukin-10, in 138 patients with mRCC treated with interleukin-2, interferon- α , or the combination. A separate study showed similar findings for plasma TIMP-1 in 63 patients in the sorafenib phase 3 TARGET trial, but not for VEGF, carbonic anhydrase IX, or Ras p21 (Pena *et al*, 2010). However, to the best of our knowledge, we are the first to report on the independent prognostic value for OS of plasma osteopontin specifically in mRCC.

Beyond their usefulness as biomarkers, CAFs are biologically active mediators with potential to affect tumour behaviour and aggressiveness and serve as surrogates for pathways or mechanisms impacting response and resistance to treatments (Tran *et al*, 2012; Zurita *et al*, 2012). Osteopontin, interleukin-6, and TIMP-1 are known to be involved in pro-inflammatory, pro-tumourigenic, pro-metastatic, and immunomodulatory processes in cancer progression (Bellahcene *et al*, 2008; Jones *et al*, 2011; Ries, 2014), which may drive some of the clinical and laboratory manifestations of RCC aggressiveness and may help explain why the CAFs show superior prognostic ability. We found moderately negative correlations for interleukin-6 and osteopontin with anaemia, and a weaker positive correlation for interleukin-6 with thrombocytosis. Interleukin-6 is considered an autocrine growth factor in mRCC (Miki *et al*, 1989), associated with decreased T-cell-mediated immunity (Narita *et al*, 2013) and a paraneoplastic systemic inflammatory response that includes fever, weight loss, elevated serum C-reactive protein, anaemia, and occasional thrombocytosis (van Rossum *et al*, 2009). However, the specific activities of osteopontin and TIMP-1 most relevant for RCC biology are not well characterised (Kallakury *et al*, 2001; Matusan *et al*, 2006).

Another important finding of our work is the prognostic value of pretreatment tumour burden, which we found stronger than that of haemoglobin, calcium, and platelets, even independent from that of the CAFs, and able to improve the C-index of the IMDC classification. Two smaller studies have evaluated the impact of baseline SLD on survival in patients with mRCC previously untreated with VEGF inhibitors, and in both cases found it significant even after adjusting for established risk scores (Basappa *et al*, 2011; Iacovelli *et al*, 2012). Even without including CAFs, a

purely clinical model that incorporates SLD together with LDH, diagnosis-to-treatment time, neutrophil count, and PS performed noticeably better than that of the IMDC, and should be considered as an alternative worthy of comparison.

Limitations of this study include its retrospective nature, missing CAF data in 21% of patients, minimal racial diversity (mostly whites), survival estimates that may not be representative of the current RCC treatment landscape with the availability of new agents, and lack of external validation. Future research integrating genomic analysis will help assess whether some of these CAFs may reflect distinct mutational profiles or their biological consequences in RCC. Regardless, our results support the use of circulating osteopontin, interleukin-6, and TIMP-1 to better stratify patients by risk and to provide more accurate counselling on treatment and prognosis. Those biomarkers, together with SLD and the selected established clinical parameters, improved predictive accuracy relative to the IMDC model and should be considered for prospective incorporation into mRCC clinical trials for independent validation. We realise, however, the difficulties involved in the routine clinical implementation of the measurement of three CAFs for prognosis. Because interleukin-6 links critical aspects of mRCC biology with response to treatment, it could be prioritised.

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CONFLICT OF INTEREST

AJ Zurita, HT Tran, RA Figlin, TE Hutson, and JV Heymach report no potential conflicts of interest. CN Sternberg reports having received personal fees from Novartis, GlaxoSmithKline, Astellas, Pfizer, and Bayer. RC Gagnon, Y Liu, AM D'Amelio Jr, and LN Pandite were employees of GlaxoSmithKline during the time of study conduct and initial manuscript development.

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